ASSESSMENT AND CHARACTERIZATION OF HUMAN BACTERIAL DIARRHEAPATHOGENS IN STREET-VENDED READY-TO-EAT MEATS IN HAVANA INFORMAL SETTLEMENT, NAMIBIA

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ABSTRACT
The study aimed to evaluate and characterize human diarrhea pathogens potentially present in street-vended ready-to-eat meats sold in Havana, Windhoek. A total of fifty (50) samples, including fresh beef, dried beef (biltong/droëwors), polony, sausages, fish, and chicken, were assessed for the prevalence of diarrhea pathogens (*Salmonella*, *Shigella*, *Escherichia coli*, and *Staphylococcus aureus*). Selective media and biochemical confirmation tests were used for the assessment of bacterial pathogens, and a multiplex Polymerase Chain Reaction system (Seeplex® Diarrhea-B1/B2 Ace detection kit) was employed for the detection and characterization of additional diarrhea pathogens (*Vibrio spp*, *Campylobacter spp*, *Clostridioides difficile* toxin B, *E. coli* O157:H7, *Clostridium. perfringens*, *Yersinia enterocolitica*, Verocytotoxin-producing *E. coli*, and *Aeromonas spp*). Results showed positive findings for aerobic plate count (80%), *E. coli* (46%), S.aureus (36%), *Shigella* (46%), and *Salmonella* (24%). Mean microbial counts were 5.15±0.32 log cfu/g for aerobic plate counts, 3.22±0.30 log cfu/g for *E. coli*, 3.27±0.54 log cfu/g for *S.aureus*, and 3.80±0.57 log cfu/g for *Shigella*. Unsatisfactory levels were recorded in 18% of the samples for aerobic plate count, *E. coli* (46%), *S.aureus* (12%), *Shigella* (46%), and *Salmonella* (24%). From the Seeplex system, additional enteric pathogens were detected, including *Campylobacter spp*., *C. difficile* toxin B, *E. coli* O157:H7, and O157, *C. perfringens*, and *Y. enterocolitica*. *Vibrio spp* was present in 2% of the Russian sausage meat sample. With the B2 system, *C. perfringens* was detected in 38% of samples, *E. coli* O157:H7 in 32%, *E. coli* O157 in 20%, and *Y. enterocolitica* in 14%.

Significant differences ($P < 0.05$) were observed between dried beef (biltong/droëwors) and fresh beef, chicken, and fish samples for aerobic plate count, with dried beef samples recording higher APC. No significant differences were observed between samples for *E. coli*, *S. aureus*, and *Shigella.*

Keywords:Human diarrhea pathogens, street-vended meats, Unsatisfactory levels, Havana- Windhoek.

