MICROBIAL PROFILING OF FOOD CONTACT SURFACES IN HOSPITALS AT MASERU LESOTHO

by

SALMINA MANKOMANE MALIEHE

Thesis submitted in fulfilment of the requirements for the degree of

MASTERS

in

ENVIRONMENTAL HEALTH

in the

Faculty of Health and Environmental Sciences

at the

Central University of Technology, Free State

Supervisor: Dr Jane Nkhebenyane

Co-supervisor: Prof. Oriel Thekisoe

Bloemfontein, South Africa

2025

DECLARATION OF INDEPENDENT WORK

I, Salmina Mankomane Maliehe, do hereby declare that this research project submitted to the Central University of Technology, Free State, for a MASTER'S degree in ENVIRONMENTAL HEALTH is my own work, and that it has not been submitted before to any institution by me or any other person in fulfilment of the requirements for the attainment of any qualification.

------------------------------------- 10/07/2025----------

SIGNATURE OF STUDENT DATE

Central University of Technology, Free State

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to **God Almighty** who has been my pillar of strength. I thank him for the love, mercy, and guidance he has given me.

I would also like to thank the Mpakanyane and Maliehe families for their support, encouragement, and prayers that carried me throughout this journey.

Mostly, I thank my grandfather, **Ramothobi Mpakanyane**, for believing that I can achieve anything even from a young age.

I wish to express my gratitude to my mother, **Mamonyaka Mpakanyane**, for her faith in me and for persevering in her vision for me. I also thank my son, **Limpho Maliehe**, who has been my beacon of hope.

I am grateful to my supervisors, **Dr Jane Nkhebenyane** and **Prof. Oriel Thekisoe,** for making this dream come true through their professional guidance, patience, and continued support throughout the years of study. I am also thankful to my friend, **Miss Letuka,** and my mentor, **Dr Oriel Taioe,** for their friendship and assistance throughout the course of my study. Thank you, guys!

I would also like to thank the CAFSaB staff and students for their individual assistance, especially **Dr Olga de Smith** for always having an open door and receiving me whenever I came to her, crying and confused.

Lastly, I would like to thank both the National Research Fund (NRF) and the Central University of Technology Research Innovation Fund for financing my studies.

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LIST OF ABBREVIATIONS

AHA	American Hospital Association
PHC	Primary health care
UNICEF	United Nations Children's Fund
WHO	World Health Organization
HACCP	Hazard Analysis and Critical Control Point
IUSS	Integrated Underwater Surveillance System
VHW	Village health workers
MDR/XDR-TB	Multidrug resistant/extra-drug resistant -Tuberculosis
TBA	Traditional birth attendants
FDA	Food and Drug Administration
TBA	Traditional birth attendants
NICD	National Institute for Communicable Diseases
NI	Nosocomial Infections
ICU	Intensive Care Unit
CDC	Centre of Disease Control
ESBL-PE	Extended-spectrum-beta-lactamases-producing Enterobacteriaceae
HIV/AIDS	Human Immuno-deficiency Virus / Acquired Immuno-deficiency Syndrome
UNAIDS	The Joint United Nations Programme on HIV/AIDS
OPD	Outpatient department

HCAIs	Healthcare Associated Infections
SARS	Sever Acute Respiratory Syndrome
MRSA	Methicillin-resistant *Staphylococcus aureus*
ESKAPE	Enterococcus, Staphylococcus aureus, Klebsiella spp. Acinetobacter spp. Pseudomonas aeruginosa and Enterobacter spp.
CHAL	Christian Health Association of Lesotho
PPE	Personal protective equipment
KAPs	Knowledge, Attitudes and Practices
TVC	Total Viable Count
VRBM	Violet-Red-Bile Mug
BRG	Brilliant green Agar
HTG	Horizontal gene-transfer
MDR	Multidrug resistance
EFSA/ECDPC	European Food Authority / European Center for Disease Prevention and Control
CRE	Carbapenem-resistant Enterobacteriaceae
MRSA/VRSA	Methicillin-resistant *Staphylococcus aureus*
VRE	Vancomycin-resistant *Enterococcus faecium*
AMR	Antimicrobial resistance
NTS	Non-Typhoid Salmonella
TMP-SMZ	Trimethoprim-Sulfamethoxazole
DDST	Double disk synergy test

CLSI	Clinical and Laboratory Standards Institute
QAC	Quaternary ammonium compounds
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration

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SUMMARY

Food handlers play a key role in ensuring food safety throughout the food production, processing, storage, and preparation chain. In food service establishments, the common sources of microbial contamination reportedly originate from dirty food contact surfaces, poor personal hygiene practices, and inappropriate storage temperatures. Contamination primarily arises from food contact surfaces such as utensils, equipment, and food handler hands or clothing.

The overall purpose of this study was to investigate microbial contamination of food contact surfaces in hospital kitchens in the city of Maseru, Lesotho. The objectives were to assess food handlers' knowledge, attitudes, and hygiene practices and to characterize prevalent hospital foodborne pathogens associated with food contact surfaces. The study also evaluated the efficacy of common disinfectants against microorganisms and investigated the production of extended-spectrum beta-lactamase (ESBL) against Gram-negative bacteria.

The study was conducted in five hospitals involving food handlers ranging between 2-7 per hospital. A total of 26 food handlers participated in the study. A structured questionnaire that had been derived from previous studies was used to assess the food handlers' knowledge, attitudes, and practices (KAPs). The microbial isolates were identified to specie level by use of biochemical test kits (Staph API, RAPID ONE and RAPID NF Plus). The identified Gram-negative bacteria were subjected to a series of antimicrobials in order to determine the susceptibility profile using the Kirby-Baur disc diffusion method. The phenotypic tests for ESBL detection were used to test for ESBL production with subsequent double disc synergy test used as a confirmation method. Isolates were further subjected to the Kirby-Baur disc diffusion method to establish susceptibility to common disinfectants. Subsequently, the minimum inhibitory concentrations and the minimum bactericidal concentrations were determined by using the in-vitro susceptibility test employing 96-well microtiter plates.

More female (n=23; 88%) than male (n=3; 12%) food handlers participated in the study. The majority of these food handlers (n=25; 96%) were aware that proper cleaning and sanitization of utensils decreases the risk of contamination. Although 54% (n=14) of the food handlers reported that they had received training, none of the food handlers were could provide formal documentation to verify their training. However, there was general consensus (n=26; 100%) that food safety training is important. Despite the evident lack of knowledge regarding refrigeration and holding temperatures for hot ready-to-eat foods, the food handlers agreed that refrigerators/freezers should be checked regularly to reduce the risk of contamination.

The microbial analysis study revealed that food contact surfaces, including food handlers' hands, yielded high counts of all detected microorganisms (TVC, total coliforms, *Escherichia coli*, *Staphylococcus* sp., *Salmonella* sp., and *Pseudomonas* sp). This indicated a discrepancy between knowledge of food processing and of environmental and personal hygiene. In natural settings, microorganisms can be introduced in various ways by means of people, food, water bioaerosols, and domestic animals. The acquisition of microorganisms such as *Stenetrophomonas maltophilia, Sphingomonas paucimobilis,* and *Myroides odorantum* that are predominantly associated with clinical infections was evidence of possible cross-contamination from the hospital wards to the kitchen.

The investigation of the extended-spectrum beta-lactamase (ESBL) production of Gram-negative bacteria revealed positive ESBL production in 43% of the microorganisms. This is an important finding given that the ESBL-producing genes can be transferred within niches and the production of these enzymes confers resistance to most commonly used antibiotics. Additionally, there was a 71% multidrug resistance among isolates and none of the microorganisms tested showed susceptibility to current antibiotics. However, a 100% resistance to penicillin was noted which affirms the growing resistance of microorganisms to common antibiotics and the need for new treatment options. The high resistance profile demonstrated by microorganisms in this study can be attributed to personal prescription of antibiotics as these antibiotics are available at pharmacies without a doctor's prescription.

The investigation of the efficacy of the disinfectants against the Gram-negative and Gram-positive bacteria revealed that both disinfectants 1 and 2 exhibited antimicrobial properties against the tested microorganisms. However, disinfectant 2 was found to have bactericidal effect against only the Gram-positive bacteria. The results therefore indicated that although there was an antimicrobial effect of disinfectant 2 against Gram-negative bacteria, disinfecting surfaces with such a disinfectant will not render the surface free from Gram-negative bacteria.

Keywords: food safety, contamination, hospital kitchen, disinfectants, extended-spectrum beta-lactamase, multidrug resistance

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1 Introduction

A hospital is an institution that focuses on health care and it is therefore mandated to render various forms of health treatment and to make intensive efforts to reduce hospital-acquired infections. According to the World Health Organization a hospital should have at least one permanent physician, can offer inpatient accommodation, and can provide active medical and nursing care. The American Hospital Association (AHA) also considers a hospital as an institution that is licenced and has at least six beds whose primary function is to provide diagnostic and therapeutic patient services during a medical condition (CDC, 2024).

In many developing countries such as Lesotho, hospitals are mainly used for clinical care and training. Therefore, depending on the health system that has been adopted in a specific area, health professionals may advise and coordinate care delivery within the community while effective and high-quality clinical services are provided in hospitals. This is achieved through the inclusion of community health workers and traditional birth attendants (Seutloali *et al*., 2018). This approach to basic service delivery is commonly known as primary health care (PHC). According to UNICEF (1978), PHC is:

> "…an essential health care based on practical, scientifically sound and socially acceptable methods and technology made universally accessible to individuals and families in the community through their full participation and at a cost that the community and country can afford to maintain at every stage of their development in the spirit of self-reliance and self-determination. It forms an integral part both of the country's health system, of which it is the central function and main focus, and of the overall social and economic development of the community."

Hospitals influence the health of communities through medical interventions and through nutritious meals served to in-ward patients during their hospital stay. During hospitalization, meals that are served to patients are a critical component of care in the quest to improve patient well-being (Fernando and Wijesinghe, 2017). Hospital patients are provided with nutritional meals and thus meal models are designed specifically for their recovery (Wilandh *et al*., 2025). To execute this mandate, consideration of the microbial risk associated with food is vital. Therefore, low microbial diets that exclude uncooked fruits, vegetables, cold cuts, undercooked eggs and meat, unsterilized water, unpasteurized milk products, and soft cheeses need to be served to patients (Hanekom *et al*., 2010; Lund, 2014).

Providing patients with safe food is a fundamental principle in hospital food services. Implementing a food safety program known as the Hazard Analysis and Critical Control Point (HACCP) is key in food services to assist in the reduction of any form of contamination of food, from the point of food production to delivery (Basalamah *et al*., 2018; Awuchi *et al*., 2021). According to Awuchi, (2023), HACCP involves the systematic study of food ingredients and products as well as the conditions of food processing, handling, storing, packaging, and distributing. It also includes the evaluation of loss of control as it is important to curb and eradicate unacceptable food safety risks. It is important to implement HACCP throughout the food chain as food can be contaminated at any point-from food reception to serving of meals to patients (Basalamah *et al*., 2018).

According to Marzano, (2011), there are two major methods of catering currently used in hospitals: (1) the conventional (or classical) cook-serve and cook-warm-hold methods, and (2) the innovative cook-freeze, cook-chill and *sour vide* cooking (cooling under vacuum) method. The cook-serve method is conventional and commonly used in hospitals by catering services (Ahmed *et al*., 2015). In this method, food is prepared and cooked for each meal and served whilst hot (IUSS Health Facility Guidelines, 2014). According to these guidelines (2014), all catering services in a hospital should be located on the same level to enable effective workflow. This means that these services should be located centrally and have a direct connection to most corridors to ensure a short food delivery time, as illustrated in Figure 1.1. Additionally, catering services should be located at the same level as the hospital wards in order to limit the movement of people.

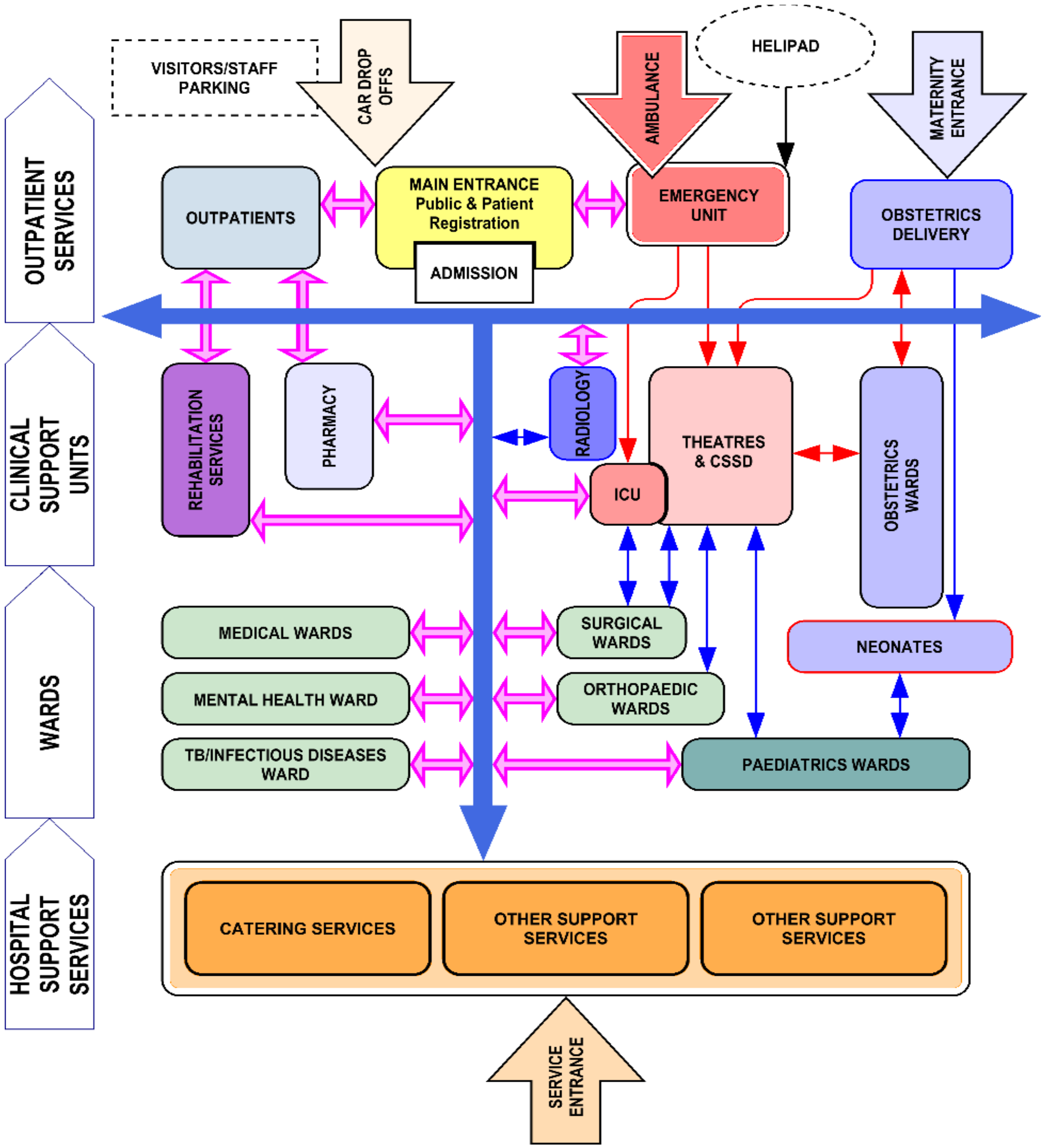

Figure 1.1: Ideal hospital layout indicating service relationships

Source: IUSS Health Facility guidelines, 2014

1.2 Literature review

1.2.1 The Lesotho health care system

The Kingdom of Lesotho is a monarchy and its borders are completely land-locked by the Republic of South Africa (Seeiso, 2009), as shown in Figure 1.2. It is situated at 28^0 to 30^0 latitude and 27^0 to 30^0 longitude and covers an area of 30 359 square kilometres. It is topographically divided into four zones based on altitude: the lowlands, the foothills, the mountains, and the Senqu River valley. The country has a population of 2213 939 citizens who are mainly Sesotho speaking. English is the official language, while Sesotho, IsiXhosa, and IsiZulu are also spoken (Mundi, 2018). It is divided into 10 districts that each falls under the administration of a District Administrator (DA) who is located in the capital of the district that is commonly known as Camp Town. There are 120 community councils and health inspectors are responsible for food safety in each municipality (Seeiso, 2009).

Figure 1.2: Map of Lesotho showing the 10 districts that are indicated by a dotted bullet. Food handlers were sampled from Maseru city in the Maseru district (highlighted in green).

The primary health care (PHC) system was adopted in Lesotho in 1978. This means that, in some parts of the country, community level health services are delivered with the cooperation of village health workers (VHWs) and traditional birth attendants (TBAs). Although traditional healers are not formally included in the health system structure, they are important to the system and are regulated by the Lesotho Ministry of Health (Public Health Order, 1970). The Lesotho government owns eight of the public hospitals and about 50% of health centres that serve 52% of the population. A conglomerate of other public health care facilities that serve 48% of the population exists mainly in rural areas. These are owned by six churches that are administered by the Christian Health Association. There are three referral hospitals (general, MDR/XDR-TB, Mental), and there is one military hospital and two private hospitals in Maseru. Two more private hospitals are located in another district while many private medical facilities are owned by doctors and nurses and these are spread across all ten districts. Private medical outlets such as pharmacies and chemists are also spread across the districts with the highest density within the Maseru urban area (MoH, 2014-2020).

1.2.2 Presentation of foodborne diseases in the community

A foodborne disease is any disease of an infectious or toxic nature caused by or thought to be caused by the ingestion of contaminated food or water (Kadariya *et al*., 2014; CDC, 2025b). This type of disease is a common cause of gastroenteritis and mainly results from the consumption of food containing pathogens such as bacteria, viruses, parasites, or food contaminated with poisonous chemicals or bio-toxins (Shimul, 2017). Although the majority of foodborne illness cases are mild and self-limiting, severe cases can occur amongst high-risk groups such as infants, young children, the elderly, and people with compromised immune systems. Severe cases of food poisoning are known to result in high mortality and morbidity in susceptible groups (Barton *et al*., 2011).

Most foodborne outbreaks are usually caused by common factors that are associated with the onset of events. These include poor personal hygiene, inadequate food holding time and temperatures, cross-contamination, lack of adequate heat treatment, improper food storage, poor sanitation, a poor attitude towards food hygiene, as well as overcrowding (Osei-Tutu and Anto, 2016; Kinyau, 2024). However, the causative agent of the majority of reported foodborne illness outbreaks is mostly unknown. Such outbreaks are due to certain issues related to outbreak investigations, including a lack of timely reporting, a lack of resources for investigations, and poor prioritising in health departments (Hall *et al.,* 2012). Additionally, determining the causative agent of a foodborne outbreak is a challenge due to limited reporting of sickness and testing of specimens.

According to the Food and Drug Administration (FDA) (2019), a foodborne illness outbreak is defined as "the occurrence of similar symptoms or illness in two or more individuals who have eaten the same contaminated food". These outbreaks are investigated systematically with the aim of finding the etiology of the disease. It is important that foodborne illness outbreak investigations are timeous and that proper environmental assessments are conducted to identify appropriate prevention strategies (Marriott *et al.,* 2018). In South Africa, the National Institute for Communicable Diseases (NICD) (2024) has developed a brief reference guide to assist in the investigation of foodborne disease outbreaks. This guide provides a summary of the essential steps in the investigation of foodborne disease incidents to be used by public health personnel in the field. It urges that investigations have to be prompt due to a limited window of opportunity following the onset on the illness. Timely investigations provide an opportunity to reduce morbidity and mortality through enforcing early and effective interventions (Shonhiwa *et al.,* 2018).

1.2.3 Common microorganisms in food processing and clinical settings

Bacteria are the most prevalent pathogens among food-related infections, although viral and fungal pathogens are also common. Therefore, it is common to isolate non-*typhoid Salmonella species*, *Campylobacter* spp., *Yersinia enterocolitica*, *Shigella* spp., and diarrheagenic *Escherichia coli* that cause gastrointestinal infections (Duda-Madej *et al*., 2025). Although most infections are acute to mild, severe cases of extra-intestinal manifestations such as pneumonia, endocarditis, meningitis, and abscesses have also been reported (Roshan-Zamir *et al*., 2025).

Nosocomial infections (NI), which develop in a patient during the patient's stay in a hospital or other type of clinical facility, are infections that were not present at the time of admission (Ahmad *et al*., 2021). They may become clinically apparent either during the hospitalization period or shortly after the patient has been discharged. NIs are common in patients admitted to the surgical intensive care unit (ICU) due to their state of immobility, surgical incisions, multiple invasive monitoring lines, urinary catheters, and mechanical ventilation (Kafazi *et al.,* 2025). Although *Salmonella* spp., rotavirus and norovirus are associated with diarrhoea in hospital ICUs, *Clostridium difficile* is the leading cause of infectious diarrhoea in ICUs (Bobo *et al.,* 2010; Polage *et al.,* 2012). Similarly, in the food environment *C. difficile* and *C. perfringens* are known to occur in food, particularly in meats, vegetables, and shellfish (Borji *et al*., 2023).

1.2.4 Contact surface cleaning and disinfection

Cleaning is a complex systematic process of removing organic and inorganic substances from surfaces by employing detergents or enzymatic products to ensure that subsequent disinfection will be effective (Rutala and Weber, 2016). Disinfection is employed to destroy most microbial forms (especially vegetative pathogens, with the exception of spores) by using physical and chemical procedures such as UV radiation, boiling, and vapouring (Kirmusaoglu, 2018). Using these processes has become a very important multi-barrier approach to preventing infections (Hausemann *et al*., 2018).

Cleaning and disinfection are processes commonly employed in both domestic and clinical settings to combat the presence of pathogenic microorganisms. For example, to avoid the occurrence and spread of existing infections between two or among many patients, hospitals employ certain infection control measures, including hand hygiene (Mathur, 2011). This is because, in the event of poor hand hygiene, health workers alternating between patients may transfer infections from contaminated surfaces to patients (Russotto *et al*., 2015). The most common disinfectants used in hospitals are quaternary ammonium compounds, chlorine containing compounds and phenolics or improved hydrogen peroxide (HP) (Rutala and Weber 2016). Disinfectants such as peroxides, peroxyacid compounds, acid anionic, and iodine are used in food premises while quaternary ammonium compounds and chlorine compounds are used in both food processing and hospital settings (Gaulin *et al*., 2011).

1.3 Rationale

1.3.1 Problem statement

Food-related infections constitute an important health problem in both developed and developing countries. Globally, foodborne illnesses that cover a wide spectrum of diseases result in morbidity and mortality, more especially in susceptible groups. In severe cases of a foodborne illness, symptoms include severe dehydration, gastrointestinal perforation, and septicaemia. Chronic symptoms (chronic sequelae) of foodborne illnesses include reactive arthritis, septic arthritis, and cardiac manifestations (Gharib *et al*., 2022; Cheeti *et al*., 2025). However, symptoms of foodborne illnesses are usually relatively mild, hence infections often go unnoticed.

According to records that were issued by the US Center of Disease Control (CDC), an estimated 76 million people get sick, more than 300 000 are hospitalized, and 5 000 die each year from foodborne illnesses in America (CDC, 2025a). In Italy, approximately 30 000 cases per year have been recorded with 55-75% of these cases due to foodborne outbreaks, of which 25-40% were traced back to homemade preparations (Langiano *et al.,* 2012). Similarly, many reports of South African residents who contracted food poisoning are issued every year. However, according to Seeiso (2009), in Lesotho there is very little information available on the true level of exposure of specific populations to potential foodborne hazards, particularly in cases where a bacterial disease has been transmitted through meal consumption.

Hospitals cater for groups of people who are especially sensitive to foodborne infections. Although it is impossible to quantify the precise extent of this influence, hospital kitchens can play a role in the spread of pathogens to patients. Therefore, as most people who are catered for within the hospital setting have a suppressed immune system, hygiene management in hospital catering must go beyond the normal measures of communal catering (Haddad, 2018). Osei-Tutu and Anto (2016) state that several factors contribute to the emergence of foodborne illnesses in different locations, and they specifically mention the spread of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-PE) during outbreaks. Common microorganisms such as *E. coli* and *Klebsiella* spp. have been proven to survive months on dry inanimate hospital surfaces (Poter *et al.,* 2024). Added to this is their ability to show resistance to various pH levels, NaCl concentrations, and numerous antimicrobial substances. This aids the ability of common microorganisms such as *L. monocytogens* to persist in the food environment (Osek *et al*., 2022; Manyi-Loh *et al*., 2025). Therefore, in both the hospital environment (Bencardino *et al*., 2021; CDC, 2025c) and the food service environment, surfaces and asymptomatic workers (health care personnel, patients, visitors, and food handlers) are sources of infection and preceding contamination.

1.3.2 Justification for the study

A hospital is a place where immunocompromised people depend on healthy meals for the improvement of their health status (Sathiaraj *et al*., 2019). However, the food that is served can be contaminated by the environment in which it is produced. Environmental contamination can occur through water, air, surfaces, and personnel (Abdolshahi and Yancheshemeh, 2020), and therefore ensuring a contamination-free environment should be a priority in food production unit in each hospital. To minimize the potential for foodborne illnesses to thrive, food handlers in Lesotho commonly have to obtain a food handler certificate. This is preceded by food safety training and a number of clinical investigations that include: (1) stool microscopy for the detection of parasites (2) a stool culture to detect *Salmonella* spp. and *Shigella* spp.; and (3) a Widal agglutination test to detect antibodies of *Shigella* sp. and *Salmonella* sp. (Meurs *et al*., 2017). However, following food handlers' safety training, their knowledge, attitude, behaviour, and practices need to be investigated to ascertain whether they might potentially compromise the health of patients.

Food hygiene is particularly important in hospitals where patients are highly vulnerable to the risks that microbiological infections and poor nutrition pose. In hospitals, a large number of people can be exposed to infections and their resultant complications. Additionally, malnutrition, food poisoning, and nosocomial infections can lengthen a patient's stay in hospital. Lesotho is a country that has been hit hard by HIV/AIDs (UNAIDS, 2018); hence many residents run the risk of hospitalization where they are prone to both hospital-acquired and community-acquired opportunistic infections. Their health condition can thus be exacerbated by foodborne infection. There is also the added risk of compromised health due to antibiotic resistance (ABR) to most microorganisms (Olaru *et al*., 2021; Salam *et al*., 2023). Therefore, it is important to identify and manage possible sources of exposure to pathogenic microorganisms, including those that can be introduced by food in hospitals.

1.3.3 Research Aim

The aim of the study was to investigate the extent of microbial contamination of food contact surfaces in hospitals in the Maseru urban area in Lesotho.

1.3.4 Research Objectives

The objectives of the study were to:

- Assess food handlers' food safety knowledge, attitudes, and hygiene practices from food preparation to food consumption.
- Characterize hospital foodborne pathogens associated with food contact surfaces in the hospitals under study.
- Investigate the production of ESBL in Gram-negative bacteria detected in the hospitals under study.

* Investigate the efficacy of common disinfectants against detected microorganisms in the hospitals under study.

1.4 References

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CHAPTER 2

ASSESSMENT OF FOOD HANDLERS' KNOWLEDGE, ATTITUDES AND PRACTICES

2.1 Abstract

A hospital environment is occupied by hospital workers and patients with compromised immune systems. Food is prepared and served to patients and staff in any hospital environment. However, hospitalized patients are more vulnerable to potential hazards than people with healthy immune systems. Therefore, the implementation of food safety measures by the hospital food service staff is a necessity for the prevention of foodborne outbreaks in this setting. Clearly, any negligence and ignorance of these principles can result in increased morbidity and mortality. The aim of this study was to investigate the level of knowledge, the attitudes, and practices of food handlers working in hospitals in Maseru, Lesotho. The study was conducted using a descriptive survey in five hospitals by administering a semi-structured questionnaire to 26 food handlers. The majority of the food handlers were above 40 years of age with a mean SD of 40 ± 10.67. Most of the respondents were female while only 12% was male. The food handlers had a high level of knowledge (92%) of cross contamination. All the food handlers were also knowledgeable about the importance of washing utensils with a suitable detergent and washing hands before handling food. However, 88% of the respondents lacked knowledge on the storage temperature of ready-to-eat food, while 81% lacked knowledge on the storage temperature of perishable food items. In general, the hospital food handlers had satisfactory knowledge (64.15: SD = 11.00), a positive attitude (88.85: SD = 10.13), and good self-reported food safety practices (70.28: SD = 11.94). Based on the findings, it is argued that there should be regular food safety training and health education programs with clear indications of the risk of pathogenic infections (e.g., HIV/AIDS) and related pathogenic infection transmission modes.

Keywords: hand hygiene, knowledge, attitudes and practices

2.2 Introduction

The prevention of food-related infections relies on a combination of good hygiene practices during food preparation as well as appropriate cooking and storage habits. Food- and waterborne infections are a serious public health problem in both developed and developing countries globally (Jones *et al*., 2017; Yang *et al*., 2012). The global estimate of annual incidences of foodborne diseases, particularly those due to diarrhoea and invasive infections caused by non-typhoidal *Salmonella enterica,* has been as high as 230 000 (WHO, 2015). Additionally, WHO (2018) reports that diarrheal disease in Lesotho is among the top ten diseases in outpatient departments (OPDs). The latter disease is commonly associated with the ingestion of microorganisms acquired directly through food handling and preparation and by eating food that was directly in contact with surfaces that harbour such organisms, or with aerosolized water (Akhondi *et al*., 2025).

Globally, healthcare-associated infections (HCAIs) are no longer seen as a minor burden but as a major barrier to delivering health. This is further compounded by the generalized acceptance that controlling organisms that cause infections, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* (*C. difficile*), and norovirus, is a community as well as a hospital problem (CDC, 2024a). Additionally, the danger of emerging infections such as HIV/AIDS and Sever Acute Respiratory Syndrome (SARS) and the burden of hygiene- and sanitation-related infectious diseases (such as diarrhoeal, vector-borne, and respiratory diseases) continue to be the most critical public health threats (De Gaetano *et al*., 2025). Therefore, cost-effective infection prevention and control programs that focus on hand and surface hygiene are important in controlling cross-infection and cross-contamination (Toney-Butler et al., 2023; CDC, 2024b).

According to Monegro *et al*. (2023), it is estimated that 648 000 patients acquire 721800 hospital acquired infections (HAI) annually in acute care hospitals. Additionally, according to the CDC, (2025) about 47.8 million illnesses, 127839 hospitalizations, and 3037 deaths occur in a year due to domestically acquired foodborne outbreaks in the United States of America (USA). All these were associated with foodborne infections (Moabi, 2016) of which the majority had been caused by *C. difficile* and organisms of the ESKAPE group (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, and *Enterobacter* spp.) (Fernandez-Garcia *et al*., 2016). These microorganisms exist naturally in the environment and are commonly found on the hands of healthy individuals from where they are transmitted to multiple surfaces (Dennissen *et al.,* 2022; Said *et al*., 2024). Consequently, the food service industry deems food handlers' hygiene practices as an important aspect in the transmission of foodborne diseases (Engdaw *et al*., 2023).

2.3 Food handlers' knowledge and their role in food safety

Hospital kitchens and canteens cater for a large population consisting of hospital staff, patients, and visitors. In this setting, food is prepared in large quantities, and it is important that food handlers maintain a high standard of food safety (Bieke *et al*., 2019; Tola *et al*., 2025). According to the Codex Alimentarius Commission a food handler is any person who handles packaged or unpackaged food and food production equipment and utensils or is in contact with any other food preparation surfaces (FAO/WHO, 2003). These handlers are therefore expected to adhere to all food hygiene requirements. In a hospital setting, food handlers are kitchen workers, nurses, and people who are involved in the preparation, distribution, and serving of food to patients.

According to Juma (2017), the risk of food contamination is associated with the health status of individual food handlers, their personal hygiene, and their knowledge and practices in maintaining high food hygiene practices. Knowledge is a person's cognitive ability based on remembering, relating to, or judging an idea or abstract phenomenon (Thanh, 2015). Dudeja *et al.* (2016) highlight that there is a positive correlation between knowledge, attitude, and practice and that training improves the knowledge of food handlers (Zhang *et al.,* 2015). However, a wealth of literature states that food handler training for improved knowledge of food safety practices does not always lead to positive changes in food handling behaviours (Ansari-Lari *et al.,* 2010; Dudeja *et al.,* 2016).

Therefore, merely completing the legal requirement to undergo food safety training does not guarantee safe food production.

2.4 Food handlers’ attitude and their role in food safety

According to a Princeton University definition, the term ‘attitude’ refers to a complex mental state involving beliefs, feelings, values, and dispositions to act in a certain way (Kumar and Lal, 2024). Food handlers’ attitudes, particularly of those who work in developing countries, are influenced by cultural beliefs, taboos, prejudices, and superstitions which could affect the nutrition and health care of consumers (Mendagudali *et al*., 2016). Consequently, a careless attitude towards food hygiene and incorrect practices by food handlers can jeopardize the safety of food and cause foodborne illnesses (Dudeja *et al*., 2016). For this reason, Nkhebenyane *et al*. (2012) argue that good hygiene practices, including proper hand washing and food handling, are essential in the reduction and prevention of the spread of infectious diseases in all health care settings.

2.5 Food handlers’ practices and their role in food safety

Prior to food-based work, experience and education are deemed important in ensuring proper food handling practices by all food handlers. According to Alqurashi *et al*. (2019), in order to ensure good hygiene practices and an understanding of the concepts related to the safety of food within healthcare facilities, a food handler must have the required knowledge and skills. This requires a training program that offers the theoretical aspect of food safety covering important topics such as proper hand washing techniques, preventing cross-contamination, and temperature and storage requirements (Alkhaldi *et al*., 2025). The program also provides practice-based training with simulation and/or role playing both in interactive and hands on manner (Barrett and Feng, 2021).

According to Castro *et al*. (2024), the combined effect of theoretical and practical training on food safety enhances the ability of food handlers to learn and their food safety practices. Additionally, Viveiros, (2021), asserts that employing supervisors who hold certificates is important in reducing non-compliance and improving the prevention of foodborne outbreaks in restaurants and, by extension, in hospitals. A wealth of literature has reported that supervisors with certificates are more proactive during food handler training than those without certificates, although the relationship between knowledge, attitudes, and practices is not always evident (Reboucas *et al*., 2017).

In Lesotho, both private and public institutions usually have food service catering units where meals are prepared and served to both clients and staff. Such institutions include schools, army or police barracks, hospitals, and prisons. To prevent outbreaks of foodborne diseases in these institutions, quality standards of hygienic and safety practices by food handlers are essential to ensure an overall food safety program (Akabanda *et al.,* 2017). Therefore, training and education are essential in ensuring that workers have the awareness and knowledge that are necessary to comply with food hygiene demands (Seaman and Eves, 2008). Training of food handlers is also an important part of the Hazard Analysis and Critical Control Point (HACCP) concept and is recognized by WHO (Garayoa *et al.,* 2011). The HACCP concept is a food safety program that ensures the control of problems in food processing before they occur (McSwane *et al.,* 2003).

2.6 Materials and methods

2.6.1 The study area

The study was conducted in five hospitals in the Maseru urban environment. These hospitals were: Maseru Private Hospital situated at Ha-Thetsane, Scott Hospital situated at Morija, St Joseph Hospital situated at Roma, and the Bots'abelo Hospital and Mohlomi Mental Hospital situated at Bots'abelo. Mohlomi Mental Hospital and Bots'abelo Hospital are government-owned specialized referral hospitals (National Health Strategic Plan, 2016), while Scott Hospital and St Joseph Hospital are owned by the Christian Health Association of Lesotho (CHAL). The Maseru Hospital is privately owned. Bots'abelo hospital houses the Senkatana Centre of Excellence for cervical cancer prevention and HIV/AIDS management. It also has a tuberculosis (TB) outpatient department (OPD) and a multi-drug resistant tuberculosis (MDR-TB) unit. In this hospital, there is a shared hospital kitchen that caters for inpatients who are accommodated in the Senkatana Centre and who are part of the MDR-TB project.

2.6.2 Sample size and study design

The study was conducted in five hospitals as mentioned previously. There are 37 - 200 beds per hospital, with Mohlomi hospital having the highest number of beds. The number of food handlers ranged from 2-7 per hospital, therefore a total of 26 food handlers were selected as the sample to participate as respondents in the study.

2.6.3 Ethical considerations

A structured peer-reviewed ethical informed consent form was developed. The document explained the study objectives and guaranteed the participants' privacy. The informed consent form was presented in the two official languages, namely Sesotho and English. The study was approved by the Faculty Research and Innovation (FRIC) and University Research and Innovations (URIC) committees at the Central University of Technology in Bloemfontein, and by the Ministry of Health Research and Ethics Committee of Lesotho, REF: ID93-2017.

2.6.4 Data collection

The questionnaire was structured into five different sections. Section 1 consisted of food handlers' demographics, Sections 2, 3, 4 elicited responses regarding the food handlers' knowledge, attitudes and practices, and Section 5 was an observational checklist. Demographic characteristics such as gender, age, level of education, and duration of employment in the food service industry were the focus in Section 1. Questions pertaining to employees' food safety knowledge, attitudes, and practices were adapted from

Akabanda *et al.* (2017). The section of the questionnaire dealing with food safety knowledge comprised 25 closed-ended questions with three possible responses: ‘true’, ‘false’, and ‘do not know’. These questions specifically dealt with the respondents’ knowledge of personal hygiene, cross-contamination, foodborne diseases, microorganisms, temperature control settings, and hygienic practices. A scale ranging from 0-25 (representing the total number of questions on food knowledge) was used to evaluate the overall knowledge of the respondents. Food handlers who obtained a total score of ≤16 points were considered to have ‘insufficient’ knowledge and those who had a total score of ≥17 points (≥ 68% accuracy) were considered to have ‘good’ knowledge of food safety.

Questions pertaining to ‘attitude’ determined the food handlers’ understanding of food safety. In this context, attitude meant the “complex mental state involving beliefs, feelings, values, and dispositions to act in certain ways” Rao *et al*., (2021). This section had 20 statements/questions that had three possible responses: ‘agree’, ‘disagree’, and ‘don't know or remember’. For assessment purposes, food handlers who answered 13 or fewer statements/questions incorrectly were assumed to have ‘insufficient’ or ‘poor’ understanding, whereas those who correctly responded to 14 or more statements/questions were assumed to have ‘good’ understanding.

The last section dealt with food hygiene practices. The hygiene practices of the respondents (institutional food handlers) were assessed and evaluated based on self-reporting of personal hygiene and other safe food handling practices. The section had 11 statements/questions with two possible responses: ‘yes’ and ‘no’. Each correct practice reported scored one (1) point. For evaluation purposes, a score of ≥70% by an individual respondent was considered as having “good” hygiene practices.

2.7 Statistical analysis

Data from the questionnaires were captured electronically in Microsoft Excel by the researcher. All further analyses were done by an experienced statistician using SAS

Version 9.2. Descriptive statistics (frequencies and percentages) were calculated for categorical data, while means and standard deviations or medians and percentiles were calculated for numerical data. The Shapiro-Wilk test was conducted to determine if the distribution of the numerical variables followed a normal distribution (p = > 0.05) pattern. Where the numerical variable did not follow normal distribution (p = < 0.05), the median and inter-quartile range were used to analyse the data. Analytical statistics based on the independent T-test were used to compare the mean values of independent two groups, and analysis of variance (ANOVA) was used to compare the mean values of three or more independent groups. A significance level (α) of 0.05 was applied.

2.8 Results and discussion

2.8.1 Food handlers' demographic characteristics

Overall population of 26 food handlers was found in these hospitals. Therefore, Total Population Sampling (TPS) which is a type of purposive sampling where the entire population is sampled was carried out. The demographics of the overall population of 26 food handlers who participated in this study (response rate of 100%) are presented in Table 2.1. Most of the food handlers (n=23; 88%) were females and three (n=3; 12%) were males (Table 2.1). A similar finding regarding gender distribution has been reported in many other studies (Akabanda *et al*., 2017; Nkhebenyane, 2018; Dora-Liyana *et al*., 2018; Letuka *et al*., 2021; Alemayehu *et al*., 2021).

Table 2.1: Demographics of hospital food handlers in Maseru, Lesotho (n=26)

Characteristics	Number of individuals	Percentage (%)	Mean ± SD	Range
Gender				
Male	3	12		
Female	23	88		
Age				
22-30	5	19		
31-39	10	38	40±10.67	22-64

≥40	11	42

Characteristics	**Number of individuals**	**Percentage (%)**	**Mean ± SD**	**Range**
Education level				
Primary	6	23		
J.C	4	15		
COSC/LGCSE	9	35		
Tertiary qualification	7	27		
Duration of working as a food handler				
<1 year	7	27		
1- 5 years	8	31		
6- 10 years	3	12		
10- 20 years	6	23		
21- 30 years	2	8	6 ± 4.41	0.5-30
Employment status				
Contractor	13	50		
Part-time	1	4		
Full time	12	46		
Hours of operation				
Morning shift	8	31		
Afternoon shift	6	23		
Straight shift	12	46		
Language				
English	1	4		
Sesotho	21	81		
Both	4	15		
Race				
Black	26	100		
Training				
No	12	46		
Yes	14	54		

The mean age of the food handlers was 40 years (SD=10.67), with the majority (42%; n=11) being in the age group ≥40 years. Approximately 73% (n=19) had worked in a food service establishment for at least one year, while 52% (n=16) had qualifications above Junior Certificate (JC) level. Of the total respondents, 46% (n=12) worked as full-time employees doing straight shifts. More than half (54%; n=14) of the respondents reported that they had attended a food safety training program at least once. However, these food handlers failed to present a food safety training certificate as proof of their training. It may be assumed that they regarded informal session and instructions in the kitchens where they worked as "trainings".

2.8.2 Hospital food handlers' food safety knowledge

The survey of the food handlers' knowledge demonstrated that they generally had sufficient knowledge of food safety and hygiene as evidenced by a score of 64.15 % ± 11.00 in this regard. Food handlers were knowledgeable in the following aspects: hygiene practices and cleaning and sanitization as evidenced by a score of more than 90% on questions related to these aspects. For example, 96% was able to give a correct answer to the statement that "proper cleaning and sanitization of utensils decreases the risk of contamination". However, it was evident that the food handlers were not aware of the difference between cleaning and sanitization, given that 35% (n=9) failed to choose the correct answer to this question.