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Abbreviations and Acronyms

ACC – Aerobic Colony Count

ACE – Auto Capillary Electrophoresis

ANOVA – Analysis of Variance

APC – Aerobic Plate Count

ATCC – American Type Culture Collection

BB – Best Before

BPA – Baired Parker Agar

BPB –

Butterfield

Phosphate

Buffer

BPW –

Buffered

Peptone Water

CDC – Centre

for Disease

Control

CFU – Colony

Forming Units

DAEC – Diffusely Adhering *Escherichia coli*

DNA – Deoxyribonucleic Acid

DPO – Dual Priming

oligonucleotide primers

EAEC –

Enteroaggregative

Escherichia coli

E-C – *Escherichia coli*

Broth

EHEC – Enterohemmorhagic *Escherichia coli*

EIEC –

Enteroinvasive

Escherichia coli

EPEC –

Enteropathogenic

Escherichia coli

ETEC –

Enterotoxigenic

Escherichia coli

EURO – European

FSANZ – Food Standards Australia, New Zealand

GEMS – Global Enteric Multicentre

HACCP – Hazard Analysis Critical Control Point

HUS – Hemolytic Uremic Syndrome

H_2S – Hydrogen Sulphide

IBM – International Business Machines Corporation

IC – Internal Control

ICMSF – International Commission on Microbiological Specifications for Foods

IDD – Infectious intestinal disease

ISO – International Standard Organization

LT – Lauryl Tryptose Broth

MKTTn – Mueller Kauffmann Tetrathionate Novobiocin

NA – Nutrient Agar

NC – Negative Control

NSW – New South Wales

NSWFA – New South Wales Food Authority

PCA – Plate Count Agar

PC – Positive Control

PCR – Polymerase Chain Reactions

RTE – Ready-to-eat

RVS – Rappaport- Vassiliadis medium with Soya

SD – Standard Deviation

SFP –

Staphylococcal

Food Poisoning

SMAC – Sorbitol

MacConkey Agar

SOPs – Standard

Operational

Procedures

SPC – Standard

Plate Count

SPSS – Statistical Package for Social Science

SSA – *Salmonella-Shigella* Agar

STEC – Shiga toxin-producing *Escherichia coli*

STX – Shiga toxin

TSI – Triple Sugar Iron Agar

UK – United Kingdom

USA – United States of America

USDA – United States Department of Agriculture

VTEC – Verotoxin/ Verocytotoxin-producing *Escherichia coli*

WHO – World Health Organization

XLD – Xylose Lysine Desoxycholate

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Dedication

I dedicate my thesis to my family and many friends. Special gratitude to my mother, Julia Shinombedi, and grand-mother Luise Kashopola, whose words of encouragement and push for tenacity ring in my ears. And to my sibling, who has never left my side and always encouraged me to move forward.

Declaration

I, **Hilma Ndinehafo Ndjabayondume**, declare hereby that this study is a true reflection of my own research and that this work or part thereof has not been submitted for a degree in any other institution of higher education.

Signature:_____ Date: _____ October 2024

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Street-vended Ready-to-eat (RTE) foods are comestibles and beverages sold or prepared by vendors in the streets and public places for immediate or deferred consumption without further processing [1]. These foods are admired by consumers for their distinctive flavours, low prices, and availability and by vendors as a source of income [1]. According to Alimi [2], rapidindustrialization and flocking to cities and towns contribute to the increased demand for street foods. Microorganisms, both harmless and pathogenic, are everywhere. Since these foods are handled in open and inadequately controlled or unhygienic environments coupled with the absence of pre or post-processing and proper preparation, potential pathogenic contamination is likely highposing significant health risks.

Many vendors prioritise immediate financial gains over consumer safety by openly exposing theirmeats on the street. These meats are not only exposed to passengers/customers but also to environmental elements such as dust, rain, and air [3]. Exposure to these atmospheric conditions results in contamination, posing a hazard when consumed. It is crucial to acknowledge that unsanitary conditions associated with exposed meat are the cause of many ailments. It raises concerns regarding the safety of these products as their consumption may lead to severe sicknesses[3].

Street-vendors have faced scrutiny for selling contaminated foods causing

diarrheal diseases. Casual observation of handling and storage practices indicates risks, and epidemiological evidence validates these observations [4]. Frequently, vending sites lack access to running water; leading to

hand and dishwashing being done in one or more buckets or pans of water, and occasionally, without the use of soap [5]. Some venders do struggle to sell their food within a day, and in some instances, they continue selling stale food beyond their designated Best Before (BB) period. According to Mosupye et al [5], safe food-storage temperatures are difficult to maintaining safe food-storage temperatures are a challenge since foods are often displayed for extended periods andmay not be reheated before serving.

Furthermore, intrinsic properties of the food are involved. Sometimes, some food may contain toxic chemicals or microbial pathogens or toxins because of the practice involved in producing the raw materials. For instance, raw meats may be already contaminated at the slaughtering stages. So, in such cases, the vendors may purchase raw materials that have already been infected with microbial pathogens. Other epidemiological observations include the preparation and handling of food and the volume of food that may be prepared [6].

Moreover, the disposal of garbage and water wastes at vending sites provides food and harbourage for insects and rodents. In African countries, where street-food vending is common, there has been little information regarding the incidence of street-food in general and especially ready-to-eat meat products, and related diseases raising concerns because the operational conditions are usually unsuitable for the preparation and selling of food-meat [7]. However, Soliman et al.[8] reported that microbiological studies on street-vended foods in American, Asian, and African countries had revealed high bacterial counts and high incidents of foodborne bacterial

pathogens in food [8].

In addition, meat is traditionally considered the vehicle for many food-borne diseases [9]. The sale of ready-to-eat meats in the streets is controversial from a health standpoint. It is reportedly due to implications of foodborne disease breaks caused by ready-to-eat meats [10][11]. According to

Magwedere [12], the primary contaminating meat pathogens (disease-causing microorganisms) are *Bacillus cereus*, *Staphylococcus aureus*, *and Listeria monocytogenes*, *Salmonella*, *Shigella,* and *E. coli 0157:H7*. People who consume street ready-to-eat meats face the risk of contracting foodborne pathogens, thus putting their health at stake [3].