The hands of food handlers can introduce foreign materials and microorganisms into food and thus contaminate it. Hence, good personal hygiene and sanitary handling practices at work are essential for food safety (Cempaka *et al*., 2019). Particularly, hand washing has been identified as one of the most important ways to prevent the spread of food-borne diseases (Soares *et al*., 2012). However, only 54% of the food handlers was knowledgeable that bloody diarrhoea can be transmitted by food. This finding is important as bloody diarrhoea is common in infections associated with enteric pathogens, the transmission of which is greatly associated with hand hygiene (Njuguna *et al*., 2016). Microorganisms, particularly *Staphylococcus aureus,* naturally inhabitant the skin and the nasal cavity of humans and animals. This pathogen is opportunistic and can cause a spectrum of infections ranging from facial skin infections to severe and even fatal diseases (Kadariya *et al*., 2014). In general, the food handlers were considered to have awareness of the importance of personal hygiene for safe food production. About 96% of

Table 2.2: Food safety knowledge of hospital food handlers in Maseru, Lesotho

Statements	Responses % (n)			Total mean score
	Correct	**Incorrect**	**Don't know/ remember**	
Washing hands before work reduces the risk of food contamination.	100 (26)	0 (0)	0 (0)	64.15 ± 11.00
Using gloves while handling food reduces the risk of food contamination	58 (15)	27 (7)	15 (4)	
Proper cleaning and sanitization of utensils increases the risk of food contamination	96 (25)	4 (1)	0 (0)	
Eating and drinking at the workplace increases the risk of food contamination	85 (22)	12 (3)	4 (1)	
Food prepared in advance reduces the risk of food contamination	42 (11)	42 (11)	15 (4)	
Reheating cooked foods can contribute to food contamination	69 (18)	23 (6)	8 (2)	
Washing utensils with detergent leaves them free of contamination	100 (26)	0 (0)	0 (0)	
Children, healthy adults, pregnant women and older individual are at equal risk for food poisoning	42 (11)	58 (15)	0 (0)	
Typhoid fever can be transmitted by food	54 (14)	4 (1)	42 (11)	
HIV/AIDS can be transmitted by food	8 (2)	92 (24)	0 (0)	
Bloody diarrhoea can be transmitted by food	54 (14)	38 (10)	8 (2)	
Salmonella is among the food-borne pathogens	46 (12)	8 (2)	46 (12)	
Hepatitis A virus is among the food-borne pathogens	8 (2)	27 (7)	65 (17)	
Swollen cans may contain the microorganism *Clostridium botulinum* which causes botulism	96 (25)	0 (0)	4 (1)	
Microbes are on the skin, in the nose, and in the mouth of healthy food handlers	73 (19)	8 (2)	19 (5)	
Clean is the same as sanitized	65 (17)	35 (9)	0 (0)	
Cross contamination is when microorganisms from contaminated food are transferred by the food handler's hands or kitchen utensils to another food	92 (24)	4 (1)	4 (1)	

Statements	Responses % (n)			Total mean score
	Correct	Incorrect	Don't know/ remember	
The correct temperature for storing perishable foods is 5 ^{0}C	19 (5)	0 (0)	81 (21)	
Hot, ready-to-eat foods should be kept at a temperature of 65^{0}C	12 (3)	0 (0)	88 (23)	
Freezing kills all the bacteria that may cause foodborne illnesses	35 (9)	50 (13)	15 (4)	
Contaminated foods always have some change in colour, odour, or taste	96 (25)	4 (1)	0 (0)	
Raw vegetables are at higher risk of contamination than undercooked beef	23 (6)	73 (19)	4 (1)	
During infectious diseases of the skin, it is necessary to take leave from work	96 (25)	4 (1)	0 (0)	
The health status of workers should be evaluated before employment	100 (26)	0 (0)	0 (0)	
The ideal place to store raw meat in the refrigerator is on the bottom shelf	96 (25)	4 (1)	0 (0)	

the food handlers was knowledgeable that, during active cases of infections of the skin, food handlers should take leave. It was therefore acknowledged that the food handler respondents understood the potential for a food handler to serve as a source of food contamination. Additionally, 85% was knowledgeable of the increased risk of contamination associated with eating and drinking while working. This finding is important because it is possible that bacteria that inhabit the nasal cavity of a human being can be introduced to the food environment through saliva deposits while eating and drinking.

It was rather surprising that 8% of the food handlers failed to answer correctly that HIV/AIDS is not a foodborne infection as dissemination of information regarding this infection had been carefully established. Additionally, only 8% and 46% of the food handlers responded correctly to the question regarding an association between Hepatitis A virus and *Salmonella* and food contamination respectively. These responses serve as indication that there is a need for educational training that includes information on potential foodborne pathogens and their role in food safety. This notion was further highlighted as more than 50% of the food handlers claimed to understand the relationship between food contamination, *clostridium botulinum,* and typhoid fever.

Recently, South Africa has experienced outbreaks of *Listeria monocytogenes*, a microorganism that is commonly found in contaminated food regardless of proper refrigeration temperatures and that is known to cause adverse outcomes during pregnancy (Baud and Greub, 2011; Segado-Arenas *et al*., 2018). It is a major concern that only 19% and 12% knew the food-holding temperatures for both perishable and ready-to-eat foods respectively, while only 42% (n=12) understood the risk contaminated food poses for susceptible groups. These results were similar to those reported a similar study (Akabanda *et al*., 2017). However, there is grave concern regarding these findings as appropriate knowledge is required to develop and execute a comprehensive HACCP program which should ensure the prevention of food contamination that may result in food-related infections originating within the food service unit. It should always be borne in mind that improper handling of food, including the abuse of time and temperature, accounts for most foodborne disease outbreaks (Anon, 2003). Additionally, these findings were considered significant given that food from hospital kitchens is mainly served to people who are susceptible to contamination and infection.

2.8.3 Food safety attitude of hospital food handlers

The attitude of food handlers towards the food they prepare and distribute is a crucial factor that influences food safety behaviour and practices either positively or negatively because, if food is handled correctly, it will decrease incidences of foodborne illnesses (Al-Shabib *et al*., 2016; Al- Kandari *et al*., 2019). Table 2.3 below presents the results of food handlers' attitude to food safety. There was generally a positive attitude towards food safety at an overall score of 88.85% ± 10.13. The result indicated a positive attitude towards proper food handling to ensure foodborne disease prevention was excellent (100%; n=26). This result was similar to the one reported by Reboucas *et al*. (2017). Additionally, the statement that the health status of food handlers should be evaluated prior to employment was approved by 96% (n=25) of the food handlers. Subsequently, 92% (n=24) of the respondents agreed that food handlers could be a source of foodborne outbreaks. These findings corroborate what Akabanda *et al*. (2017) discovered in their study, which was that 64% (n=151) affirmed that food handlers could be a source of foodborne outbreaks. According to the Food and Drug Administration (FDA) (2018), food handlers with diseases or with cuts and wounds on their hands should stop working immediately and leave the food establishment to prevent the spread of foodborne illnesses. In the current study, 88% (n=23) of the respondents agreed with the statement that food handlers with wounds or cuts on their hands should not be handling food without gloves Zanin *et al*. (2015) and Al-kandari *et al*. (2019) reported a similar result. All these studies agreed that food handlers were aware of the danger of handling food with cuts on their hand or fingers. However, according to Al-Kandi *et al*. (2019), it is a common practice for food handlers to continue to work when wounds, cuts, or burns are properly covered with water-proof coverings such as a finger cot, disposable gloves, or the application of a dry and durable tight-fitting bandage.

Table 2.3: Food safety attitudes of hospital food handlers in Maseru, Lesotho

Statement	Agree	Disagree	Don't know	Total mean score
Well cooked foods are free of contamination	96 (25)	4 (1)	0 (0)	88.85± 10.13
Proper hand hygiene can prevent foodborne diseases	100 (26)	0 (0)	0 (0)	
When cleaning products are closed, they can be stored with cans and jars of food that are also closed	15 (4)	85 (22)	0 (0)	
Raw and cooked foods should be stored separately to reduce the risk of food contamination	88 (23)	12 (3)	0 (0)	
It is necessary to check the temperature of refrigerators/ freezers periodically to reduce the risk of food contamination	81 (21)	8 (2)	12 (3)	
Defrosted foods should not be frozen	85 (22)	15 (4)	0 (0)	
The health status of workers should be evaluated before employment	96 (25)	4 (1)	0 (0)	
The best way to thaw chicken is in a bowl of cold water	35 (9)	65 (17)	0 (0)	
Wearing masks is an important practice to reduce the risk of food contamination	77 (20)	23 (6)	0 (0)	
Wearing caps and adequate clothing is an important practice to reduce the risk of food contamination	100 (26)	0 (0)	0 (0)	
Safe food handling is an important part of my job responsibility	100 (26)	0 (0)	0 (0)	
Learning more about food safety through training courses is important to me	100 (26)	0 (0)	0 (0)	
Beards could contaminate food with foodborne pathogens	88 (23)	4 (1)	8 (2)	
Long and painted fingernails could contaminate food with foodborne pathogens	96 (25)	0 (0)	4 (1)	
Food handlers can be a source of foodborne outbreaks	92 (24)	0 (0)	8 (2)	
Eggs must be washed immediately after delivery	77 (20)	15 (4)	8 (2)	
Dish towels can be a source of food contamination	100 (26)	0 (0)	0 (0)	
Knives and cutting boards should be properly sanitized to prevent cross-contamination	100 (26)	0 (0)	0 (0)	
Food handlers who have abrasions or cuts on their hands should not touch foods without gloves	88 (23)	0 (0)	12 (3)	
It is possible for infectious pathogens from the hospital wards to be imported into the kitchen and into the food	92 (24)	8 (2)	0 (0)	

Most of the respondents (n=20; 77%) were in agreement that eggs should be washed immediately after delivery. According to Whiley and Ross, (2015), such a view is erroneous as *Salmonella* is one of the leading causes of foodborne illnesses while egg and eggshell contamination has been identified as one of the major causes of foodborne *Salmonella* (Howard *et al*., 2012). Therefore, the Food and Safety Authority of Ireland (FSAI) (2010) advises against washing eggs as it compromises the natural barrier found on the eggshell. If this barrier is washed off, it will potentially enable *Salmonella* spp. to penetrate and contaminate the egg. Therefore, if eggs are washed before storage and use, they may be contaminated and will result in food poisoning, especially if the egg is not thoroughly cooked.

According to Nkhebenyane (2018) and Sani and Siow (2014), refreezing thawed food is a serious health risk as this practice has the potential to provide an environment that is conducive for the proliferation of contaminating bacteria. The majority (85%; n=22) of the respondents presented a positive attitude towards avoiding the refreezing of defrosted food. This was significantly higher than the 66% reported in a study by Al-Kandari *et al*. (2019), who found that food handlers agreed that defrosted food could be refrozen at least once. In the current study, although the food handlers lacked knowledge of the food storage and holding temperatures, most (81%; n=21) affirmed the necessity to check the temperatures of refrigerators and freezers periodically to reduce the risk of food contamination. The results therefore revealed that the food handlers under study had a positive attitude towards time and temperature controls when storing food to prevent microbial growth.

The food handlers also had a positive attitude towards personal hygiene and the use of adequate personal protective equipment (PPE). This was evidenced by the fact that all the food handlers agreed that wearing a head covering and adequate PPE was an important practice to reduce the risk of food contamination. A similar result was reported by Faour-Klingbeil *et al*. (2015), who found that food handlers were confident that wearing PPE would reduce the risk of food contamination. In the current study, food handlers also affirmed the importance of hand hygiene, which correlated with their positive attitude towards food handling. For instance, 85% agreed that long and painted nails and beards could contaminate food.

2.8.4 The self-reported food safety practices of hospital food handlers

The self-reported food safety practices of food handlers are presented in Table 2.4. These results show that the food handlers maintained good hygienic practices with a mean score of 70.28% ± 11.94. However, many food handlers (69%; n=18) reported that they did not use gloves, while 58% (n=15) did not wear masks when distributing unpackaged food. All the food handlers reported that they wore aprons while working, while some concern was raised as only 58% of the food handlers demonstrated knowledge of the importance of wearing gloves while handling food. In general, however, the food handlers had satisfactory personal hygiene practices as more than 60% practised sound cleanliness. For example, 69% (n=18) reported that they did not eat at their work stations, while 92% (n=24) reported that they did not wear nail polish when handling food. These results are important given that hand hygiene is considered vital in the control of pathogens (Al-kandari *et al*., 2019), while improper food handling is a significant cause of foodborne illnesses (Codex Alimentarius Commission, 2003).

Food handlers' practices with regards to the use of sanitizers were also somewhat satisfactory. All the food handlers reported that they used a sanitizer when washing service utensils, but they did not use a sanitizer when washing fruit. These results were not surprising given the fact that the use of sanitizers in washing water is common in large-scale farming business (Lawton and Kinchla, 2015). Additionally, sanitizers are commonly used in hospitals for handwashing, laundry, rinsing dishes, food baths, cleaning floors, washing bedding, cleaning latrines, and handling corpses (String *et al*., 2020). Therefore, it is important that users are knowledgeable about the proper use of sanitizers, as various sanitizers in a variety of concentrations are used for different applications (CDC, 2018b).

Table 2.4: Food safety knowledge of hospital food handlers in Maseru, Lesotho

Questions	Response		Total mean score
	Yes	No	
Do you use gloves during the distribution of unpackaged food?	31 (8)	69 (18)	70.28% ± 11.94
Do you wear an apron while working	100 (26)	0 (0)	
Do you wear a mask when you distribute unwrapped foods?	42 (11)	58 (15)	
Do you eat or drink at your workplace	31 (8)	69 (18)	
Do you wear nail polish when handling food	8 (2)	92 (24)	
Do you prepare a meal in advance (i. e, from one shift to another)?	12 (3)	88 (23)	
Do you properly clean the food storage area before storing new products?	81 (21)	19 (5)	
Do you use sanitizer when washing service utensils (plates, mugs and spoons)?	100 (26)	0 (0)	
Do you use the sanitizer when washing fruits?	0 (0)	100 (26)	
Do you check the shelf life of foods at the time of delivery?	100 (26)	0 (0)	

2.8.5 Food handlers' KAPs and demographics

Table 2.5 below shows the relationship between the food handlers' knowledge, attitudes and self-reported practices. Age was the only factor that significantly ($p < 0.05$) influenced the knowledge, attitudes, and practices of the food handlers ($p < 0.0478$; $p < 0.0346$; $p < 0.0546$ respectively). Conversely, there was no significant relationship between educational level, work duration, and exposure to food safety training and the food handlers' KAP ($p > 0.05$ and $\alpha>0.05$ respectively). These findings differed from those in similar studies (Cakiroglu and Ucar, 2008; Sharif *et al*., 2013) where educational levels and professional experience significantly affected the KAP, while average scores increased with the level of education. A study by Ansari *et al.* (2010) found a significant association between food handler practices and the duration of work (r_s=040; p=0.001), while Buccheri *et al. (*2010) reported that continued attendance of food training courses contributed to increased knowledge. The findings of the current study were not surprising as, although the food handlers were cognisant of Lesotho Public Health Order of 1970, none were able to produce a food handler certificate during the course of this study. Therefore, it was apparent that these food handlers had not been exposed to any official food safety training and it can only be surmised that they regarded their initial in-service training as sufficient.

Table 2.5: Association between food handler demographics and food safety knowledge, attitude and practices (KAP)

	Knowledge			Attitudes			Practices		
Predictors	**Mean**	**SD**	**P-value**	**Mean**	**SD**	**P-value**	**Mean**	**SD**	P-value
Age									
22-30	56.00	11.66		88.00	18.37		65.45	7.61	
31-40	70.00	8.89		88.00	6.32		77.27	7.73	
>40	62.55	10.32		93.64	4.52		66.12	14.14	
ANOVA			0.0478			0.0346			0.0546
Education									
C.O.S.C	58.00	9.72		84.17	17.15		72.73	8.13	
J.C	68.00	7.30		91.25	8.54		72.73	19.64	
Primary	61.33	10.58		92.78	4.41		67.68	13.72	
Tertiary	70.86	11.71		86.43	8.02		70.13	8.65	
ANOVA			0.1296			0.3703			0.8549
<1 year	63.43	10.44		88.57	8.52		68.83	13.74	
1-5	57.50	9.55		82.50	14.39		68.18	11.90	
6-10	72.00	14.42		91.67	2.89		72.73	9.09	
11-20	66.00	9.38		95.00	3.16		72.73	15.21	
>20	76.00	5.66		92.50	3.54		72.73	-	
ANOVA			0.1266			0.2102			0.9481
Employment									
Contractor	65.23	10.51		88.46	5.91		75.52	7.77	
Full-time	63.67	12.12		89.17	13.95		65.15	13.89	
Part-time	56.00	-		90.00	-		63.64	-	
ANOVA			0.7221			0.9801			0.0754
Race									
Black	64.15	11.00		88.85	10.13		70.28	11.94	
Gender									
Female	64.35	11.44		88.91	10.55		70.75	12.55	
Male	62.67	8.33		88.33	7.64		66.67	5.25	
ANOVA			0.8091			0.9280			0.5878

Predictors	Knowledge			Attitudes			Practices		
	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
Operation hours									
Afternoon shift	66.00	7.48		92.50	6.89		72.73	16.26	
Morning shift	63.50	13.26		89.38	4.96		70.45	7.87	
Straight shift	63.67	11.63		86.67	13.54		70.45	7.87	
ANOVA			0.9030			0.5261			0.7920
Language									
Both	65.00	8.87		93.75	2.50		72.73	7.42	
English	76.00	-		75.00	-		54.55	-	
Sesotho	63.43	11.47		88.57	10.62		70.56	12.50	
ANOVA			0.5476			0.2523			0.3996
Training									
No	63.00	13.23		85.42	12.15		70.45	9.59	
Yes	65.14	9.07		91.79	7.23		70.13	14.01	
T- test			0.6302			0.1114			0.9466

2.9 Conclusions

Food handlers are the undisputed custodians of food hygiene and food safety due to their participation in food procurement, preparation, and distribution to consumers. Ensuring safe food handler behaviour is essential as food handler practices influence the quality of the product served to immune-compromised patients. The findings of this study revealed that the food handlers recruited from the five selected hospitals in Maseru had substantial levels of knowledge and a positive attitude towards appropriate food handling practices. Overall, there was sufficient knowledge pertaining to hand hygiene practices and cleaning and sanitization procedures. These findings are noteworthy as hand hygiene is the most basic and critical criterion for ensuring the safety of food.

Food handlers are employed and allowed to work in hospital kitchens without producing a food handler certificate. Therefore, there is a need that the Lesotho Ministry of Health through the District Health Management Team (DHMT) strictly enforces the pre-employment and continued monitoring of food handler certification. Hospital management should ensure that without a certificate no one is allowed to work in the hospital kitchen as this is required by law for any one intending to work in any food establishment. Food handlers should be encouraged to acquire certificates in food safety short courses to improve on their knowledge and understanding of safe food handling and to promote a culture of accessing food safety information from various information sources.

Although hospitals are known to practise the cook-serve method of catering, it was obvious that the food handlers (57%; n=15) were generally of the opinion that preparing food in advance would reduce the risk of food contamination. However, it seemed that there was confusion amongst the food handlers regarding food spoilage and food contamination, as 96% (n=25) of the respondents failed to choose the correct answer regarding the change in taste, colour, and smell of contaminated food. This was an important finding as although the aesthetics of contaminated food may not change, such food is unsafe for human consumption. Serving contaminated food in a hospital may result in outbreaks of foodborne infections that may affect hospital staff, the patients, and visitors.

The food handlers were of the opinion that freezers/refrigerators should be checked regularly to reduce the risk of contamination. However, there was an overall lack of sufficient knowledge regarding refrigeration and holding temperatures of hot ready-to-eat foods. This is concerning given that when food holding temperatures are poorly managed, food becomes a conducive environment where microorganisms procreate and produce toxins. Although the food handlers had reportedly received some training, there was no significant relationship ($\alpha>0.05$) between training, educational level, and work duration ($p>0.05$) and the food handlers' knowledge, attitudes, and practices. This finding supports the notion that knowledge on its own does not always translate to positive attitudes and good practices (Park *et al*., 2010).

It is important that independent food safety training agencies prioritise general food safety knowledge training with emphasis on the existing knowledge gaps. Behaviour based training should be in cooperated in food safety training in order to attain transformation and positive food handling behaviour. Food service managers and floor supervisors should be more engaged in guidance during the production process and in motivating workers by providing recognition and rewards.

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CHAPTER 3

THE PREVALENCE OF MICROBIOTA ON FOOD CONTACT SURFACES IN HOSPITAL KITCHENS IN MASERU, LESOTHO

3.1 Abstract

In hospitals immunosuppressed patients are highly vulnerable to infections from contaminated food rendering food safety a top priority. The high prevalence of diarrhoeal diseases in many developing countries is indicative of underlying food safety problems. However, limited data are available to quantify incidences of foodborne illnesses in Africa. Therefore, the purpose of this study was to evaluate the prevalence of foodborne microorganisms on food contact surfaces in hospital kitchens in the city of Maseru in Lesotho. The targeted microorganisms were total viable counts (TVC), total coliforms, *Escherichia coli*, *Staphylococcus* sp., *Salmonella* sp., and *Pseudomonas* sp. These organisms were identified at specie level using commercialized biochemical test systems, namely Staph API, RAPID ONE, and RAPID NF PLUS. The total viable counts ranged from 5.4 cfu.cm^{-2} to 9.9 cfu.cm^{-2} on food handlers' hands and tables, while food contact surfaces had TVCs in a range of 7.4×10^{3} cfu.cm^{2} to 1.4×10^{-5} cfu.cm^{2}. Five species of organisms from the family Staphylococacaeae were identified, including *Staphylococcus aureus,* and six organisms of the family Enterobacteriacea were detected, including *Acinetobacter* sp. Microorganisms that are commonly implicated in hospital-associated infections could indicate the possibility that food contact surfaces contributed to the spread of these infections when they occurred. Based on the findings, the study highlights the importance of investing in good food preparation practices, with specific attention to hand washing using antimicrobial and not ordinary soap.