Research from the Centres for Disease Control and Prevention [13] estimates that food-borne illnesses cause 48 million illnesses, 128 000 hospitalizations, and 3000 fatalities in the United States per year. The issue is made worse in nations where improper food handling procedures, insufficient food safety regulations, lax regulatory frameworks, a lack of financial resources to purchase safer equipment, and a lack of food safety education are widespread. Additionally, the study defines ‘’street food’’ as a broad range of foods with both plant and animal origins; these meals are frequently consumed in numerous nations where unemployment is high, wages are poor, and there are few job possibilities and social programs and where food borne illness prevalence ishigh due to the contamination of food articles with pathogens [14].

Around Windhoek, Street-vended ready-to-eat foods are sold in public places, industrial areas, and informal settlements and play a vital role in everyday life in Namibia. Foodborne diseases can cause short-term symptoms, such as nausea, vomiting, and diarrhea (known as food poisoning). Still, they can also cause longer-term illnesses, such as cancer [3]. In a previous study by Shiningeni et al. on the prevalence of pathogenic bacteria in the street-vended meat in Windhoek, Havana emerged to have

unsatisfactory levels of aerobic plate count, *Enterobacteriaceae* and *E. coli*, with *Salmonella* and *Shigella* having being detected in several samples, which is unacceptable for human consumption. Thus, this study assessed and characterized human diarrhea pathogens in street-vended ready-to-eat meats in Havana Settlement, Windhoek, Namibia.

1.2 Statement of the problem

The phenomenon of mushrooming informal settlements due to rural-urban migration is a significant issue observed not only in Namibia but worldwide. In Windhoek alone, the population has nearly doubled in size over the past decade (from 270 000 in the last national census in 2011 to estimates ranging widely from 420 000 to 600 000), manifesting in about 68 000 new shacks

[15] . This trend leads to a high-density population in these settlements, exacerbating the problems associated with poor sanitation. Lack of proper waste management systems further compounds the problem, which contributes to contamination of street-vended meats, posing health hazards. Improper waste disposal in public areas attracts pests, contaminating food and endangering consumers. Local residents have expressed concerns about street vendors operating near the Havana four-way stop, citing hazardous conditions due to improper waste disposal, which poses risks to the area's cleanliness and safety [16]. Havana Informal Settlement is characterized by overcrowding, inadequate infrastructure, and lack of services such as clean water, sanitation, and electricity[17]. Additionally, the high dependence of informal settlement residents on open market-vended foods and ready-to-eat meats for consumption and small-scale business setups, increases the risk of exposure to pathogenic contaminated foods. This reliance raises concerns about food safety and hygiene practices. Diarrhea pathogens pose significant hazards to human health and can lead to various complications if not properly addressed. Street vendors in informal settlements frequently store

raw meats and cooked foods together, promoting cross-contamination. This dangerous practice heightens the risk of foodborne illnesses as bacteria from raw meats can easily transfer to ready-to-eat foods [18]. Previous studies conducted in Havana informal settlement, Namibia, revealed unsatisfactory levels of *Enterobacteriaceae* in roasted beef samples, indicating unacceptable contamination levels or under-processing of food. *Salmonella* and *Shigella* were also

detected, rendering the food unsuitable for human consumption. These findings necessitated an extended study focusing on the Havana informal settlement to better understand the extent of the problem and provide data for focused interventions.

1.3 Objective(s) of the study

1.3.1 Main Objective

1.3.1.1 The main objective of this study was to assess the safety of street-vended-ready-to-eat meats in the Havana informal settlement in Windhoek, Namibia. The study focused on human bacterial diarrhea pathogens (*E. coli*, *Salmonella*, *S. aureus*, and *Shigella*) and their potential implications for public health. The assessment will include characterising these pathogens and evaluating their presence and levels in street-vended-ready-to-eat meats. Additionally, the study will consider the socio-economic conditions and other contextual factors that could impact the safety of these meats in the informal settlement.

1.3.2 Specific Objectives

1.3.2.1 To determine the prevalence of human diarrhea pathogens (*E. coli, S. aureus, Shigella, and Salmonella*) in various street-vended ready-to-eat meat such as polony, beef, and chicken in Havana informal settlement in Windhoek, Namibia.

1.3.2.2 To identify and characterize human diarrhea pathogens (*E. coli, Salmonella, S. aureus, Shigella, and Salmonella*) in

street-vended ready-to-eat meats using culture- dependent methods.

1.3.2.3 To detect the presence of human diarrhea pathogens in street-vended ready-to-eat meats using culture-independent methods (multiplex PCR).