Keywords: cross-contamination, hand hygiene, food safety, hospital food, foodborne illnesses

3.2 Introduction

One of the core businesses of a hospital is the provision of safe food to patients who are immune compromised and therefore prone to infections. In hospital settings, food is commonly prepared in the kitchen and distributed to patients throughout different hospital wards (Baghapour *et al*., 2015). According to WHO (2015a), the number of people that become ill due to the consumption of unsafe food has become matter of global concern. Concern has also been expressed for the inordinate presence of physical and chemical contaminants and microorganisms that are associated with infections and illnesses that are contracted in hospitals. The microbiological contamination of food can be due to naturally contaminated raw materials or cross-contamination during handling and preparation in unhygienic settings (Osimani *et al*., 2014). Although it would be ideal to assess and control the burden of food-related diseases, the ability of pathogens to adapt and mutate changes the structural characteristics and alters sensitivity to antibiotics resulting in change in treatment options (Watford and Warrington, 2023).

Bacteria exist naturally in nature and they are normally harmless; however, some may be opportunistic or symbiotic while others are pathogenic to humans. According to Khelissa *et al*. (2017), most pathogenic bacteria, such as *Listeria monocytogenes, Staphylococcus aureus, Salmonella* sp., *Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus cereus* are found in hospitals as well as in industrial, natural, and domestic environments. The presence of these pathogenic organisms on inanimate surfaces such as food and water has led to human infections globally (Kramer and Assadian, 2014). This phenomenon has been attributed to deficiencies in food handling practices such as using insufficiently cleaned surfaces, processing contaminated ingredients, and infected food handlers who may also act as a source of the contamination of foodstuff (WHO, 2015b).

3.3 Food safety and security in Lesotho

Food safety and nutrition are important in ensuring food security and are therefore major concerns in Lesotho, which is still a developing country. According to Perez-Escamilla

(2017), the organisers of the World Food Summit in Rome in 1996 were of the opinion that “food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs as well as food preferences for an active life”. However, it was estimated that 24% of Lesotho’s population would face high food insecurity in May to September 2019, with the projection that food insecurity would increase by 30% between October 2019 to March 2020 (IPC,2020). The food insecurity state in Lesotho has persisted though the years and a prediction that 22% of the population in the rural areas are to be in crisis phase 3 in October 22 - March 2023 (IPC,2023).

Lesotho is a developing country that has been hard hit by the human immunodeficiency virus (HIV), which is a virus that infects the immune system and results in impaired immune function, and this often exacerbates opportunistic infections among infected individuals (CDC, 2019). According to Kotler *et al*. (1984), the most affected part in untreated HIV- infected persons is the gastrointestinal tract. For example, Munnink *et al*. (2014) found that, despite the observation of dual infections in HIV patients, 54% of infections could be attributed to viral, 17% to bacterial, and 21% to parasitic infections.

The pursuit of providing safe food to consumers is a global ideal; however, it is marred by controversies regarding routine medical examinations of food handlers in various countries globally. According to Lesotho Public Health Order of 1970 and 1973 the health status of food handlers should be examined prior to employment. Therefore, food handlers are prohibited from continuing work when suffering from a communicable or venereal disease. Food handlers are therefore screened for food-related infections such as *Salmonella* and *Shigella* as well as food and water-related parasites such as *Cryptosporidium.* if a food handler knowingly disregards the legislation and continues work during active communicable or venereal disease infection, they are liable to a fine or imprisonment or both (Government of Lesotho,1970,1973). However, in South Africa pre-employment and routine medical examinations of food handlers are not required; instead, emphasis is placed on the health education of food handlers (Budavari, 2016) and investigations that evaluate the general hygiene of food premises and transport by taking swab samples (Food Authority. 2015). In light of this oversight, the current study aimed to evaluate the prevalence of foodborne microorganisms on food contact surfaces in hospital kitchens in Maseru, Lesotho.

3.4 Materials and methods

3.4.1 Sampling Site

Bacterial samples were collected from food preparation surfaces and food handlers' hands in five hospitals in the city of Maseru. The hospitals included in the study were Scott Hospital, Mohlomi Mental Hospital, Bots'abelo Hospital, St Joseph's Hospital, and Maseru Private Hospital. Available beds in these hospitals range from 37 to 200 beds per hospital, with Mohlomi Hospital having the highest number of beds. Each hospital employs a minimum of two food handlers and therefore two food handlers were recruited from each hospital, so a total of 10 food handlers participated in this study.

3.4.2 Sampling protocol

Samples were collected at the above mentioned hospitals in duplicate from tables, chopping boards, spoons, knives, and plates found in the hospitals' respective kitchens. Samples were also taken from the hands of two food handlers per hospital using sterile swabs, and the sampled material was transferred to 55mm Rodac contact plates (Rodac Nunc Denmark). Samples were collected from the thumb and three index fingers of both hands of the food handlers (Nkhebenyane, 2010). Samples were also obtained from a surface area of 2×2 cm^2 and the utensils used by the food handlers (Mokoena, 2013). A total of 90 samples were collected over the duration of the study. All the samples were kept on ice during transportation to the laboratory and processed without delay.

3.4.3 Sampling procedure and microbiological analysis

Samples were collected according to the following procedures: (1) Microbial samples were collected from food handlers' hands and the tables where they worked. The samples were appropriately processed and then quantified using 65mm Rodac plates (Nhlapho, 2014). (2) Samples were collected from spoons, knives, plates, and chopping-boards. These samples were collected using cotton swabs that had been sterilised in 5ml of peptone water. Upon arrival at the laboratory, samples were diluted to 10^{-3} and spread-plated according to previously described selected media. Plates were then placed in an invert position at suitable temperatures for periods ranging from 24-48hrs (Mokoena, 2013). After the desired period of incubation, colonies were counted and the figures recorded as colony-forming units per square centimetre. An agar growth medium was used to investigate the presence rates of TVC, total coliform, *Escherichia coli*, *Staphylococcus* sp., *Salmonella* sp., and *Pseudomonas* sp as follows;

3.4.3.1 Total viable counts (TVC)

In the food processing environment, it is generally accepted that the total viable count (TVC) indicates the level of contamination of particular foodstuff as well as the presence of pathogens in that particular food processing environment (Department of health, 2000). For detection and enumeration of TVCs, plate count agar (PCA) plates (Merck South Africa) were prepared according to the manufacturer's instructions and incubated at 37ºC for 24-48 hours. This was done upon arrival at the laboratory after sampling.

3.4.3.2 *Staphylococcus* sp.

Staphylococcus aureus is generally associated with contaminated hospital kitchen surfaces and for causing hospital-acquired infections (Donkor, 2019). Baird-Parker agar (Biolab, SA) with 50ml egg-yolk tellurite emulsion (Merck, SA) was used and the plates were incubated at 35ºC for 48 hours (Nkhebenyane, 2010). An *S. aureus* (ATCC 25923) positive control plate was incubated at 35ºC for 48 hours and used for colony morphology comparison.

3.4.3.3 *Salmonella* sp.

The *Salmonella* species is a leading cause of gastroenteritis and its prevalence has become an important public health concern, particularly in developing countries (Popa and Papa, 2021). Brilliant green agar (BRG) was used and plates were incubated aerobically at 35ºC for 18-48 hours (Public Health England, 2017). A plate with *Salmonella typhimurium* (ATCC 14028) that was used as the positive control plate, incubated at 35ºC for 18-48 hours and was used for colony morphology comparison.

3.4.3.4 *Pseudomonas* sp.

The organism *Pseudomonas* is recognised as a food spoiler and a psychotropic bacterium that easily develops in food. It has also been flagged as a major contaminant of different surfaces (Stella *et al*., 2017). *Pseudomonas* agar (Merck, SA) was added to cold water, supplemented with 5ml glycerol, and further prepared according to the manufacturer's instructions. Plates were incubated at 37ºC for 24-48 hours and a plate with *Pseudomonas aeruginosa* (ATCC 9027) was used for colony morphology comparison.

3.4.3.5 *Escherichia coli* and total coliforms

Escherichia coli spp. are generally regarded as indicators of pathogenic microbiota that originate from faecal matter and their presence is associated with poor sanitation during the processing of food (Department of Health, 2000). Violet-Red-Bile Mug (VRBM) agar was used for the enumeration of *E. coli* and coliforms, and plates were incubated at 37ºC for 18-24 hours. The *E. coli* colonies were dark red while coliforms appeared as pink colonies. All plates were incubated at 37ºC for 18-24 hours and the positive control plate with *E. coli* (ATCC 25922) was used for colony morphology comparison.

3.4.4 Biochemical test identification

Colonies from selective media were further used for biochemical identification. If fewer than five colonies appeared for a particular organism on a plate, they were ignored. When more than five similar colonies were counted on the plate, then five isolated colonies were carefully picked one by one using a 10µl loop. They were then inoculated into buffered peptone water on sterile microtitre plates. Cultures from each microtitre well were re-inoculated onto a nutrient agar (Merck, SA) to obtain pure growth. Organisms that were identified to be isolate from the microtiter wells were grouped as one isolate from the food contact surfaces (Feglo and Sakyi, 2012). The pure cultures (growth of not more than 24 hours) were then used for biochemical identification using Staph API, RAPID ONE and RAPID NF Plus (Merck, SA).

3.4.5 Analysis of the data

Following differentiation and morphological characterization of colonies, enumeration was done using a Symbiosis aCOLade colony counter (Vacutec, Johannesburg, South Africa). The results were expressed as colony-forming units (CFU/cm^2). For the purpose of this study, all the results were evaluated using the British Columbia Centre for Disease Control (BCCDC) Guide for Environmental Health Officers. This guide is articulate with the units ranging from ‘satisfactory’ (<5 CFU/cm^2) to ‘acceptable’ (5 CFU/cm^2 to 10 CFU/cm^2) and ‘unsatisfactory’ (>10 CFU/cm^2) (BC Centre for Disease Control, 2010), whereas the South African R. 918 offers only the guide of 100 CFU/cm^2 (Department of Health, 2000). Colonies formed after incubation were counted (30-300) and the data were used to determine the colony-forming units per square centimetre (cfu/cm^2).

3.5 Results

3.5.1 Total viable counts

Total viable counts are used as indicators of the general degree of contamination after microbial analyses have been performed (Nhlapho *et al*., 2014). Figures 3.1 (A) and (B) depict TVCs on various food preparation surfaces after lunch meal sessions in the hospital under study. The average bacterial counts on various food preparation surfaces after lunch sessions were calculated and recorded. The average bacterial counts on food handlers' hands and kitchen tables ranged from 5.4 cfu.cm^2 to 9.9 cfu.cm^2. High average counts of 9.8 cfu/cm^2 were found on tables, while the righthand counts showed 9.9 cfu.cm^2 and 7.9 cfu.cm^2 on the right thumb and on the index finger respectively.

A B

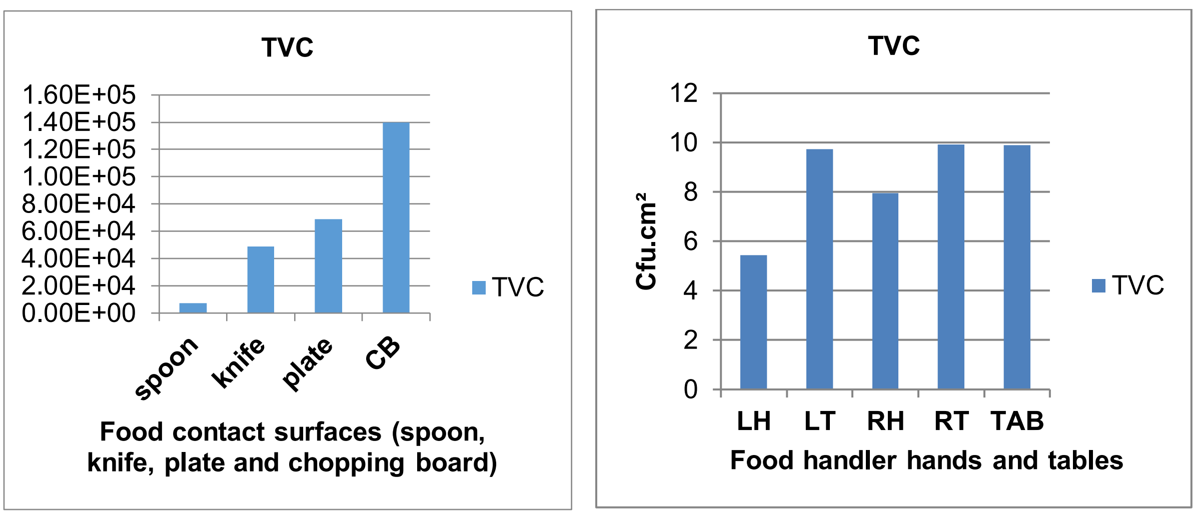


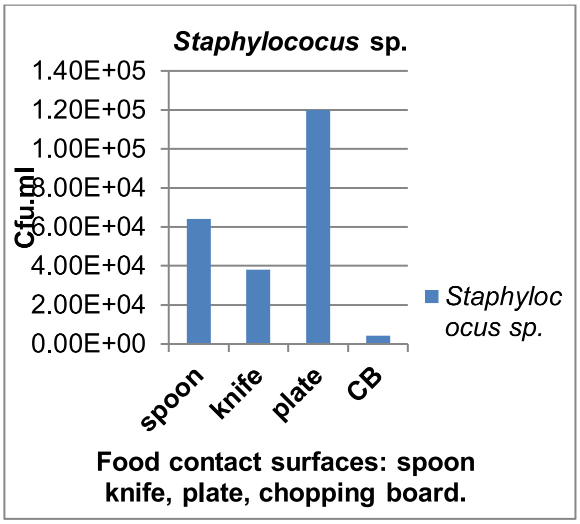
Figure 3.1: Total viable counts (TVC) of food contact surfaces. (A): swab samples; (B): Rodac plates, CB: chopping boards; LH: left hand; LT: left thumb; RH: right hand; RT: right thumb; TAB: table.

Readings of the left hand showed a 9.7 cfu.cm^2 count on the thumb and a 5.4 cfu.cm^2 count on the index finger. The total viable counts emerging from swab samples indicated that chopping boards had the highest average count amongst the food contact surfaces at 1.4×10^{-5} cfu.cm^2. The counts were 4.9×10^4 cfu.cm^2, 6.9×10^4 cfu.cm^2, and 7.4×10^3 cfu.cm^2 for knives, plates, and spoons respectively.

3.5.2 *Staphylococcus* sp.

The results depicted in Figure 3.2 (A) show and average count of 1.2×10^{-5}cfu.cm^2 for *Staphylococcus* sp. that was found on plates in this study. Additionally, the average counts were 6.4×10^4 cfu.cm^2, 3.8×10^4 cfu.cm^2, and 4.3×10^3 cfu.cm^2 for spoons, knives, and chopping boards respectively.

A



B

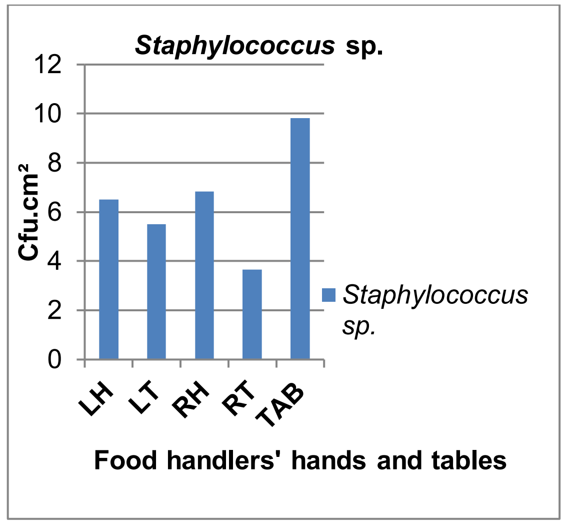


Figure 3.2: *Staphylococcus* sp. isolated from food contact surfaces A: swab samples and B: Rodac plates, CB: chopping board, LH: left hand, LT: left thumb, RH: right hand, RT: right thumb and TAB: table

Figure 3.2 (B) shows average counts of 9.8 cfu.cm^2 and 5.5 cfu.cm^2 for the index finger and the thumb on the left hand. Counts were 6.8 cfu.cm^2 and 3.6 cfu/cm^2 for the index finger and the thumb of the right hand respectively. The table surface count for *Staphylococcus* sp. was 6.5 cfu/cm^2.

3.5.3 *Salmonella* sp.

Figure 3.3 (A) shows the results for the *Salmonella* species. Chopping boards had a high average count of 4.3×10^{-5} cfu.cm^2, while the counts for plates, knives, and spoons were 1.2×10^{-5} cfu.cm^2, 3.4×10^{-5} cfu.cm^2, and 6.1×10^{-5} cfu.cm^2 respectively.

A B

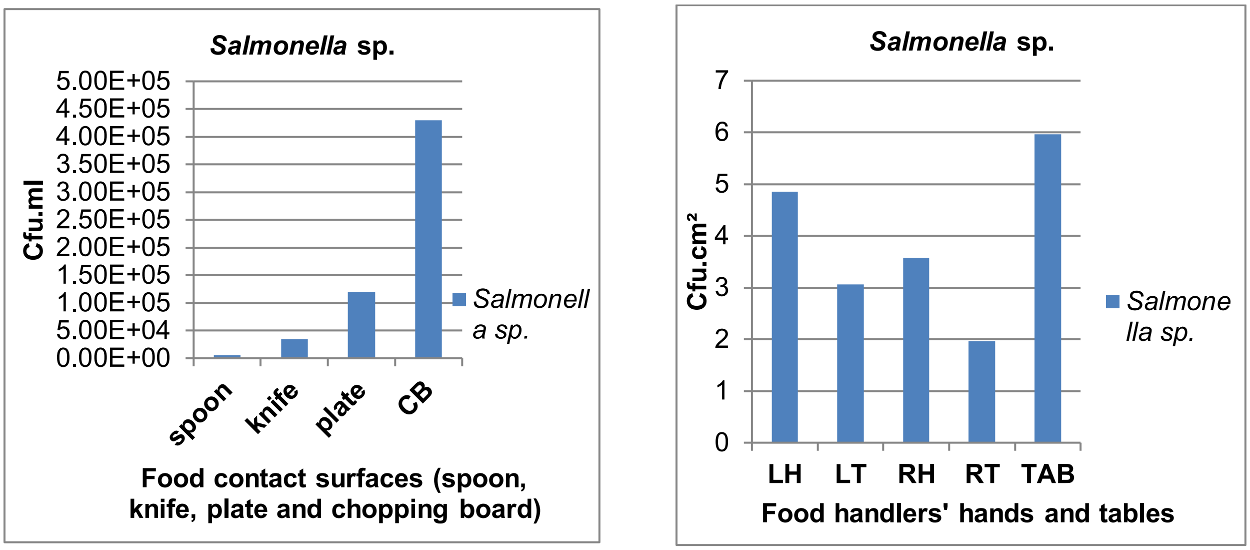


Figure 3.3: *Salmonella* sp. isolated from food contact surfaces A: swab samples and B: Rodac plates, CB: chopping board, LH: left hand, LT: left thumb, RH: right hand, RT: right thumb and TAB: table

Figure 3.3 (B) shows the results for the *Salmonella* species found on food handlers' hands and on table surfaces. The left hand presented high average counts of 3.0 cfu/cm^2 while a count of 4.8 cfu/cm^2 was obtained for the left thumb. The right hand presented an average count of 1.9 cfu/cm^2 for the thumb and 3.5 cfu/cm^2 for the index finger.

3.5.4 *Pseudomonas* sp.

Figure 3.4 (A) presents the results for *Pseudomonas* sp. that was found on the food handlers' hands and on the table surfaces. The latter resulted in a count of 6.7 cfu.cm^{-2} for *Pseudomonas* sp. The average count count for food handlers' hands was 2.9 cfu.cm^{-2}, with 4.9 cfu.cm^{-2} accounting for the index finger and thumb of the left hand. Moreover, 3.4 cfu.cm^{-2} and 4.8 cfu.cm^{-2} counts of *Pseudomonas* sp. were found on the index finger and on the thumb of the right hand.

A B

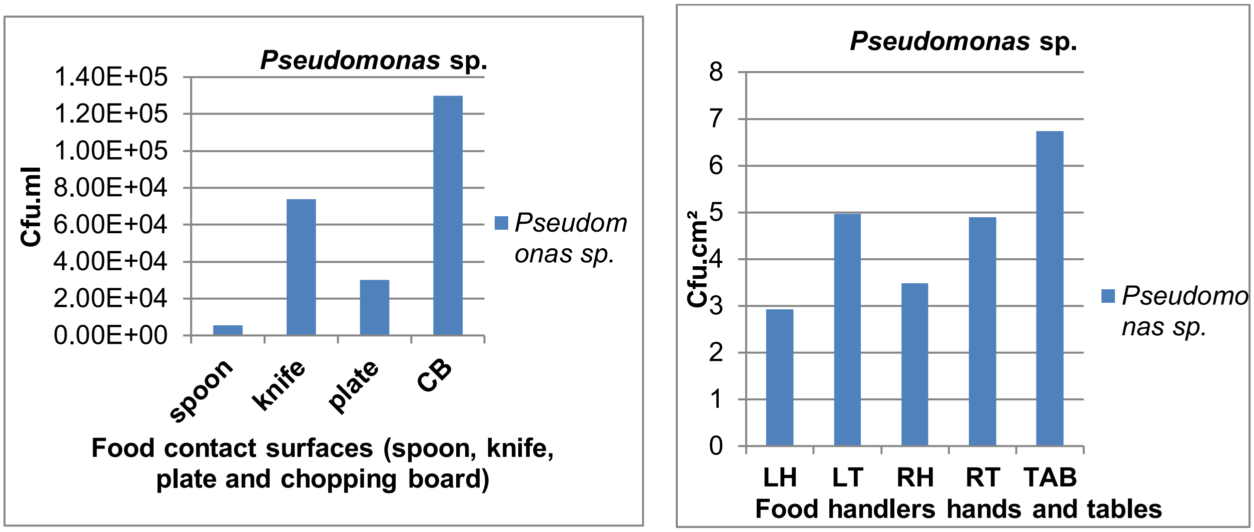


Figure 3.4: *Pseudomonas* sp. isolated from food contact surfaces A: swab samples and B: Rodac plates, CB: chopping board, LH: left hand, LT: left thumb, RH: right hand, RT: right thumb and TAB: table

Subsequently, Figure 3.4 (B) shows the results for food contact surfaces (spoons, knives, plates, and chopping boards). Microbiological counts of 5.4×10^{-3} cfu.cm^2, 7.4×10^{-4} cfu.cm^2, 3.0×10^{-4} cfu.cm^2, and 1.3×10^{-5} cfu.cm^2 of *Pseudomonas* sp. were found on spoons, knives, plates, and chopping boards respectively.