1.4 Significance of the study

The study will contribute to a comprehensive understanding of the microbiology safety status of commonly consumed street-vended ready-to-eat meats in Havana informal settlement, Windhoek, Namibia. This study will further provide valuable information for public health intervention and regulatory measures to enhance food safety in informal settlements.

1.5 Limitation(s) of the study

The sample size in this study was constrained by the availability of resources. Culture-dependent and culture-independent methods used in this study may have detection limits, potentially leading to underestimation or overestimation of pathogen prevalence. Pathogen prevalence can vary over time due to seasonal factors, changes in food handling practices, or evolving public awareness

While a prior investigation revealed unsatisfactory levels of *L. monocytogenes* in ready-to-eat (RTE) meats from the Havana informal settlement, the present study did not assess these pathogens due to resource limitations. The study targets specific pathogens (*E. coli*, *Salmonella*, *S. aureus*, *Shigella*) but may not cover all potential contaminants or emerging pathogens.

1.6 Delimitation(s) of the study

The analysis concentrated solely on samples collected from the Havana informal settlement. It is crucial to acknowledge that the findings from this

specific informal settlement may not be generalisable to other regions or settings. Hence, future research should focus on microbial dynamics in diverse settings to ensure broader applicability.

CHAPTER 2: LITERATURE REVIEW

2.1 Microbiological quality of ready-to-eat food

Maintaining the good microbiological quality of ready-to-eat food is an essential priority for both vendors and consumers [19], because microbiological quality is a primary concern in food industries due to its acute risk to health posed by pathogens. The recent global shift towards ready- to-eat (RTE) consumption has exponentially increased due to its prompt availability and nutritious value [20]. Stephan [21] and Fellows [22] support that RTE foods' safety and microbiological quality have always been questionable, especially when considering local retailers.

In a study conducted in South Africa, out of fifty-one (51) food samples analysed, *Bacillus cereus* was detected in 22%, *Clostridium perfringens* in 16%, *Salmonella spp*. in 2%, and *E. coli* (non- O157:H7) in 2%. Whereas *L. monocytogenes, Staphylococcus spp.,* were not detected. Therefore,the food samples analysed in the study were of acceptable quality and safety [23].

However, in a study by Mamun et al. [24] in Bangladesh, foodborne pathogens and high microbial counts were found in different street-vended ready-to-eat meats, and about 35% of the street meat samples were unsatisfactory and were not fit for human consumption. The presence of *B. cereus,*

S. aureus, and *E. coli* was detected in 12.5%, 2.5%, and 22.5% of the street meat samples, respectively.

2.1.1 Classification of microbiological quality of ready-to-eat food

As explained by the centre for food safety [25], satisfactory test results indicate good microbiological quality. Borderline test results are not unsatisfactory but also unacceptable basedon the upper limit of acceptability and indicate the potential for developing public health problems

and unacceptable risk. Unsatisfactory aerobic plate count results may be considered, which showsinvestigating reasons for the high count. For hygiene indicator organisms, test results require

remedial action. To identify test results at levels that indicate a product that is potentially injurious to health and unfit for human consumption, and to determine the appropriate corrective actions, Table 1 and 2 can be consulted for references [25].

According to the New South Wales Food Authority [26], there are four microbiological categories for assessing food quality. These categories are: good, acceptable, unsatisfactory, and potentially hazardous. However, the Hong Kong food safety centre [25] only described three types: satisfactory, borderline, and unsatisfactory.

Table 1: Potential action based on the microbiological category of ready-to-eat foods [25]**.**

	Result category	Interpretation	Action
Pass	Good	Results are within expected microbiological levels for this product (lower range) and present no food safety concern.	None
	Acceptable	Results are within expected microbiological levels for this product (upper range) and present no food safety concern.	None

Fail	Unsatisfactory	Results are outside the expected microbiological levels for this product and present no food safety concern but	Further samples are taken for testing. If these return good or acceptable results, no action is

		might indicate poor food handling practices.	taken. If these return unacceptable results, the business is inspected to determine if food handling controls and hygiene practices are adequate. A product withdrawal may be considered while further testing occurs.
	Potentially hazardous	Results are outside the expected microbiological levels for this product and present a potential food safety concern.	Inspect supplier to determine if food handling controls and hygiene practices are adequate; consider withdrawal of the product from sale

Table 2: Summary of suggested actions (not exclusive) to be taken by officers for each class in response to the results of Aerobic Colony Count (ACC), hygiene indicator organisms, and specific foodborne pathogens in ready-to-eat food in general [25]**.**

	ACC	Hygiene indicator organisms	Specific foodborne pathogens
Satisfactory	No action is required.		
Borderline	Consider the source of the food (producer/ retailer etc.) and the stage of shelf life before determining action. Further investigation may be appropriate if other samples from the same source are also of borderline quality.	Parties concerned (e.g., vendors) should be advised to review cooking and all hygiene procedures, including cleaning. Consider taking investigative food samples. Action should be proportional to the levels detected.	Risk will increase proportionally to the levels detected. Parties concerned (e.g., vendors) should be advised to investigate the causes and adopt measures to improve the situation. Consider taking investigative food samples

Unsatisfactory	Consider investigating the reasons for the high count.	Parties concerned (e.g., vendors) should be advised to review cooking and all hygiene procedures, including cleaning. Take investigative food samples.	Immediate investigation; Parties concerned (e.g., vendors) should be instructed to stop the sale of the food item in question, investigate immediately and find out the causes, and adopt measures to improve the situation. Take investigative food samples. In addition, warning letters, source tracing, and other enforcement actions should be considered.

2.1.2 Microbiological quality criteria for ready-to-eat food in general

Some ready-to-eat foods are regarded as “potentially hazardous,” meaning they can support the growth of pathogenic (food poisoning) bacteria and must be kept at certain temperatures depending on the product to minimize the development of any pathogens that may be present in the food or to

prevent the formation of toxins in the food [26].

Food contains a variety of microorganisms, many of which are harmless. However, even those that can cause illness can be found in foods at low levels, and even if consumed, it is unlikely to cause an infection [27]. As ready-to-eat food varies widely, the decision on what to test will vary from product to product. Some factors influencing the choice of the test include the type of ingredients in the finished product, the status of the ingredient (i.e., cooked or raw), the type of cooking or processing undertaken, etc. [28]. The components of microbiological RTE foods include standard plate count, hygiene indicator organisms, and specific foodborne pathogens [24].

2.1.2.1 Standard Plate Count/Aerobic Colony Count

Aerobic colony count (ACC), also known as total viable count or standard plate count, is the total number of bacteria able to grow in an aerobic environment at 25 ° C. ACC is an indicator of food quality, and it cannot predict the safety of food products, and the storage conditions of the product can influence it. Furthermore, ACC cannot directly contribute to the safety assessment of ready- to-eat food, and it can only provide a general indication of the microbiological quality of food [26][25]. A high standard place count may indicate that the product may be highly infected, prepared unhygienically, or stored inappropriately. The product type, an ingredient presents in the food, and the type of processing may influence this. When assessing for microbiological quality of food, three categories can be used [28]. Category A – applies to ready-to-eat foods in which allcomponents are fully cooked for immediate sale or consumption (e.g., meat pie). Category B – applies to ready-to-eat foods that are fully cooked with further handling or

processing before consumption (e.g., custard-filled pastry). Category C – food that contains uncooked fermented ingredients or fresh fruits and vegetables (e.g., takeaway fruit and dried products) [28].

2.1.2.2 Indicator Organisms

Enterobacteriaceae and *E. coli* are used as hygiene indicator organisms in the microbial analysis of ready-to-eat food. The *Enterobacteriaceae* group of bacteria is found in many different environments, such as the intestinal tract of humans and animals. They are also found in soil, vegetable matter, and marine environments. Both pathogenic and non-pathogenic bacteria can be found in this group. *Enterobacteriaceae* indicate either post-processing contamination or inadequate cooking in ready-to-eat foods that are fully cooked. As they can be found in raw foods, their detection may not indicate any processing failure. It would be inappropriate to test ready-to-eat foods containing raw components (mainly fresh fruits and vegetables) for *Enterobacteriaceae*[26].

E. coli is an organism that is part of the normal microflora of the intestinal tract of humans and warm-blooded animals. As such, their presence in ready-to-eat foods (fully cooked or those containing raw fruits or vegetables) can indicate poor hygiene and sanitation or inadequate heat treatment.

The table below (Table 1) has been developed based on the guidance documents developed by both the UK Food Protection Agency and Food Standards Australia New Zealand (FSANZ)[29]. Moreover, the table gives guidance on the status of the food when determining the microbiological quality of ready-to-eat foods.