3.5.5 *Escherichia coli*

Figure 3.5 (A) shows the results for *E. coli* that was found on food handlers' hands and table surfaces. Microbial counts of 2.9 cfu.cm^2 and 3.0 cfu.cm^2 were found on the index finger and on the thumb of the left hand respectively, while counts of 2.9 cfu.cm^2 and 2.5 cfu.cm^2 were recorded respectively for the index finger and thumb of the right hand. For coliforms, a count of 2.9 cfu.cm^2 was found for table surfaces. Figure 3.5 (B) depicts the results of *E. coli* found on food contact surfaces (spoons, knives, chopping boards and plates). These counts were respectively 6.6×10^{-5} cfu.cm^2, 3.7×10^{-5} cfu.cm^2, 7.0×10^{-4} cfu.cm^2, and 1.4×10^{-5} cfu.cm^2.

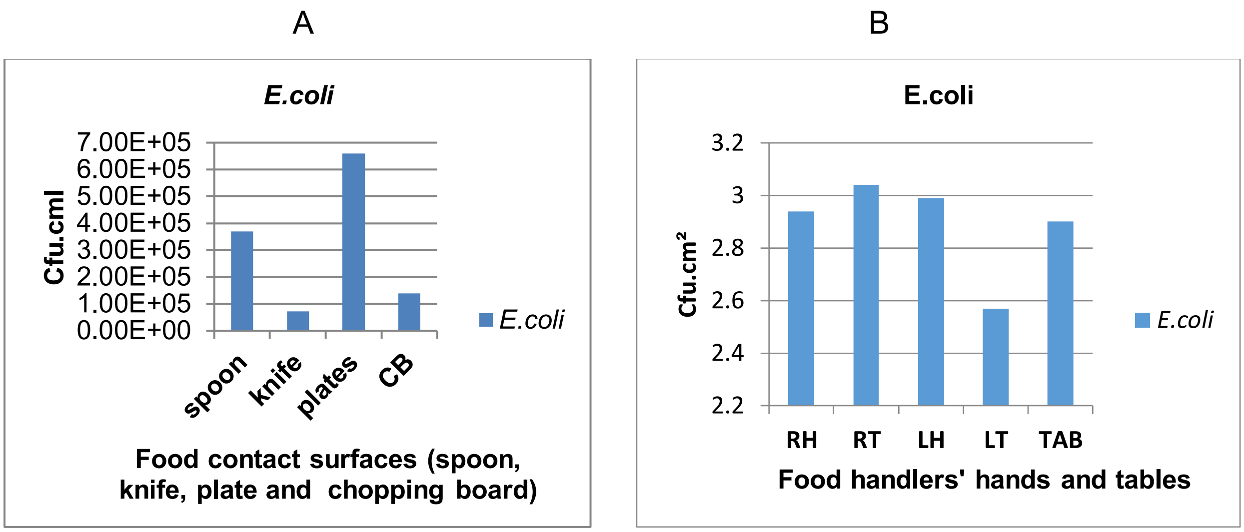


Figure 3.5: *E. coli* isolated from food contact surfaces. A: swab samples, B: Rodac plates, CB: chopping board, LH: left hand, LT: left thumb, RH: right hand, RT: right thumb, and TAB: table

3.5.6 Total coliforms

Figure 3.6 (A) shows the results for coliforms on the food handlers' hands and on table surfaces. On average, 9.6 cfu.cm^2 coliform counts were found on these surfaces. Conversely, 1.4 cfu.cm^2 and 3.3 cfu.cm^2 counts of coliforms were found on the index finger and the thumb of the left hand. Also, 5.5 cfu.cm^2 and 5.4 cfu.cm^2 coliform counts were found on fingers and thumbs of the right hand. Figure 3.6 (B) shows the results for coliforms found on food-related contact surfaces (spoons, knives, plates, and chopping boards). Coliforms were found on these surfaces at rates of 4.9×10^{-5} cfu.cm^2, 7.9×10^{-4} cfu.cm^2, 1.6×10^{-6} cfu.cm^2, and 5.2×10^{-6} cfu.cm^2 respectively.

A B

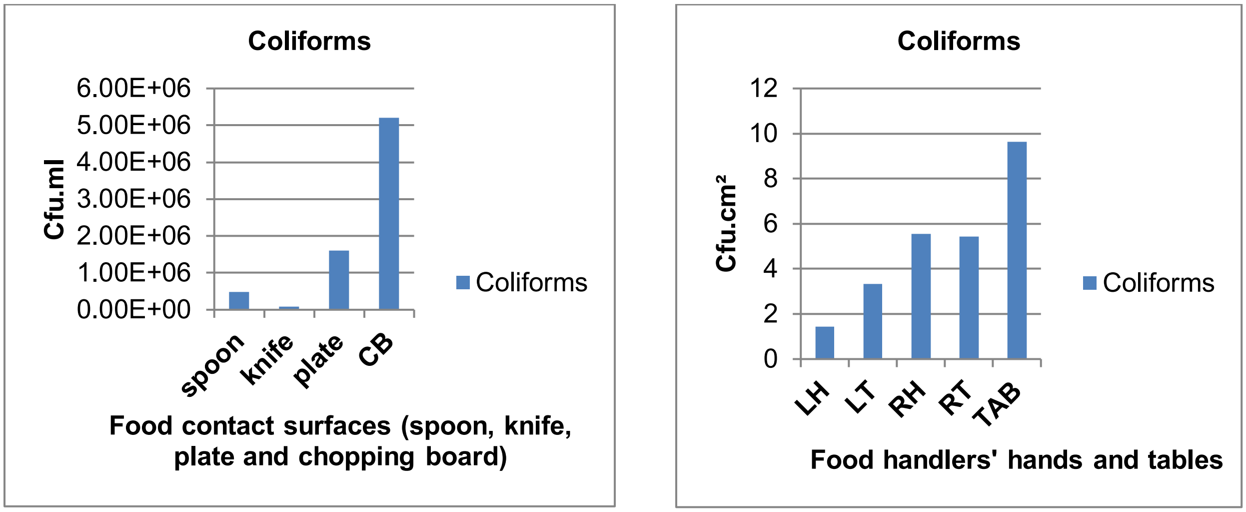


Figure 3.6: Total coliforms isolated from food contact surfaces. A: swab samples, B: Rodac plates, CB: chopping board, LH: left hand, LT: left thumb, RH: right hand, RT: right thumb and TAB: table

3.5.7 Bacterial strains identified by biochemical test kits

Staph API, RAPID ONE and RAPID NF PLUS were identified. The results of the biochemical tests revealed five species of organisms from the family Staphylococcaceae (77.8%) on the food contact surfaces that were tested. The microorganisms included *Staphylococcus aureus* (14%), *Staphylococcus lentus* (9%), *Staphylococcus capitis* (14%), *Staphylococcus xylosus* (8%), and *Staphylococcus hominis* (11%). Eight species of organisms of the family Enterobacteriaceae were detected, namely; *Pseudomonas oryzihabitans* (24%), *Stenetrophomonas maltophilia* (9%), *Sphingomonas paucimobilis* (17%), *Acinetobacter* sp. (17%), *Myroides odorantum* (12%), *Enterobacter cloacae* (24%), and *Enterobacter asburiae* (24%). These were detected on 55.6% of the food contact surfaces.

3.6 Discussion

Microbial analysis has been demonstrated as an effective method for the evaluation of cleaning and disinfection practices (Casin *et al*., 2018). When the microbiological qualities of the contact surfaces in hospital kitchens in Maseru Lesotho were evaluated, the total viable counts of the organisms on the food handlers' hands and on table surfaces were within an acceptable range of 5.4 $cfu.cm^2$ to 9.9 $cfu.cm^2$. However, the count on the chopping boards was 1.4×10^5 $cfu.cm^2$. This high total level of viable counts on the chopping boards could be attributed to the development of cracks and scratches on the surfaces of these boards due to continued use. Additionally, the floor, walls, and ceilings of the hospital kitchens were not free of visible dust as many holes and cracks were noted during the sampling rounds. These conditions can result in difficulty in cleaning, thereby retaining organic residue from food processing and creating a suitable environment for the accumulation and growth of pathogenic microorganisms (Stellato *et al*., 2017). Of particular concern was the acquisition of microorganisms on the food handlers' hands. The potential for contamination was exacerbated as they admitted in the self-report questionnaire that they did not use gloves when distributing unwrapped food.

Staphylococcus aureus is a Gram-positive coccal bacterium of the Staphylococcaceae family (Jamali *et al*., 2015). This pathogen is deemed important due to the Staphylococcal enterotoxins (SEs) produced in food by some *S. aureus* strains (Hennekinne *et al*., 2012). Although the *Staphylococcus* sp. counts in Figure 3.2 (B) were found to be within acceptable limits as proposed by the BCCDC, this microorganism was found to have high counts (Figure 3.2 A). The high count of *Staphylococcus* sp. in the hospital food environment suggested poor personal hand washing hygiene practices, which is a risk factor that may lead to food poisoning, especially in cases of excessive food handling by food handlers. The high counts of *Staphylococcus* sp. should be regarded as a major point of concern as *S. aureus* counts above 10^5cfu/g produce heat stable toxins that are responsible for Staphylococcal food poisoning (Malavi *et al*., 2018).

According to the Department of Health (2000), 10^5/g of *Salmonella* is highly suggestive of possible food poisoning. Additionally, levels ranging from 10^7-10^9 cells/g of *Salmonella* are adequate for the causation of salmonellosis. Foodborne salmonellosis often follows the consumption of contaminated animal products, fruits, and vegetables, while it is also transmitted from person to person through the faecal-oral route (WHO, 2018). Therefore, the detection of *Salmonella* in the hospital food environment was especially noteworthy given that food handlers in Lesotho who are contaminated with *Salmonella* are excluded from employment according to food handlers' certification requirements (Government of Lesotho, 1970).

Pseudomonas sp. is notorious as a food spoiler (Marchand *et al*., 2012; Doulgeraki *et al*., 2012). The bacteria of this species are psychotropic and easily develop in food such as meat, fish, milk, and dairy products stored aerobically and at low temperatures (Remenant *et al*., 2015). The detection of this microorganism in the hospital food environment was a matter of deep concern as this microorganism compromises the aesthetics, quality, and the acceptability of the affected food (Andreani *et al*., 2015; Casaburi *et al*., 2015). Although food handlers' hands are an important source of food contamination, structural defects are also a factor that exacerbates food contamination (Sethlare *et al*., 2013). This includes harbourage and growth of food spoilage microorganisms that are a threat to food security. The prevalence of food spoilage microorganisms in the hospital food environment is especially concerning in developing countries where food security and access to food are major concerns (Rant'so and Seboka, 2019).

E. coli is primarily found in the intestines of both humans and animals, hence detecting this microorganism in the hospital food environment is generally considered as an indication of faecal contamination (Lambrechts *et al*., 2014). Although some strains of *E. coli* are non-pathogenic, there are *E. coli* strains that produce toxins that are pathogenic to humans (Garcia, 2010). Pathogenic *E. coli* strains such as *E. coli* 0157:H7 are associated with severe cases of diarrhoea, haemorrhagic colitis, and haemolytic-uremic syndrome (HUS) (Ammer *et al*., 2018). Therefore, the presence of *E. coli* on food contact surfaces and on food handlers' hands may be attributed to improper hand washing hygiene, mainly after the use of a toilet. During the sampling rounds, it was observed that hand washing facilities were not equipped with appropriate liquid soap, while in some hospital kitchens there were bars of soap at hand washing facilities. These were important findings as the use of antimicrobial soap is vital for the mechanical removal and killing of both transient and resident flora (Larson, 1989).

The absence of coliforms is associated with hygienic environments, hence the detection of these organisms in large quantities on the food preparation surfaces could be attributed to insufficient cleaning which, in turn, could have resulted in contamination (Tominaga and Ishii, 2020). The presence of coliforms was indicative of the presence of a number of Enterobacteriaceae microorganisms such as *Citrobacte*r, *Enterobacter*, *E. coli*, *Klebsiella,* and *Salmonella*. In this study, members of the Enterobacteriaceae family *(P. oryzihabitans*, *S. maltophilia*, *S. paucimobilis*, *Acinetobacter* sp., *M. odorantum*, *E. cloacae,* and *E. asburiae*) were identified using appropriate biochemical tests. Moreover, the detection of microorganisms that are not typically associated with the food processing industry (e.g., *S. maltophilia, S. paucimobilis,* and *M. odorantum*) was a matter of grave concern as they would lead to cross-contamination. This affirms the need for the urgent implementation of the HACCP plan in the hospital under study.

The results of the study affirm the important role that food preparation surfaces play in patient care. Additionally, the study highlights the threat that insufficiently cleaned surfaces and inadequate personal hygiene pose as this tardiness exacerbates the presence of opportunistic pathogens and the possibility of patients contracting a foodborne illness. For instance, *Staphylococcus aureus* food poisoning has been traced back to food contamination during food preparation due to factors such as poor personal hygiene (Bintsis, 2017). The presence of the pathogen *Staphylococcus aureus* in the hospital food environment was also disconcerting given the fact that infections with this microorganism have become difficult to treat due to antibiotic resistance (ABR). The emergence of multidrug resistant strains, particularly methicillin resistance in *S. aureus* (MRSA), has been highlighted by authors such as Kulkarni *et al*. (2019). Therefore, it is important to ensure that all food contact surfaces are free from pathogenic microorganisms through proper and careful implementation of both personal and environmental hygiene procedures.

3.7 Conclusions

The kitchen is a critical food production and processing environment as it houses all food preparation stages until consumption. In this environment, food handlers are key participants in food safety as they process, prepare, and deliver food to consumers who are patients. Therefore, it is the responsibility of all food handlers to ensure food safety and security by employing safe food handling practices.

The study found that food contact surfaces were generally contaminated with common food-related microorganisms. All the investigated food pathogens, namely *Staphylococcus* sp., *Salmonella* sp., *Pseudomonas* sp., *E. coli,* total coliforms, and total viable counts were found on all the food contact surfaces that were surveyed. Subsequently, the biochemical test kit identification revealed six species of organisms from the family Enterobacteriaceae. Among these were pathogens that are predominantly associated with the clinical environment. These findings were therefore an indication of potential patient contamination in the wards of these hospitals. Moreover, the detection of species of the family Staphylococcaceae in the food environment was an indication of poor personal and hand hygiene.

The findings highlighted the role of food contact surfaces in the transmission of foodborne pathogens and the possible transmission of hospital-acquired infections. The fact that microbial populations were found in the hospital kitchens during this study underscores the importance of surveillance and monitoring of kitchen surfaces to ensure minimal contamination of food products. The detection of *Salmonella* sp. affirmed the importance of routinely monitoring food handler certification as it was apparent that the investigated microorganisms had been acquired post-employment. Moreover, the importance of investing in good food preparation practices was evident. This requires proper food handling practices as well as personal, environmental, and hand hygiene habits. It is also argued that hands should be washed using antimicrobial soap. Moreover, the Lesotho Health Department should invest in continuous formal training and education pertaining to hand washing. These programmes should also stress the importance of the use of antimicrobial soap for the reduction of microbial populations. Training should encompass the role of food handlers in food safety and security and should predominantly focus on the prevention of cross-contamination. The importance of environmental, personal, and hand hygiene in the food preparation department should also be stressed. The Ministry of Health Lesotho and Christion Health Association of Lesotho (CHAL) through the hospital management should conduct regular infrastructure monitoring. This infrastructure audits will assist in the prevention of environmental and structural factors that can lead to contamination, such as worn-out equipment and infrastructural flaws that can exacerbate the retention of food residue and dust.

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CHAPTER 4

EVALUATION OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCTION OF GRAM-NEGATIVE BACTERIA STRAINS

4.1 Abstract

There is global concern regarding antibiotic resistance among pathogenic microorganisms of food origin as it is argued that they have the capacity to produce extended-spectrum beta-lactamases (ESBLs) with few treatment options as a consequence. The aim of this study was to investigate the antimicrobial susceptibility profile and production of extended-spectrum beta-lactamases (ESBLs) amongst microorganisms isolated from food contact surfaces in the kitchens of hospitals in Maseru, Lesotho. In vitro susceptibility of various isolates (*Stenotrophomas maltophilia, Sphingomonas paucimobilis, Acinetobacter* spp., *Pseudomonas oryzihabitans, Enterobacter cloacae,* and *Enterobacter asburiae*) was investigated against seven antibiotics (chloramphenicol [30mg], gentamicin [10mg], ceftpodoxime [30mg], cefotaxidime [30mg], amoxicillin [10mg], penicillin [5mg], and nalidixic acid [30mg]. This was done by employing the Kirby-Bauer disc diffusion method. The phenotypic detection of ESBL was confirmed using the double disc synergy test and the combined disc diffusion test. Resistance to more than three antimicrobial classes was detected and reported as multidrug resistance. About 71% of the microorganisms exhibited multidrug resistance (MDR) against antibiotics, while 43% was found to be positive ESBL producers. None of the microorganisms in this study were susceptible to all the tested antibiotics. Based on the high prevalence of MDR and ESBL producers, the importance of including the ESBL production test as part of routine antibiotic testing is recommended. The study also highlights the importance of reinforcing hand washing as a measure to reduce the spread of ESBL-producing bacteria.

Key words: antibiotic resistance, extended-spectrum beta-lactamase, food contact surfaces

4.2 Introduction

It has been generally acknowledged that the use or over-use of antibiotics is the primary cause of global antibiotic resistance amongst microorganisms. The reduction in the effectiveness of antimicrobial drugs in curing a disease or condition is referred to as antibiotic resistance (Li and Webster, 2018; Shaikh *et al*., 2015). This lack of effectiveness of antibiotics could also be attributed to their short generation time and the intrinsic resistance of certain bacteria to antibiotics (Laxminarayan *et al*., 2013), whereas the escalating use of antibiotics as growth promoters in the farming industry also adds to this phenomenon (Ventola, 2015).

Bacteria may develop resistance to antibiotics through mutation or acquisition of resistant genes from other bacteria through a mechanism known as 'horizontal gene transfer' (HGT) (Habbosh and Guzman, 2023). Horizontal gene transfer occurs between and among bacterial strains, species, genera, and through the transfer of non-pathogenic to pathogenic bacteria (Capita *et al*., 2016). These microorganisms acquire multiple antimicrobial-resistance genes and this results in multiple resistance functions that enable the organism to resist multiple antibiotics (Zhang *et al*., 2019). Antibiotic resistance mechanisms include efflux pumps, enzyme production and cell wall modification, degradation of antimicrobial compounds, and the use of alternative metabolic pathways (Belay *et al*., 2024; Elshobary *et al*., 2025).

In the pursuit to alleviate the burden and spread of antimicrobial resistant bacteria in food, various interventions and measures have been employed. Interventions to combat the burden of antibiotic resistance include restricting antibiotic use as a growth promoter and limiting antibiotic use for non-therapeutic applications within animal rearing settings (Tang *et al*., 2017). Additionally, in order to reduce the dissemination of MDR bacteria through the food chain, there have been efforts to improve farm biosecurity, enhance the development of alternative treatment strategies, and ensure increased hygienic conditions and practices along the food chain. Education programs that are directed mainly at veterinarians, farmers, and food handlers have been developed and surveillance systems on antibiotic resistance have been established for human and animal linkage purposes (Rocha *et al*., 2015). Additionally, critical antibiotics for human health should be clearly identified and the use of such antibiotics must be restricted to human use only to avoid cross resistance. It is in this regard that the World Health Organization (WHO) has issued a list of critical antibiotic agents for human use so that they can be avoided in non-human interventions (WHO, 2011; EFSA and ECDPC, 2015).

4.3 Antimicrobial resistance of foodborne pathogens: A public health concern

Food such as dairy, meat and fish are a nutritional necessity for human beings, yet they are also an ideal culture medium for microbial growth which poses a significant risk to humans and animals alike (Urban-Chmiel *et al*., 2025). When suitable conditions exist for bacterial chemical reactions, the aesthetics of food is altered as food is used as an energy and carbon source. Some bacteria cause foodborne intoxication and infection (Pundir and Jain, 2011), and therefore food that is contaminated with bacteria that release toxins is unsafe for human consumption (WHO, 2015a). Contaminated food is responsible for foodborne illnesses as well as outbreaks caused by antimicrobial resistant bacteria (FAO and WHO, 2018). Food-related antibiotic resistant microorganisms are spread to humans through the food chain by means of food products, food handlers, and environmental pollutants such as farming effluent (Oldeyemi and Sani, 2016). Bacteria that emerge on farms are often maintained throughout the food production chain and are subsequently transmitted to consumers. When ingested, these bacteria cause foodborne infections (EFSA and ECDPC, 2015).