Table 3: Guideline levels for determining the microbiological quality of ready-to-eat foods

[29]

Test	Microbiological result (cfu/g unless otherwise stated)			
	Good	Acceptable	Unsatisfactory	Potential hazardous
Standard Plate Count				
Category A	$<10^4$	$<10^5$	$\geq 10^5$	N/A
Category B	$<10^6$	$<10^7$	$\geq 10^7$	N/A
Category C	N/A	N/A	N/A	N/A
Indicators				
Enterobacteriaceae	$<10^2$	10^2 to $<10^4$	$\geq 10^4$	N/A
E. coli	<3	3 to $<10^2$	$\geq 10^2$	N/A
Pathogens				
Coagulate +ve *Staphylococcus*	$<10^2$	$10^2 < 10^3$	$10^3 < 10^4$	$\geq 10^4$
C. perfringens	$<10^2$	$10^2 < 10^3$	$10^3 < 10^4$	$\geq 10^4$
B. cereus	$<10^2$	$10^2 < 10^3$	$10^3 < 10^4$	$\geq 10^4$
Salmonella spp.	Not detected in 25g			detected in 25g

2.2 Microbiological quality and safety of street-vended meat

Abdalla et al. [30] states that consumers appreciate street foods. However, a report by Kotzekidou

[10] and Rane [31] states that the significant risk factors that lead to the production of unsafemicrobiological street-vended RTE meats are

inadequate food safety knowledge, insufficient basic

hygiene, operation in unsanitary environments and lack of public awareness. Angelidis et al (2006). also reported that the microbial load on raw materials is a crucial factor that increases with the transportation, handling, processing, and displaying of ready-to-eat meats [32].

These foods can be consumed without heat treatment and cause foodborne illness, a health problem in developing countries [6]. Moreover, meat, being a significant food source rich in proteins, provides favorable conditions for the proliferation of numerous microorganisms on its cut surfaces.[33].

The common foodborne pathogens associated with street-vended food include *C. perfringens*, *E. coli*, *Shigella spp*., *C. jejuni*, *S. aureus*, *Salmonella spp*., and *B. cereus* [34]. Manguiat and Fang
[3] reported similar results: most street-vended ready-to-eat meats have a high level of coliform bacteria and pathogenic bacteria such as *E. coli* (O157:H7), *Salmonella spp*., *Staphylococcus aureus*, *Bacillus cereus, Listeria monocytogenes, Clostridium perfringens,* and *Vibrio cholera*. Similarly, the Foodborne Disease Active Surveillance Network [35] report stated that *Salmonella spp.*, *Campylobacter spp.*, *Shigella spp*., *Cryptosporidium spp*., and *Shiga* toxin-producing *Escherichia coli O157* continued to be the primary causes of the number and incidence of laboratory-confirmed foodborne infections in the United States [36].

Bacteriological quality of ready-to-eat meats report carried out in Iran reported that 62% of semi-cooked samples contained more than 10^2 cfu/g coliforms, while *S. aureus* was more than 10^2 cfu/g in 35% and 40% of samples, respectively [37]. Also, 28% of cooked samples and 44% of semi-

cooked samples contained *E. coli*, and *Salmonella* contaminated 14% of all samples and hence was microbiologically unsatisfactory.

An investigation conducted in South Africa [38], *Bacillus cereus* emerged as the most prevalent bacterium, detected in 23 (17%) out of 132 street food samples. Additionally, within the same study, *C. perfringens* was identified in a single raw chicken sample, while *S. aureus* was found intwo raw beef samples and two stew samples of street-vended foods.

In a similar study conducted in Namibia [11], a total of 96 street vended ready-to-eat meats whereassessed for microbiological quality, and revealed that hat 42%, 15%, 6%, 52% and 83% of the samples were positive for *E. coli*, *L. monocytogenes*, *Shigella*, *S.aureus* and *Enterobacteriaceae* respectively. Unsatisfactory microbial levels were 32% for aerobic plate count, 26% for *Enterobacteriaceae*, 35% for *E. coli*, 11% for *L. monocytogenes*, 7% for S. aureus and 6% for *Shigella*.

2.2.1 Street vended ready-to-eat meats

Street-vended ready-to-eat meats are a popular form of street food in urban areas and informal settlements globally, offering convenience, affordability, and reflecting local culinary heritage [39]. Culturally, street food consumption is deeply ingrained in many societies. It provides an opportunity for people to experience a wide range of flavours, ingredients, and cooking styles, often reflecting the diversity of the local population [40]. Street food vendors often use traditionalrecipes and cooking methods, preserving culinary traditions and passing them down through generations [40]. The consumption of street food also fosters a sense of community, as people gather to eat and socialize in public spaces.

Economically, street food vending provides a source of income for many people, particularly in informal settlements where formal employment opportunities may be limited [41]. Street food vendors often operate small businesses that require minimal capital investment, making it an

accessible option for entrepreneurs. The low overhead costs associated with street food vending allow vendors to offer affordable meals, which can be especially important for low-income consumers [41].