Antibiotics are natural, synthetic or semi-synthetic substances that interfere with the growth of or kill microorganisms, specifically bacteria. Antibiotics are used to treat or prevent infections in humans and in animals (O'Neill, 2015; WHO, 2015b). According to Wall *et al*. (2016), the extensive use of antibiotics as growth promoters for the rapid growth of food-producing animals and fish exacerbates the emergence of antibiotic resistance. The emergence of antibiotic resistance in the food chain is considered a cross-sectional problem as antibiotics are widely used in aquaculture, livestock production, and crop culture. Consequently, antibiotic resistant bacteria and antibiotic resistant genes (ARGs) can easily spread at each stage of the food production chain and subsequently cause infections in humans (Chang *et al*., 2015).

According to WHO (2017), most critical pathogens in hospital-acquired infections (HAI) are stratified into three groups based of the global threat they pose and the urgency of the action required. The first and the second groups (urgent and high priority pathogens) encompass the ESKAPE pathogens which are the vancomycin resistant *Enterococcus faecium* (VRE), methicillin-resistant and vancomycin-resistant *S. aureus* (MRSA/VRSA), and the carbapenem resistant and third-generation cephalosporin-resistant *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp. These pathogens are associated with high morbidity and mortality HAIs due to their acquired resistance towards a large number of antibiotics, including last-resort antibiotics such as carbapenems and colistin (Santajit and Indrawattana, 2016; Ma *et al*., 2020). Infections involving these pathogens are therefore associated with serious illnesses that result in increased healthcare costs, prolonged hospital stays, high costs due to the need for second-line drugs, and even treatment failures (ECDC, 2017; Shrestha *et al*., 2018). For example, a survey in the US revealed that antimicrobial resistance and a 20-billion-dollar surplus in direct healthcare cost this country about 35 billion dollars in loss of productivity annually (CDC, 2018).

4.4 Antimicrobial resistance within the hospital environment

Food-related pathogens such as *Salmonella*, pathogenic *E. coli, Campylobacter, Staphylococcus* spp., *Enterococcus* spp., and extended-spectrum beta-lactamase (ESBL) producing Gram-negative bacteria have been the main focus of the AMR phenomenon (Cahill *et al*., 2017). Infections with resistant strains of bacteria are normally complicated and difficult to treat as these bacteria are often resistant to multiple antimicrobials (Zhang *et al*., 2019). For example, there are reports of 1.29% non-typhoid *Salmonella* (NTS), 12.5% and 2.43% Shiga toxin-producing *Escherichia coli* (*stx* only and *stx* + *eae*) and 4.46% campylobacter species resulting in hospitalisation (Teshale *et al*., 2025). Of these, NTS were previously treated with ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole (TMP-SMZ). However, significant resistance to these antibiotics has been reported over the years with resistance rates of 49% Ampicilin, 31.3% Chloramphenicol 29.4% trimethoprim-sulfamethoxazole (TMP-SMZ), 75% Ampicilin and 36% trimethoprime salfamexazole (Yue *et al*., 2022; Chang *et al*., 2021). Consequently, new treatment options that include azythromycine, meropenem and piperacillin/tazobactum are currently administered. The escalating resistance of bacteria even to these treatment options is currently an important public health concern (Rasheed *et al*., 2020).

Food-related pathogens are mainly responsible for extra-intestinal infections in the hospital setting. For example, AMR strains of *E. coli* are a common cause of urinary tract infections and are associated with failure in treatment therapy (Nordstrom *et al*., 2013). Additionally, there is a significant pandemic potential regarding the emergence of antibiotic resistance in bacteria such as colistene resistance in *E. coli* (Liu *et al*., 2016) and methicillin resistance in *S. aureus* (Price *et al*., 2012). Although these strains are recognised as being of animal origin, they are adaptable to multiple hosts (Fernandes *et al*., 2016; Liu *et al*., 2016), virulence mechanisms (Ewers *et al*., 2012; Price *et al*., 2012), and high gene exchange (Liu *et al*., 2016). These organisms are a serious threat to public health as they could lead to the emergence of new resistant, virulent, and mobile strains that may be unknown to the human immune system (van de Honert and Hoffman, 2018; Founou *et al*., 2016). It is for this reason that the World Health Organization deems antibiotic resistance of zoonotic foodborne pathogens as one of the many global challenges mankind faces (Chavan and Vashishth, 2025).

4.5 Materials and methods

4.5.1 Antimicrobial susceptibility testing

The bacterial strains *Stenotrophomas maltophilia*, *Sphingomonas paucimobilis Acinetobacter* spp., *Myroides odorutum*, *Pseudomonas oryzihabitans*, *Enterobacter cloacae*, and *Enterobacter asburiae* were subjected to a battery of antimicrobials in order to determine their susceptibility profile using the Kirby-Bauer disc diffusion method according to interpretive criteria as recommended by CLSI guidelines (CLSI, 2012). The antimicrobial agents used in this study were as follows: chloramphenicol (30mg), gentamicin (10mg), ceftpodoxime (30mg), cefotaxidime (30mg), amoxicillin (10mg), penicillin (5mg), and nalidixic acid (30mg) ((Merck, SA).

Briefly, after preparation of bacterial suspension to 5ml, the turbidity was adjusted using 0.5 McFarland standards. The strains were subsequently inoculated on Mueller-Hinton agar. After overnight incubation at 37ºC, diameters of inhibition zones were measured and the results were interpreted as susceptible, intermediate, and resistant. The *Escherichia coli* ATCC strain 25922 was used for quality control (Seyedjavadi *et al*., 2016).

4.5.2 Phenotypic tests of ESBL detection

ESBL production was confirmed phenotypically by the double disk synergy test (DDST) and the combined disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) criteria for ESBL screening (CLSI, 2012).

4.5.2.1 Double disc synergy test

The double disc synergy test (DDST) was carried out concurrently with the susceptibility test of each isolate by placing a susceptibility disc of amoxicillin-clavulanic acid (10mg/20mg) at the centre of the plate while the discs containing cefotaxime (30mg) and cefpodoxime (30mg) were placed 30mm (centre to centre) from the amoxicillin-clavulanic acid disc. Plates were then incubated aerobically at 37ºC for 18-24 hours. A clear extension of the edge of the inhibition zone of cephalosporin towards amoxicillin-clavulanic acid disc was interpreted as positive ESBL production (Ibrahim *et al*., 2013).

4.5.2.2 Combined disc diffusion method

The disc diffusion method was conducted using both cefotaxime (30mg) and cefpodoxime (30mg) discs alone and as a combination of clavulanic acid (30mg/10mg) according to the CLSI protocol. Discs were placed 25mm apart from each other on Mueller-Hinton agar inoculated with 0.5 McFarland suspension of the test isolate. The increase of more than or equal to 5mm in the zone diameter of a combination of clavulanic acid and the tested antimicrobial agent was compared to the zone diameter found when the tested antimicrobial was tested alone and was interpreted as ESBL production (Seyedjavadi *et al*., 2016).

4.6 Results

4.6.1 Antimicrobial susceptibility and multidrug resistance strains' profile

The antimicrobial susceptibility profile of strains isolated from hospital kitchen surfaces are presented in Table 4.1. *Stenetrophomonas maltophilia* was found to be susceptible to chloramphenicol (24mm) and gentamicine (20.9mm). However, resistance of *S. maltophilia* to amoxicillin (11.9mm), cefotaxime (12.1mm), and cefpodoxime (9.2mm) was also observed. These results were similar to those reported by Chung *et al*. (2012), who found that *S. maltophilia* had the lowest susceptibility rates to chloramphenicol and ceftazidime (3rd generation cephalosporine). *Sphingomonas paucimobilis* was found to be susceptible to chloramphenicol (24.8mm), nalidixic acid (23.1mm), cefpodoxime (25.0mm), gentamicine (24.2mm), and cefotaxime (35.6mm) and resistant only to penicillin (8.6mm). According to Lugito *et al*. (2016), *S. paucimobilis* is commonly known for its susceptibility to carbapenems, aminoglycosides, trimethoprim-sulfamethozole, and

Table 4.1: Antimicrobial susceptibility and multidrug resistance profile of strains isolated from hospital kitchen surfaces

Organism	β-lactam antibiotic resistances				Resistance to common antibiotics		
	P (mm)	AMX (mm)	CTX (mm)	CPDX (mm)	C (mm)	NA (mm)	CN (mm)
Stenetrophomonas maltophilia	8,6 (r)	11,9 (r)	12,1 (r)	9,2 (r)	24,4 (s)	17,0 (r)	20,9 (s)
Sphingomonas paucimobilis	8,6 (r)	17,6 (i)	35,6 (s)	25,0 (s)	24,8 (s)	23,1 (s)	24,2 (s)
Acinetobacter spp.	7,1 (r)	14,5 (i)	12,3 (r)	18,1 (r)	24,7 (i)	6,2 (r)	19,6 (i)
Myroides Odoratum	7,2 (r)	24,1 (s)	35,4 (s)	20,9 (r)	26,6 (s)	22,3 (s)	20,5 (i)
Pseudomonas Oryzihabitans	7,4 (r)	16,2 (r)	27,4 (s)	18,6 (r)	24,8 (s)	20,7(s)	19,4 (s)
Enterobacter cloacae	15,3 (r)	20,5 (i)	25,0 (i)	18,6 (r)	30,3 (s)	10,8 (r)	25,6 (s)
Enterobacter asburiae	8,4 (r)	14,7 (r)	10,9 (r)	17,4 (r)	24.1 (s)	21,3 (i)	27,1 (s)

P: penicillin; AMX: amoxicillin; CTX: ceftaxidime; CPDX: cefpodoxime; C: chloramphinecol; NA: nalidixic acid; CN: gentamicine

r: resistance, s: susceptibility, i: intermediate piperacillin-tazobactam, although it is resistant to both penicillin and first generation cephalosporines.

Enterobacter spp. are a common cause of bacterial infections in humans. *Enterobacter cloacae* and *Enterobacter aerogenes* represent the most common *Enterobacte*r species with newer species such as *Enterobacter asburiae* emerging as pathogens that cause severe infection (Koth *et al*., 2012). In the current study, *E. cloacae* was found susceptible to chloramphenicol (30.3mm) and gentamicin (25.6mm). However, *E. cloacae* was also found to be resistant to penicillin (5.3mm), cefpodoxime, and nalidixic acid (10.8mm). On the other hand, *E. asburiae* was found to be susceptible to gentamicine (24.1mm) and chloramphinacol (27.1mm). A similar result regarding the susceptibility of *E. asburiae* to gentamicine was found in a study by Mardaneh *et al*. (2014).

Acinetobacter spp. are associated with severe nosocomial infections and hospital outbreaks (Almasaudi, 2018; Brandy *et al*., 2023). Many infections with this microorganism involve multidrug resistant strains and occur in intensive care units where severely ill patients are treated with broad-spectrum antibiotics (Yadav *et al*., 2020). In the current study, *Acinetobacter* spp. were found not to be susceptible to any of the test antibiotics as they had an intermediate response to amoxicillin (14.5mm), chloramphenicol (24.7mm), and gentamicine (19.6mm).

According to Panagopoulos *et al*. (2016), *Pseudomonas oryzihabitans* is rarely a cause of human infection and is rarely resistant to antibiotic therapy, hence it is easy to treat. However, *P. oryzihabitans* was observed to be resistant to penicillin (7.4mm), amoxicillin (16.2mm), and cefopodoxime (18.6mm).

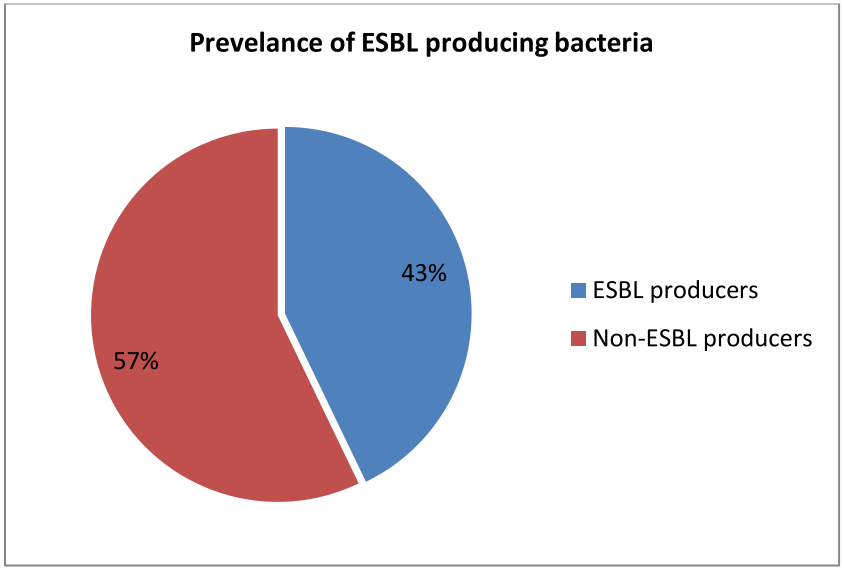
Developing standardized definitions to classify and characterize bacteria that are resistant to multiple antimicrobials could assist in ensuring the reliability of epidemiological surveillance data collected and compared across health settings and counties. For example, in this study, MDR refers to non-susceptibility to one or more antimicrobials on three or more known antimicrobial classes, as proposed by Magiorakos *et al*. (2012) and

Cheesman *et al*. (2017). In this study, most of the microorganisms (71%) were found to be multidrug resistant, namely *S. maltophilia*, *Acinetobacter* spp., *M. odorutum*, *E. cloacae,* and *E. asburiae*. Among the MDR bacteria, *S. maltophilia* exhibited most resistance at 71% with 28% susceptibility to the test antibiotics. Additionally, *Acinetobacter* spp. presented a 57% resistance to test antibiotics and there was no susceptibility to antibiotics observed.

4.6.2 Prevalence of antibiotic resistance in EBSL-producing bacteria

Figure 4.1 shows the prevalence of ESBL-producing bacteria, while Figure 4.2 shows the antimicrobial profile of ESBL-positive bacteria. Figure 4.3 depicts the positive ESBL production of isolates. A proportion of about 43% positive extended-spectrum beta-lactamase (ESBL) producing *Acinetobacter* spp*., E. cloacae,* and *E. asburiae* was found in this study*.* The extended-spectrum beta-lactamase production exhibited in this study was significantly higher than the 6.5% and 30% reported respectively in similar studies (Kader and Kumar, 2004; Ibrahim *et al*., 2013). However, AL-Agamy *et al*. (2006) reported a significantly high ESBL production rate of 60.9% among isolates.

It is apparent in Figure 4.2 that the ESBL-producing organisms were susceptible only to chloramphenicol at a 66% response rate. ESBL-producing organisms also presented an intermediate response to amoxicillin, cefotaxime chloramphenicol, and nalidixic acid with the highest response rate at 66% on amoxicillin. However, isolates were resistant to amoxicillin, penicillin, cefpodoxime, cefotaxime, and nalidixic acid with a 100% resistance to penicillin and cefpodoxime. Singh *et al*. (2017) found a similar resistance response among ESBL-producers.



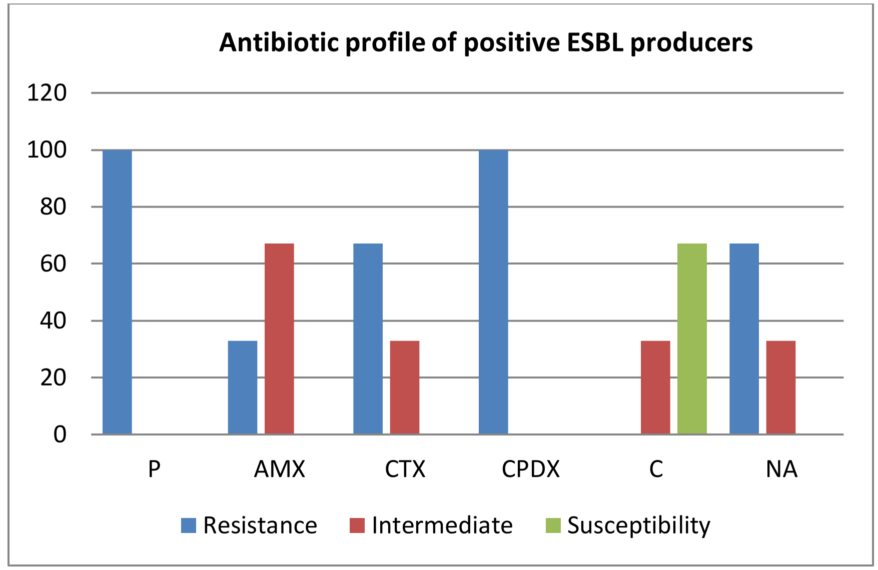


Figure 4.1: Prevalence of extended-spectrum beta-lactamase producing bacteria.

Figure 4.2: Antibiotic profile of extended-spectrum beta-lactamase (ESBL) producers. P: penicillin; AMX: amoxicillin; CTX: cefotaxime; CPDX: cefpodixim; C: chloramphenicol; NA: nalidixic acid; CN: gentamicin.

A B C

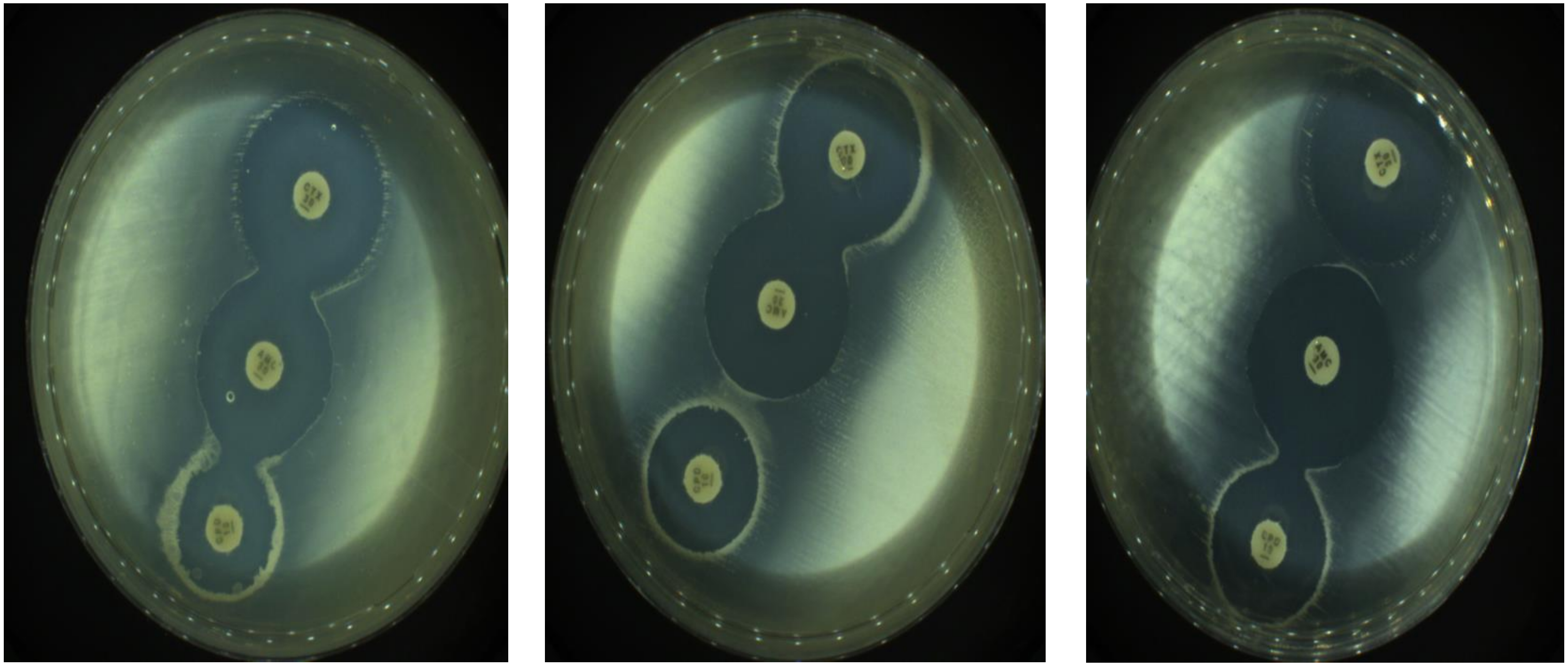


Figure 4.3: Positive ESBL-producing *E. asburiae* (A), *E. cloacae* (B), and *Acinetobacter* spp. (C). Antibiotic discs contain cefotaxime (30mg), cefpodoxime (30mg), and amoxicillin/clavulanic acid (amoxicillin 10mg/clavulanic acid 20mg)

4.7 Discussion

The prevalence of multiple antimicrobials within bacterial communities has become a challenge to antibiotic therapy and in the treatment of infections. Microorganisms, particularly Gram-negative bacteria, acquire resistance to multiple antimicrobials and are able to transfer the resistant genes within the microbial niches. This has become a global challenge and has raised the urgent need for development of newer treatment options. In this study, *Acinetobacter* spp. were found to present susceptibility to none of the test antibiotics, which could be attributed to the intrinsic resistance to many antibiotics associated with this microorganism (Kyriakidis *et al*., 2021). Additionally, there was evidence of multiple resistance to antibiotics (71%) and 100% resistance to penicillin among the tested microorganisms. It is possible that this outcome occurred as a consequence of the dispensation of these antibiotics by pharmacies without a physician's prescription.