However, concerns arise regarding food safety, as studies have found contamination of street- vended foods with foodborne pathogens such as bacteria [42]. Street food vendors may lack access to proper facilities for food preparation, leading to a higher likelihood of contamination [42]. Additionally, the presence of *S. aureus* in street-vended foods poses health risks [43]. Aflatoxin, apotential public health risk, has also been implicated in street-vended foods [44]

To address these concerns, it is imperative to examine the current state of street-vended ready-to- eat meats in specific informal settlements, in Havana, Namibia. Targeted literature reviews have revealed a scarcity of studies focusing on street food vending and food safety in these specific contexts. Previous reports have hinted at the prevalence of foodborne pathogens in street-vended ready-to-eat meats in Havana and Namibia, though comprehensive data is lacking [45]. Moreover, emerging pathogens or unique challenges pertinent to these regions necessitate further investigation.

Exploring literature specific to informal settlements reveals a wide range of unique challenges andrisk factors associated with food safety. Studies have identified factors such as limited access to clean water, inadequate sanitation facilities, and poor hygiene practices as significant contributorsto increased food safety concerns [46]. The informal nature of these

settlements often means that regulatory oversight and enforcement of food safety standards are lacking, further exacerbating the risks [47]. Additionally, the transient nature of informal settlements and the lack of infrastructure may hinder the implementation of proper food storage and handling practices, leading to an increased likelihood of contamination [48].

Moreover, emerging pathogens such as Salmonella, Escherichia coli, and Listeria monocytogenespose significant risks to consumers of street-vended ready-to-eat meats in both Havana and Namibia [45,49] . The unique climatic conditions and cultural practices in Namibia may also contribute to the prevalence of certain pathogens, necessitating tailored interventions and mitigation strategies [50].

A critical analysis of existing literature reveals both strengths and weaknesses. While some studies provide valuable insights into the broader issues of food safety in informal settlements, there is a notable lack of research specifically examining street-vended ready-to-eat meats in Havana and Namibia. Furthermore, existing studies often rely on small sample sizes and qualitative methodologies, limiting the generalizability of findings. Future research should aim to address these gaps by employing rigorous study designs and focusing on the specific challenges and risk factors associated with street-vended meats in these contexts.

2.2.2 Methods for determining the food microbiological quality.

Several methods are employed to assess the microbiological quality of food, aiming to identify and quantify microorganisms that may impact the safety and integrity of the product. The selectionof methods depends on the type of food, the microorganisms of interest, and the specific analysisrequirements. The choice of method depends on factors like the type of food product, regulatory requirements, and the specific microorganisms of concern. Different tests can be used to determinethe microorganisms present in food

[25]. The techniques can be categorized as to whether they arequantitative or qualitative methods of analysis. A combined approach can be applied to determinethe microbiological quality of ready-to-eat foods, namely culture-dependent and culture- independent.

As stated by Block [51] Culture- dependent methods have been proposed to provide a less biased picture of the richness of bacterial communities than culture-dependent methods because of the selective pressure imposed by the requirement for the growth on a solid substrate, leading to the isolation of a plate-growth-adapted subpopulation from the communities.

In some cases, [52], it is likely that many species may fail to grow at all the plate surfaces of the given medium or show few Colony Forming Units (CFU). Additionally, an artificial homogeneous medium typically allows the growth of only a tiny fraction of the organism. When complex microbial communities are under investigation, enumerating bacteria by traditional microbial culturing techniques may produce erroneous results [52].

Apart from selectivity allowing the growth of some species and suppressing the growth of others, the community composition of the culturable fraction is distorted during culturing because replication times may vary, with fast-growing species out-competing others. Culture-independent methods may detect species missed by plating, provided that the amplification efficiency is high enough [53].

However, because of its selectivity, the culture-dependent method focuses mainly on the microorganisms of interest. It accesses living (culturable) microbes and can recognize viable cells in a sample [53]. Therefore, it is of equal importance to combine both approaches (culture- dependent and culture-independent) for accurate, more comprehensive and precise results.

Ensuring the safety and quality of food requires a various approach,

particularly when it comes to monitoring microbial activity. One of the essential aspects of this process is the careful enumeration of yeasts and molds [54]. This is typically accomplished using specialized agar platesthat provide an optimal environment for their growth. Through this process, the abundance of these

organisms in food samples can accurately be quantified, gaining crucial insights into potential spoilage and the prevalence of fungal contamination [54].