Bacteria may present multiple mechanisms of resistance to antibiotics such as an enzymatic resistance by ESBL production in combination with changes in the permeability of the outer membrane and activity of the efflux pumps. In this study, 43% of the tested microorganisms (*Acinetobacter* spp. and *Enterobacter* spp.) was found to produce ESBLs. Although there was a 67% susceptibility to chloramphenicol among ESBL-producing microorganisms, ESBLs are known to carry genes that are resistant to antimicrobials such as aminoglycosides, fluoroquinolones, trimethoprime-sulfamathoxazole, tetracyclines, and chloramphenicol (Kidsley *et al*., 2018). Extended-spectrum beta-lactamases enzymes are carried in plasmids and can be transferred among bacterial organisms (Kaur and Sigh, 2018; Husna *et al*., 2023). Therefore, it is important to distinguish between resistance due to ESBL production and non-ESBL production among bacterial strains to prevent cross-transmission of ESBL-producing bacterial strains among patients (Jahani *et al*., 2017).

4.8 Conclusions

Antimicrobial resistance continues to be of great concern in developing countries such as Lesotho where ESBL production tests are not routinely. Pathogenic microorganisms are found throughout the food production chain from farm level to where food is served to consumers. These microorganisms are mostly implicated in nosocomial infections that result in prolonged hospital stays and complications such as infections and the re-infection of patients. This exacerbates the difficulties in treatment consequent to the acquired resistance to multiple antibiotics.

Resistance of bacteria to antimicrobials is facilitated by various mechanisms, one of which is the production of beta-lactamase enzymes. In this study, all ESBL producers presented no susceptibility to amoxicillin and 33% intermediate response to cefotxime, chloramphenicol and nalidixic acid. Furthermore, there was 66% resistance cefoxamine and 100% resistance to penicillin. This means that all the microorganisms tested in this study were resistant to penicillin. Overall, there was no antibiotic to which all the microorganisms were susceptible; however, resistance to multiple antibiotics was evident in 71% of the microorganisms. These findings are important as not much information has been reported on the production of ESBLs among microorganisms in hospitals in Lesotho. The findings should thus raise interest in future studies that should investigate the resistance patterns of microorganisms in developing countries. Such studies should be cognisant of the fact that pathogens can easily spread resistance genes and that this results in treatment failure and high treatment costs (Benner *et al*., 2014).

It is therefore important to include ESBL production tests in routine antibiotic testing as this will ascertain the susceptibility of microorganisms to prescription antibiotics and avoid possible treatment failure. Additionally, testing for ESBL production will provide insight into the resistance patterns of bacterial strains in a geographical area and subsequently guide appropriate antibiotic use. It is also important to conduct further in-depth studies on antibiotic resistance as this will assist in formulating empirical antibiotic policies to address treatment of infections. Moreover, updated antibiotic policies will assist in avoiding the misuse and overuse of antibiotics. Antimicrobial resistance policy should include restrictions on over the counter dispensation of antibiotics. Additionally, hospital staff and patients should be educated on the importance of regular and proper hand washing as this has been shown to be a positive measure in reducing the spread of ESBL-producing bacteria. These recommendations are in line with the global action plan of WHO to address antimicrobial resistance (WHO, 2015b).

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CHAPTER 5

EFFICACY OF CHLORHEXIDINE ISOPROPANOL AND SODIUM HYPOCHLORITE AGAINST GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA ISOLATED FROM FOOD CONTACT SURFACES IN HOSPITAL KITCHENS IN MASERU, LESOTHO

5.1 Abstract

The processes utilised in cleaning and disinfecting surfaces where food is prepared are of the utmost importance in both the food industry and clinical environments. These processes involve the use of detergents and/or disinfectants that are critical in the prevention and control of pathogens. Use of these products provides a break-point between preparation processes, thereby preventing cross-contamination and cross-infection which could result in the spread of pre-existing infections. Therefore, the aim of this study was to investigate the efficacy of chlorhexidine and sodium hypochlorite on *Stenetrophmonas maltophilia, Sphingomonas paucimobilis, Acinetobacter* spp., and *Staphylococcus aureus.* These microorganisms were isolated from hospital food preparation contact surfaces in Maseru, Lesotho. The antimicrobial activity of disinfectants was evaluated using the disc diffusion method by measuring the diameter zone of inhibition. The minimum inhibitory concentrations and minimum bactericidal concentrations were determined by using 96-well microtiter plates. Disinfectant 1 contained sodium hypochlorite while disinfectant 2 contained chlorhexidine with ispophyl alcohol. Both disinfectants exhibited antimicrobial activity and inhibitory activity against the tested organisms. Disinfectant 1 showed the highest zone of inhibition at 32,0 ± 1,62mm against *S. aureus* and the highest zone of inhibition against all microorganisms selected for this study. However, disinfectant 2 exhibited the highest bactericidal effect against *S. aureus* while there was no bactericidal effect exhibited against the Gram-negative bacteria using this disinfectant. Therefore, this study established the efficacy of disinfectant 1 against both Gram-positive and Gram-negative bacteria, while disinfectant 2 was effective only against Gram-positive bacteria.

Key words: Cleaning, disinfection, sodium hypochlorite, chlorhexidine, gram-negative bacteria, gram-positive bacteria

5.2 Introduction

The hospital environment is commonly associated with infections caused by pathogenic microorganisms such as *Staphylococcus aureus,* vancomycin resistant *Enterococcus faecalis*, *Clostridium difficile,* and *Acinetobacter baumannii* (Robakowska *et al*., 2021). Infections acquired in hospital settings have resulted in grievous morbidity and mortality rates globally, with narrow treatment options due to heightened antibiotic resistance among pathogenic microorganisms (Oliveira *et al*., 2017). Consequently, the hospital environment is considered an important source of infection and cross-infection (Dancer, 2014).

In the pursuit to combat the prevalence and spread of pathogenic microorganisms, cleaning and disinfection processes are commonly employed in both domestic and clinical settings. This type of cleaning is a complex and systematic process in which organic and inorganic substances are removed from surfaces by employing detergents and/or enzymatic products to ensure that disinfection will be effective (Lantagne *et al*., 2018). Disinfection is generally effective in destroying most microbial forms using physical and chemical procedures such as UV radiation, boiling, and/or vaporising. However, these processes are usually effective on vegetative pathogens and not on spores (Kirmusaoglu, 2018), and therefore they are increasingly applied in domestic and hospital food preparation environments (Hughes and Ferguson, 2017).

5.3 Cleaning in the food service industry

Cleaning and disinfection procedures are part of good food manufacturing and preparation practices within the food service industry. These procedures involve the use of detergents that are designed to break down residual soil in food and remove microbial contaminants on food contact surfaces. This process is then followed by the application of disinfectant antimicrobials that reduce the viability of any remaining microorganisms (Fazlara and Ekhtelat, 2012). Food preparation areas and poor personal hygiene of food service workers (mainly improper hand washing) have been found to contribute significantly to the risk of contracting foodborne diseases (Lambrechts *et al*., 2014). Therefore, a combination of washing, rinsing, and disinfection is employed to ensure that food contact surfaces, such as tableware and cutting boards, are not the sources of cross-contamination (Monnin *et al*., 2012). Proper cleaning and hand washing practices require the use of a detergent and/or disinfectant to reduce the threat of food contamination associated with food production.

5.4 Cleaning and disinfecting food preparation surfaces in hospitals

The hospital environment is known to harbour potential pathogens due to a large number of sick patients, the speed and acuity of patient care by healthcare workers, and the complexity of hospital surfaces and the medical equipment that require routine cleaning (Doll *et al*., 2018). For example, frequently touched hospital surfaces are commonly contaminated with drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Lin *et al*., 2016), vancomycin-resistant *Enterococcus* (VRE) (Knelson *et al*., 2014), carbapenem-resistant Enterobacteriaceae (CRE) (Weber *et al*., 2015), *Acinetobacter* spp. (Weber *et al*., 2010), and *Clostridium difficile* (Sitzlar *et al*., 2013). Surfaces involved in food preparation are deemed 'critical areas' due to their potential for cross-transmission of pathogens and they should therefore be routinely and appropriately cleaned and disinfected (Dancer, 2014).

Adherence to standard infection prevention practices is an essential part of disease control and prevention in clinical settings. According to Doll *et al*. (2018), surface cleaning is not a substitute for other infection control practices such as hand washing, room ventilation, isolation, and gowning and gloving where indicated. Therefore, routine measures to decrease the overall microbiological prevalence in the hospital environment through appropriate cleaning practices is the foundation on which other, more in-depth decontamination regimens are built.

5.5 Monitoring of environmental cleanliness

There are five systems that, if applied as recommended, can be used to objectively monitor cleaning practices. These systems include microbiological methods such as preparing and evaluating agar slide cultures and swab cultures. Non-microbiological methods can also be applied, such as direct observation, fluorescent markers, and adenosine triphosphate bioluminescence (Doll *et al*., 2018; Furlang *et al*., 2019). However, these systems have substantial limitations, hence the Centre of Disease Prevention (CDC) recommends that each hospital should carefully evaluate the advantages and limitations of each method and then choose a system or systems that best meet the needs of the particular institution (Carling and Bartley, 2010; Guh and Carling, 2010).

Direct practice observation has been practised by environmental service managers as a traditional method to monitor the cleanliness of health facilities (Doll *et al*., 2018). However, numerous studies have questioned the accuracy of visual inspection compared to other methods (Mulvey *et al*., 2011). Sitzlar *et al*. (2013) assert that, in order for the environmental cleaning evaluation method to be an acceptable monitoring strategy, data that are presented in a clear and meaningful manner and that are useful for consideration by the staff should be provided. Additionally, feedback to the staff based on the gathered data regarding the quality and efficacy of their cleaning practices will improve the effectiveness of their cleaning attempts. However, deterioration of any practice will occur in an effective model does not guide the post-intervention period (Smith *et al*., 2014).

5.6 Mode of action of common disinfectants

5.6.1 Quaternary ammonium compounds (QACs)

Quaternary ammonium compounds (QACs), such as chlorhexidine, are cationic surface-active substances used in clinical and industrial environments to reduce environmental microbial populations (Jansen *et al*., 2013). QACs are characterized by a lack of selective toxicity and target specificity (Denyer and Steward, 1998). The cationic surface of QACs is adsorbed into the anionic bacterial cell wall from where is subsequently diffuses through the cell wall into the cytoplasmic membrane (Hegstad *et al*., 2010). This process disrupts the cell membrane, thereby causing leakage of the cell contents which results in cell lysis (Jansen *et al*., 2013).

Chlorhexidine, commonly known as chlorhexidine gluconate, is a broad-spectrum, cationic, antiseptic agent with low toxicity and residual activity (Anderson *et al*., 2010). It is effective against both Gram-negative and Gram-positive bacteria through attachment to and disruption of the bacterial cytoplasmic membrane (Grare *et al*., 2010). Chlorhexidine gluconate has various mechanisms of action including cell membrane damage, the collapse of membrane potential, leakage of cell contents, and coagulation of cytosol at high concentrations (Hughes and Ferguson, 2017).

5.6.2 Sodium hypochlorite

Chlorine is widely used as a disinfectant in the food and water industries as well as in hospital settings. Amongst the chlorine-based compounds, sodium hypochlorite is the most convenient because it is an aqueous product and relatively stable in its concentrated form. Depending on the concentration, this oxidizing antimicrobial agent has a broad-spectrum effect and is classified as a low-intermediate level biocide (SCENIHR) (Kohler *et al*., 2018).

The mechanism of action of sodium hypochlorite has been widely studied. However, there is no common understanding of the processes involved. What is understood is that the mechanism of action of sodium hypochlorite is associated with the chemical dynamic balance exhibited by this substance in water ($NaOCl^{+}H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^{+} + OH^{-} + H^{+} + OCl^{-}$). In water, sodium hypochlorite ionizes to produce the sodium ion Na^{+} and the hypochlorite ion OCl^{-} to establish equilibrium with hypochlorous acid (HOCL) (Bloomfield,1996).

Hypochlorous acid is considered the most active compound responsible for bacterial inactivation by chlorine-producing agents (McDonnell and Russell, 1999). According to McDonnell and Russell (1999), chlorine exists primarily as hypochlorous acid at low pH levels (pH 4-7). At a high pH level, this substance is found predominantly as hypochlorite ions. Although there is minute contribution in comparison with hypochlorous acid, the hypochlorite ions also contribute to bacterial deactivation.

Three chemical reactions occur between sodium hypochlorite and organic tissue, namely saponification, neutralization of amino acids, and chloramination. According to Estrela *et al*. (2002), it has been observed that sodium hypochlorite acts as an organic and fat solvent that degrades fatty acids into fatty acid salts (soap) and glycerol (alcohol). This process reduces the surface tension of the remaining solution which is referred to as saponification. During the neutralization reaction, sodium hypochlorite neutralizes amino acids which results in water and salts. This process also results in the release of hypochlorite ions which, in turn, lead to a decrease in pH levels. Additionally, the hypochlorous acid acts as a solvent in the presence of organic tissue and releases chlorine which, when combined with protein groups, forms chloramines. Chloramines are the product of chloramination which is the reaction between chlorine and amino groups, and this process interferes with cell metabolism. Chlorine, which is a strong oxidizing agent, results in antimicrobial action that inhibits bacterial enzymes, leading to an irreversible oxidization of the sulphydryl group of crucial bacterial enzymes. The aim of this study was to investigate the efficacy of chlorhexidine and sodium hypochlorite on the *Stenetrophomonas maltophilia*, *Sphingomonas paucimobilis*, *Acinetobacter* spp., and *Staphylococcus aureus* organisms that isolated from food contact surfaces in hospital kitchens in Maseru, Lesotho.

5.7 Materials and methods

5.7.1 Preparation of disinfectants

Two commercially manufactured antimicrobials were purchased from a local supermarket in Bloemfontein, South Africa. For the purpose of this study, these products are referred to as disinfectant 1 (sodium hypochlorite) and disinfectant 2 (chlorhexidine with isoprophyl alcohol). To prepare stock solutions of 3.5% sodium hypochlorite, a volume of 26.25g of sodium hypochlorite was diluted in 750ml of water. To prepare 0.5%:75% of chlorhexidine isoprophyl, 75% isoprophyl alcohol was prepared and added to 0.005g of chlorhexidine in a 750ml cylinder. This was to obtain the concentrations in which these disinfectants are purchased in the market (Golus *et al*., 2016).

5.7.2 Inoculum preparation

An inoculum was prepared for each microorganism according to the CLSI direct colony suspension method. This was achieved by inoculation of pure colonies of each microorganism into 5ml of sterile Mueller-Hinton broth and subsequent incubation at 37ºC for 24hrs. The turbidity was adjusted to 0.5 McFarland Standard corresponding to 1-2×10^8 colony forming units (CFU) ml^{-1} (Golus *et al*., 2016).

5.7.3 Disc diffusion agar method

The standard paper blank disc diffusion assay (CLSI, 2003) was used to test the susceptibility of *Staphylococcus aureus*, *Stenetrophomonas maltophilia, Sphingomonas paucimobilis,* and *Acinetobacter* spp. to the two selected disinfectants. One ml of each isolate from MHB was transferred to new plates and stricken by use of sterile swabs to create a lawn. Sterile filter paper discs (6mm diameter) were placed on Muller-Hinton agar (MHA) plates at constant distances. Volumes of 20µl of 3.5% sodium hypochlorite and 0.5% chlorhexidine with 75% isoprophyl alcohol were loaded on the discs (Golus *et al*., 2016). The plates were observed for clear zones after a 24hr incubation period at 37ºC, and zones of inhibition were measured in mm. The entire diameter of the zone of inhibition was measured including the disc, and the end-point reading was taken as complete inhibition of growth to the naked eye. All experiments were conducted in duplicate (Selim *et al*., 2015).

5.7.4 The determination of MIC and MBC

In vitro susceptibility tests were performed using a 96-well microtiter plate to determine MICs of sodium hypochlorite and chlorhexidine isopropanol against the previously isolated microorganisms *Staphylococcus aureus*, *Acinetobacter* spp., *Sphingomonas paucimobilis,* and *Stenotrophomonas maltophilia* using standard broth microdilution methods with an inoculum of 0.5 McFarland standard (CLSI, 2012). A two-fold dilution of the disinfectant's active agent, sodium hypochlorite (0.5mg-70mg) chlorhexidine isopropanol (0.078mg-10ug:1117-15000mg) was mixed with a suspension of 0.5 McFarland standard of the test organism in Mueller-Hinton broth. Microtiter plates were inoculated with 20µl of freshly prepared inoculum, 20µl disinfectant, and 100µl of Muller Hinton broth. The plates were incubated at 37ºC for 18-24hrs. After incubation, the MIC was measured to determine the lowest concentration of antimicrobial agent resulting in complete inhibition of visible growth.

To determine the MBC,100µl from the wells without visible growth and 100µl from the growth control wells were plated on Mueller-Hinton agar and placed in an incubator until growth was detected on the growth control plates. The MBC was defined as the lowest concentration of antimicrobial agent in which there was no visible growth (Golus *et al*., 2016; Nillian *et al*., 2016).

5.8 Results

5.8.1 Antimicrobial susceptibility testing of the selected disinfectants

The antimicrobial activity of the two commercial disinfectants (sodium hypochlorite and chlorhexidine isoprophyl alcohol) was investigated at concentrations of 3.5% for sodium hypochlorite and 0.5% for chlorhexidine with 75% isoprophyl alcohol (Table 5.1). The investigation was done against *S. aureus*, *S. maltophilia, S. paucimobilis*, and *Acinetobacter* spp. by measuring zones of inhibition using the disc diffusion method. Although these microorganisms were found in the kitchens of hospitals included in this study, *S. maltophilia* and *S. paucimobilis* are mostly associated with clinical infections. Both disinfectants exhibited antimicrobial properties against all isolates; however, superior inhibition zones were associated with disinfectant 1 in all isolates. The highest zone of inhibition was detected for disinfectant 1 at 32,0 ± 1,63 against *S. aureus,* whereas the zone of inhibition was at 30,00 ± 1,41 for both *S. maltophilia,* while *S. paucimobilis*. *Acinetobacter* spp. had the lowest zone of inhibition at 25,67 ± 1,89 against disinfectant 1. Similarities in the zones of inhibition at 15,67 ± 1,69 were demonstrated by disinfectant 2 against *S. aureus* and *S. paucimobilis*.

Table 5.1: Average diameter zone of inhibition of disinfectants on *Acinetobacter* spp., *S. aureus*, *S. paucimobilis,* and *S. maltophilia*

	Zone of inhibition (mm)			
Disinfectant	***Staphylococcus Aureus***	***Acinetobacter* spp.**	***Sphingomonas paucimobilis***	***Stenotrophomonas maltophilia***
Disinfectant 1	32,0 ± 1,63	25,6 ± 1,89	30,0 ± 1,41	30,00 ± 1,41
Disinfectant 2	15,6 ± 1,69	14,3 ± 1,15	15,6 ± 1,69	19,6 ± 0,47

*Acinetobacte*r spp. and *S. maltophilia* showed inhibition zones at 14,3 ± 1,15 and 19,6 ± 0,47 against disinfectant 2 respectively.

5.8.2 Determining the minimum inhibitory concentrations of sodium hypochlorite and chlorhexidine isoprophyl alcohol

Table 5.2 depicts the MIC of the tested antimicrobial agents with disinfectant 1 showing high MIC concentrations of 0,437% for *Acinetobacter* spp., *S. paucimobilis,* and *S. maltophilia. Staphylococcus aureus* was found to have a low MIC concentration at 0,218% for disinfectant 1. Disinfectant 2 presented a low MIC value of 0,003% for *S. maltophilia* and a high MIC value of 0,062 for *S. aureus*. A low MIC value of 0,007% was presented for *Acinetobacter* spp. and *S. paucimobilis* for this disinfectant.

Table 5.2: Minimum inhibitory concentrations (MIC) (%v/v) values of disinfectants on *S. aureus Acinetobacter* spp., *S. paucimobilis,* and *S. maltophilia*

Disinfectant	***Staphylococcus aureus***	***Acinetobacter* spp.**	***Sphingomonas paucimobilis***	***Stenetrophomonas maltophilia***
Disinfectant 1	0,218	0,437	0,437	0,437
Disinfectant 2	0,062	0,007	0,007	0,003

5.8.3 Determining the minimum bactericidal concentration of sodium hypochlorite and chorhexidine isoprophyl alcohol

Table 5.3 shows the MBC results for disinfectant 1 and disinfectant 2. The MBC value for the microorganisms *S. aureus*, *Acinetobacter* spp., and *S. paucimobilis* was 3.5% for disinfectant 1, whereas a 0.175% MBC value was recorded for *S. maltophilia* for this disinfectant. There were also observations of 0.25% MBC for *Acinetobacter* spp. and *S. paucimobilis* and of 0.125% for *S. aureus* and *S. maltophilia* for disinfectant 2.

Table 5.3: Minimum bactericidal concentration (MBC) (% v/v) values of disinfectants on *S. aureus, Acinetobacter* spp., *S. paucimobilis and S. maltophilia*

Disinfectant	***Staphylococcus aureus***	***Acinetobacter* spp.**	***Sphingomonas paucimobilis***	***Stenetrophomonas maltophilia***
Disinfectant 1	3.5	3,5	3.5	1,75
Disinfectant 2	0,125	0,25	0,25	0,125

5.9 Discussion

Many kinds of disinfectant can be used for cleaning surfaces, such as alcoholic and hypochloric solutions that contain sodium hypochlorite, peracetic acid, alcohol, and quaternary ammonium compounds such as chlorhexidine isoprophyl alcohol. Most of these antimicrobials are cationic and have a high binding affinity for bacterial cells. The surface of the bacterial cell wall carries a net negative charge, and it is often stabilized by the presence of divalent cations. This is associated with the teichoic acid and polysaccharide elements of Gram-positive bacteria and the lipopolysaccharide of Gram-negative bacteria as well as the cytoplasmic membrane itself (Falzana and Ekhtela, 2012). Therefore, cationic antimicrobials require a strong positive charge together with a hydrophobic region in order to interact into the cytoplasmic membrane (Gilbert and Moore, 2005).

Due to the diversity of food-related pathogens and the different disinfectant options and varying susceptibility profiles, it is important to conduct susceptibility tests for disinfectants that are widely used against common microbial pathogens to prevent the development and spread of food-related infections. According to Elexson *et al*. (2014), the minimum inhibitory concentration is an important factor to be considered when choosing a disinfectant. This is because MIC shows the effectiveness of a disinfectant against pathogenic microorganisms. In this study, low MIC presentations for both sodium hypochlorite and chlorhexidine isoprophyl were recorded among the microorganisms. According to Andrews (2001), a low MIC value indicates good antimicrobial activity against microorganisms. The low MIC value of the disinfectants against *S. aureus* can be associated with the high antibacterial activity of these disinfectants in inhibiting the growth of microorganisms and the lack of cell wall barrier typical in Gram-positive bacteria (Nillian *et al*., 2016). For instance, Erikson *et al*. (2017) found that, at low concentrations, disinfectant 1 reduced the viable mass of both Gram-positive and Gram-negative bacteria (*S. aureus, A. calcoaciticus,* and *P. aeruginosa*). Tawari *et al*. (2018) argue that it is also possible that, due to the strong oxidizing effect of chlorine compounds, disinfectant 1 could interfere with structural and functional entities associated with bacterial integrity and metabolism.

Quaternary ammonium compounds are known to actively reduce populations of both Gram-negative and Gram-positive bacteria. In this study, disinfectant 2 had low MIC values, particularly when used on Gram-negative bacteria. The results could be attributed to the various mechanisms of action including cell membrane damage, the collapse of membrane potential, leakage of cell contents, and coagulation of cytosol associated with this disinfectant (Jansen *et al*., 2013). For example, although Gram-negative bacteria resistance mechanisms are complex due to the specific roles of these microorganisms' inner and outer membranes (the outer membranes modulate the accessibility of a cell to external molecules), antimicrobials generally act non-specifically and/or multifunctionally at use concentrations (Fazlara and Ekhtelat, 2012).

It is generally accepted that the MBC values of a particular antimicrobial agent are higher than the MIC values. For instance, Nillian *et al*. (2016) argue that an antimicrobial agent is considered bactericidal if the MBC value is not more than four times higher than the MIC value. Therefore, in this study disinfectant 1 was found to have a bactericidal effect against all microorganisms, whereas disinfectant 2 was found to be bactericidal only against *S. aureus*. These findings correspond with those reported by Yosefimashouf *et al*. (2014). Additionally, Reichel *et al*. (2014) reported adaptation to quaternary ammonium compounds with greater activity among Gram-positive bacteria compared to Gram-negative bacteria.

5.10 Conclusions

Hospital-acquired infections are considered a serious problem around the world, and therefore preventing and maintaining control over these infections are extremely important to ensure the safety of patients and hospital staff. For this reason, there has been intensive activity towards the prevention of the occurrence of diseases in hospital settings, including the establishment of appropriate disinfection policies (Ekizoglu *et al*., 2016). These policies provide a standard for the determination of appropriate disinfection concentrations, implementation methods, and their application (Coates and Hutchinson, 1994; Harbarth *et al*., 2014).

According to Hughes and Ferguson, (2017), when the more extensive use of biocides is considered, it is important to monitor the emergence of non-susceptibility to the agents because increased non-susceptibility may indicate selection pressure from sub-lethal concentrations through dilution or resistance. This study found that both disinfectant 1 and disinfectant 2 exhibited antimicrobial properties against the tested microorganisms. Disinfectant 1 had bactericidal effect against both Gram-negative and Gram-positive bacteria, whereas disinfectant 2 had the highest bactericidal effect against the Gram-positive bacteria only. These were also the only bacteria against which it had bactericidal effect. The variation in the activity of the disinfectants can be attributed to the mode of their action, their concentrations, and the nature of the microorganisms' cell structure. For example, the Gram-positive bacteria cell wall has a thick layer of peptidoglycan and lacks an outer membrane, and these factors allow for easy ionisation of the cell wall leading to cell lysis. Considering the results of testing the two disinfectants, it is safe to conclude that using disinfectant 2 was only effective against Gram-positive bacteria, with the Gram-negative bacteria strains showing continued growth.

5.11 References

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CHAPTER 6

GENERAL CONCLUSIONS

6.1 Introduction

Providing consumers with nutritious and safe meals is a principal objective but also a major concern within food production industries, particularly in hospitals. Although food safety is a worldwide problem in both domestic and industrial settings, individuals with compromised immune systems continue to suffer due to food poisoning. Moreover, developing countries such as Lesotho are hard hit by HIV/AIDs and food insecurity and their citizens are therefore vulnerable to a lack of food safety and resultant foodborne infections. It is a common practice in Lesotho for food to be prepared in hospital kitchens from where it is distributed to patients and staff. The diversity of people and their health status thus mandate hospital catering staff to ensure that the food they prepare and serve is healthy and safe. Against this background, the aim and objectives of this study were as follows:

The aim of the study was to investigate microbial contamination of food contact surfaces in hospitals in Maseru, Lesotho. In brief, the research objectives were to:

- Characterize hospital foodborne pathogens associated with food contact surfaces in the hospital under study
- Assess food handlers' food safety knowledge, attitude, and hygiene practices from food preparation to food consumption; and
- Investigate the production of ESBL in gram-negative bacteria detected in the hospitals under study
- Investigate the efficacy of common dis infectants against detected microorganisms in the hospitals under study

To address the aim and achieve the objectives, a semi-structured questionnaire was administered to selected food handlers to determine their knowledge, attitudes and practices (KAPs) regarding food safety in hospital settings. Two selected disinfectants available in the market were investigated to determine their efficacy in microbiological pathogen control on food contact surfaces. The food contact surfaces that were investigated included food handlers' hands as well as food contact surfaces (tables, spoons, knives, and chopping boards). The identified microorganisms were profiled for their susceptibility to common antibiotics and the production of extended-spectrum beta-lactamases (ESBLs), while further investigations tested the efficacy of common disinfectants against the detected microorganisms. This study report is presented in six chapters:

Chapter 1: General introduction and discussion on information from the literature regarding the topic under investigation, with emphasis on food contamination and the impact this has on hospital-acquired infections.

Chapter 2: Assessment of food handlers' hygiene, food safety knowledge, attitude, and practices in hospitals in the city of Maseru, Lesotho.

Chapter 3: The prevalence of microbiota on food contact surfaces within hospitals in the city of Maseru, Lesotho.

Chapter 4: Extended-spectrum beta-lactamase production of Gram-negative bacterial strains.

Chapter 5: Efficacy of chlorhexidine isopropanol and sodium hypochlorite against gram-negative and gram-positive bacteria Isolated from food contact surfaces in hospital kitchens in Maseru Lesotho.

Chapter 6: Conclusion and recommendations.

6.2 Concluding remarks on the preceding chapters

In Chapter 2, the selected food handlers' hygiene and food safety knowledge, attitudes, and self-reported practices were discussed. The findings indicated that the food handlers demonstrated good knowledge, positive attitudes, and appropriate food safety practices regarding food safety. However, despite the high scores obtained for these aspects, it was observed that educational levels, work duration, and lack of exposure to appropriate food safety training had no influence on the food handlers' KAPs. It was also found that the food handlers had overall sufficient knowledge of hand hygiene practices and cleaning and sanitization procedures.

Although food handlers were knowledgeable of good hand hygiene practices, the discovery of high levels of microbiota reported in Chapter 3 suggests that there was a discrepancy between the food handlers' reported cleaning of food contact surfaces and the washing of hands. The microorganisms that were detected were associated with resistant strains of the families Enterobacteriaceae and Staphylococcaceae. Organisms (such as *S. aureus*) that were found on the contact surfaces where food was prepared are of pathogenic importance in both the food service industry and in clinical environments. The latter microorganism is known to cause food poisoning preceded by gastroenteritis emesis with or without diarrhoea. The presence of clinically important pathogenic microorganisms in the hospital kitchens may be attributed to possible cross-contamination from the hospital wards to the kitchen and is therefore a reason for further investigation.

Chapter 4 reported on the organisms of the family Enterobacteriaceae that were investigated for susceptibility to common antibiotics and for extended-spectrum beta-lactamases (ESBL) production. Beta-lactamases are enzymes that are produced by Gram-negative bacteria that facilitate the resistance of these bacteria to beta-lactam antibiotics. These enzymes are produced by genes embedded in the chromosomes that can be transferred among bacterial populations. In this study, the majority of the isolates (71%) exhibited multi-drug resistance while 43% was positive ESBL producers. The ESBL producers exhibited 100% resistance to penicillin and 66% resistance to amoxicillin. None of these isolates were susceptible to all antibiotics, which highlights the need for new therapeutic interventions as they pose a serious threat to public health if their prevalence is not eradicated, particularly in hospital settings.

Chapter 5 reported on the findings of two commercially available household disinfectants whose efficacy was tested against the detected microorganisms discussed in Chapter 3. It was found that both sodium hypochlorite and chlorhexidine exhibited a bactericidal effect against the tested isolates. Sodium hypochlorite exhibited bactericidal effect against all the microorganisms tested, while chlorhexidine achieved bactericidal effect only against the Gram-positive bacteria. Therefore, it is important that further investigations on these disinfectants are conducted to determine which factors influence or diminish their efficacy. These investigations should include testing the efficacy of disinfectants within a certain period of time and on different surfaces where food is prepared.

6.3 Recommendations

Food handlers in hospitals are pivotal in achieving and maintaining food hygiene as they handle food from the acquisition point to the final food distribution point to consumers, who are often immune-compromised patients in hospital settings. Therefore, it is important that food handlers are trained on food safety-related issues to avoid cross-contamination, especially during periods of active infection. Given the results pertaining to the impact of training (or the lack thereof) on food handlers' KAPs, it would be wise to implement continued in-service food safety training of food handlers. Additionally, public health workers and hospital management teams should conduct frequent health education programs that focus on educating food handlers on the risks associated with HIV/AIDS, foodborne diseases, hospital-acquired infections, and susceptible groups.

Customized and recurrent food safety training courses are essential in optimising food safety outcomes. These should acquaint food handlers with the importance of food safety, cross-contamination, and safe food handling practices such as correct food holding temperatures and personal and hand hygiene should be pivotal in these programmes. Hand hygiene is particularly important in food safety measures and infection control. A case in point is that proper hand washing was identified as a key precautionary measure in the control of the spread of COVID-19. Therefore, proper hand hygiene practices by all food handlers in hospitals will curb the spread of both food-related and non-food related pathogenic microorganisms to already immune-suppressed hospitalized patients. The

HACCP system should be incorporated and consistently monitored as part of the food safety program to identify possible hazards throughout the food production route.

The food and clinical services are in need of effective, accurate, and real-time protocols for the constant evaluation of environmental hygienic conditions. The majority of health institutions reportedly use out-dated visual observation and conventional methods to evaluate the cleanliness of food preparation surfaces. However, visual observation is an insufficient method of evaluation as it only focuses on the aesthetics of the health environment and does not consider the microbiological state of food contact surfaces. In hospitals and any other food preparation setting, the microbial quality of a surface or of an item is vital in the emergence and transmission of pathogenic microorganisms. In this regard, Law *et al*. (2015) proposes the use of the ATP hygiene system for the evaluation of environmental cleanliness as it is suitable for the detection of pathogens that are present even in low numbers. This proposal is supported by the current study. According to Nante *et al*. (2017), the ATP bioluminescence assay will assess hospital surface cleanliness using multiple ATP benchmark values relative to light units (RLU). Therefore, the feedback provided by the ATP bioluminescence can be quantified and results can easily be interpreted and communicated to all stakeholders.

It is important that surveillance systems are developed to identify ESBL production among pathogenic Enterobacteriaceae. Extended-spectrum beta-lactamase production is a major global concern; hence ESBL surveillance and tracking systems will provide information to be used in developing/contributing to the ESBL production global mapping system which is useful in predicting the occurrence and spread of particular resistance patterns among pathogenic bacteria. It is therefore important that antibiotic tests pertaining to ESBL production are included in routine monitoring protocols to ascertain the susceptibility of microorganisms to prescription antibiotics and to avoid treatment failure.

Cleaning and disinfection are practices implemented in the prevention and control of infectious diseases within both clinical and domestic environments. Various products are available in the market that contain chemical substances and they are known as detergents and disinfectants. However, determining the efficacy of cleaning and disinfection substances on bacterial isolates is important if the most suitable disinfectant or detergent is to be selected for use in a particular environment. To ascertain the efficacy of available chemical substances against pathogenic microorganisms, their efficacy should be continually investigated. This will assist in understanding the mode of action of various cleaning and disinfection substances and the resistance patterns of microorganisms against them, which will allow for an informed and adequate choice. It is vital that disinfectants are selected not only for their availability, but also for their efficiency in eradicating microorganisms on food preparation surfaces.

6.4 Conclusions

A kitchen is either a domestic or health facility environment where food is processed and meals are prepared to be served to consumers. During food preparation, food handlers are in contact with food in processes of reception and preparation before the food is finally served to consumers. This means that food handlers are the main custodians of safe food production. Food handlers' behaviour towards food should therefore include proper hand washing, proper storage, and maintaining a sanitary kitchen/food environment for safe food production and the control/eradication of foodborne infections.

This study has the potential to improve food handlers' food safety knowledge as well as their attitude and practices by informing them of the importance of appropriate food handling behaviours. Moreover, through continued food safety training, food handlers will remain informed of the advantages of improved food handling practices which will result in less food contamination and food poisoning. Through the publication of the findings in academic journals, the study also has the potential to influence hospital domestic cleaning programs through improved evaluation of cleanliness and informed decisions on the choice of disinfectants to be used. Additionally, the study has highlighted the importance of interventions to combat the emergence and spread of antibiotic resistant bacteria along the food production chain, with specific focus on ESBLs. Therefore, implementing and sustaining surveillance strategies to determine extended-spectrum beta-lactamase contamination using routine antibiotic evaluation tests will assist in deciding on appropriate treatment therapies. Such measures will also inform the food preparation industry in hospital settings of the importance of developing new treatment options when required.

APPENDICES

Appendix 1: Ethical Clearance

LESOTHO

Ministry of Health
PO Box 514
Maseru 100

REF: ID93-2017

Date: 25 July 2017

Category of Review:
[x] Initial Review
[ ] Continuing Annual Review
[ ] Amendment/Modification
[ ] Reactivation
[ ] Serious Adverse Event
[ ] Other ________________

To
Ms.M.S Maliehe
Masters of Science candidate in Environmental Health
Central University of Technology
Free State, RSA

Dear Ms.M.S Maliehe,

RE: Microbiological contamination of food contact surfaces in hospitals at Maseru, Lesotho

This is to inform you that on 18 July 2017 the Ministry of Health Research and Ethics Committee reviewed and **APPROVED** the above named protocol and hereby authorizes you to continue the study according to the activities and population specified in the protocol. Departure from the approved protocol will constitute a breach of this permission.

This approval includes review of the following attachments:
[x] Protocol dated 18 May 2017
[x] English consent forms
[ ] Data collection forms in Sesotho
[x] Data collection forms in English
[ ] Participant materials *[insert types, versions, dates]*
[x] Other materials: Checklist for PPE
This approval is **VALID** until 24 July 2018.

Please note that an annual report and request for renewal, if applicable, must be submitted at least 6 weeks before the expiry date.

All serious adverse events associated with this study must be reported promptly to the MOH Research and Ethics Committee. Any modifications to the approved protocol or consent forms must be submitted to the committee prior to implementation of any changes.

We look forward to receiving your progress reports and a final report at the end of the study. If you have any questions, please contact the Research and Ethics Committee at rcumoh@gmail.com (or) 22226317.

Sincerely,

Dr. Nyane Letsie
Director General Health Services

Dr. Amelia Ranotsi
Chairperson NH-IRB

Appendix 2: Food Handler Certificate

FOOD HANDLER CERTIFICATE IN TERMS OF PUBLIC HEALTH REGULATIONS 1973 GOVERNMENT GAZETTE NO. 27 OF 6 th JULY 1973. LEGAL NOTICE NO. 25 OF 1973.

NAME………………… SURNAME……………..

DATE………………… VILLAGE…………………

EMPLOYER…………... COMPANY……………..

MEDICAL EXAMINATION (should include) CHEST X-RAY, WIDAL, STOOL and OTHERS.

CERTIFIED TO WORK AS FOOD HANDLER

[yes] [no]

NAME OF MEDICAL OFFICER……………………..

SIGNATURE…………………………………………..

DATE STAMP…………………………………………

DATE OF EXPIRY OF CERTIFICATE……………..

Appendix 3: Consent form

I acknowledge that I understand all the information that was provided by the researcher and I agree to participate in the study. I also understand that I am free to end my participation at any time and I will not be forced to do anything that may feel uncomfortable to me, harm my business in any way or put me in any danger. I am also entitled to total confidentiality through the study.

Signed ___________________on this day_________at___________

The Sesotho translation of the consent form

Ke nka kano ea hore ke utloisisa tsohle tseo monga lipatlisiso a nthlaloselitseng tsona, mm eke lumela ho ba karolo boitutong ba hae ele hona ho mo thusa. Ke utloisisa hore ke lokolohile ho emisa hob a karolo lipatlisisong tsena kaofela nako eohle ha ke utloa ke se ke sa khotsofala kapo hosena ts'ebelisano mmoho. Monga lipatlisiso o itlama ho boloka boitsebiso baka ele lekunutu hofihlela a qetile ka boithuto ba hae.

Tekeno________________kala ______________sebakeng sa___________________

Appendix 4: Food handler questionnaire

Gender	M	F

Age	22-30	31-39	≥40

Educational Level	Primary	J.C	C.O.S.C	Tertiary qualification

Duration of working period	<1 year	1-5 years	6-10 years	11-20 years	>20 years

Employment status	Contractor	Part- time	Full-time

Hours of operation	Morning shift	Afternoon shift	Straight shift

Language	English	Sesotho	Both

Race	Black

Training	Yes	No

Food handler knowledge	Correct	Incorrect	Don't know
Washing hands before work reduce the risk of food contamination.			
Using gloves while handling food reduces the risk of food contamination			
Proper cleaning and sanitization of utensils increases the risk of food contamination			
Eating and drinking at the workplace increases the risk of food contamination			
Food prepared in advance reduces the risk of food contamination			
Reheating cooked foods can contribute to food contamination			
Washing utensils with detergent leaves them free of contamination			
Children, healthy adults, pregnant women and older individual are at equal risk for food poisoning			
Typhoid fever can be transmitted by food			
AIDS can be transmitted by food			
Bloody diarrhoea can be transmitted by food			
Salmonella is among the food-borne pathogens			
Hepatitis A virus is among the food-borne pathogens			
Swollen cans may contain the microorganism, *Clostridium botulinum*, which causes botulism			
Microbes are on the skin, in the nose and mouth of healthy food handlers			
Clean is the same as sanitized			

Central University of Technology, Free State

Cross contamination is when microorganisms from contaminated food are transferred by the food handler's hands or kitchen utensils to another food			
The correct temperature for storing perishable foods is 5 ^{0}C			
Hot, ready-to-eat foods should be kept at a temperature of 65 ^{0}C			
Freezing kills all the bacteria that may cause food-borne illness			
Contaminated foods always have some change in colour, odour or taste			
Raw vegetables are at high risk of contamination than undercooked beef			
During infectious diseases of the skin, it is necessary to take leave from work			
The health status of workers should be evaluated before employment			
The ideal place to store raw meat in the refrigerator is on the bottom shelf			

Food Handler Attitude	Correct	Incorrect	Don’t Know
Well cooked foods are free of contamination			
Proper hand hygiene can prevent foodborne diseases			
When cleaning products are closed, they can be stored with cans and jars of food that are also closed			
Raw and cooked foods should be stored separately to reduce the risk of food contamination			
It is necessary to check the temperature of refrigerators/ freezers periodically to reduce the risk of food contamination			
Defrosted foods should not be frozen			
The health status of workers should be evaluated before employment			
the best way to thaw chicken is in a bowl of cold water			
Wearing masks is an important practice to reduce the risk of food contamination			
Wearing caps and adequate clothing is an important practice to reduce the risk of food contamination			
Safe food handling is an important part of my job responsibility			
Learning more about food safety through training courses is important to me			
Beards could contaminate food with foodborne pathogens			

Long and painted fingernails could contaminate food with foodborne pathogens			
Food handlers can be a source of foodborne outbreaks			
Eggs must be washed immediately after delivery			
Dish towels can be a source of food contamination			
Knives and cutting boards should be properly sanitized to prevent cross-contamination			
Food handlers who have abrasions or cuts on their hands should not touch foods without gloves			
It is possible for infectious pathogens from the hospital wards to be imported into the kitchen and into the food			

Food Handler practices	Yes	No
Do you use gloves during the distribution of unpackaged food?		
Do you wear an apron while working		
Do you wear a mask when you distribute unwrapped foods?		
Do you eat or drink at your workplace		
Do you wear nail polish when handling food		
Do you prepare a meal in advance (i. e., from one shift to another)?		
Do you properly clean the food storage area before storing new products?		
Do you use sanitizer when washing service utensils (plates, mugs and spoons)?		
Do you use the sanitizer when washing fruits?		
Do you check the shelf life of foods at the time of delivery?		