Simultaneously, the measurement of water activity plays a critical role. It offers a glimpse into theenvironmental conditions that encourage microbial proliferation. High water activity levels indicate a higher risk of spoilage, while lower levels help preserve food integrity [55].

In the modern food industry, remarkable revolution in microbial detection and analysis have been observed. Techniques like Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), and quantitative PCR (qPCR) have played a significant role in this transformation. ELISA excels in identifying specific proteins or antibodies, allowing for swift and precise identification of allergens or pathogenic agents [56]. Meanwhile, PCR and qPCR techniques provide researchers with the ability to detect and quantify DNA sequences associated with spoilage organisms or pathogens with unprecedented speed and accuracy [57].

The advent of biosensors has further enhanced the ability to monitor microbial activity in real- time. These biosensors leverage biological responses to generate electrical signals for detection, offering unparalleled sensitivity and responsiveness [58,59]. This technology equips food safety professionals with a powerful tool to uphold product quality standards and safeguard consumer health [58,59].

The integration of advanced methodologies with traditional techniques enables the proactive identification and mitigation of microbial risks throughout the food supply chain. This approach ensures the safety and integrity of food products for consumers worldwide, embodying a comprehensive commitment to food safety and quality.

2.3 Food Pathogens

Worldwide, there is a great deal of concern about food-borne infections. Various techniques for bacterial growth control, including heating, sterilization, and antibiotics, have been used to reducemicrobial populations as techniques and maintain them at levels below their infection rates. According to the Centre for Health Statistics [60], 31 different pathogens can cause food-borne illnesses. Although there have been advancements over the past ten years, the burden of food- borne illnesses still exists [61], resulting in human misery, productivity losses, and a significant increase in food production and healthcare prices [62].

The global threat of foodborne diseases, especially those associated with *Salmonella*, *E. coli*, *S. aureus,* and *L. monocytogenes* has increased [63]. CDC [64] states that the most commonly recognized food-borne pathogens include bacteria (e.g., *Campylobacter, Salmonella, E. coli, L. monocytogenes, Shigella, C. botulinum, C. perfringens, S. aureus, B. cereus*); viruses (*Norovirus,Hepatitis A* virus, *Rotavirus*); parasites (*Taenia solium*, *Taenia saginata*, *Echinococcus*, *Trichinella spiralis*, *Ascaris lumbricoides*, *Fasciola*, *Cryptosporidium parvum*, *Entamoeba histolytica, Toxoplasma gondii*); mycotoxins and marine biotoxins.

2.3.1 Human diarrhea pathogens

Human diarrhea is a prevalent gastrointestinal disorder that can be traced back to infections caused by a spectrum of pathogens, including bacteria, viruses, and parasites [65]. These microorganisms can be transmitted

through multiple routes, such as the ingestion of contaminated food or water, contact with infected surfaces, interpersonal contact, and animal-to-human transmission [66]. A comprehensive understanding of these transmission routes and their association with foodborne illnesses is critical for the effective prevention and management of diarrhea outbreaks [67].

Bacterial pathogens, exemplified by *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Clostridium difficile*, are commonly transmitted through the consumption of contaminated food or water, particularly when food is undercooked or stored improperly. For instance, *E. coli* O157:H7, a virulent strain of *E. coli*, has been frequently associated with the consumption of undercooked ground beef, unpasteurized milk, and raw vegetables. Similarly, *Salmonella* infections are often linked to the consumption of contaminated poultry, eggs, and raw produce [68]. *Campylobacter*, commonly found in poultry, unpasteurized milk, and untreated water, is another significant cause of bacterial diarrhea. It can be transmitted through the consumption of contaminated food or water, particularly foods that have been improperly prepared or stored [69].

Viruses such as Norovirus and Rotavirus, known for their high infectivity rates, can also cause acute gastroenteritis, characterized by diarrhea, vomiting, and abdominal cramps. These viruses are often transmitted through contaminated food or water, with shellfish and ready-to-eat foods being common culprits [70,71]. The highly contagious nature of these viruses makes them a significant public health concern [71] .

Parasitic pathogens like *Giardia lamblia* and *Cryptosporidium* are frequently transmitted through the ingestion of contaminated water, especially untreated water. *Giardia lamblia*, a protozoan parasite, can cause giardiasis, characterized by diarrhea, abdominal cramps, and bloating [72]. *Cryptosporidium*, another protozoan parasite, can cause cryptosporidiosis, characterised by diarrhea, abdominal cramps, and fever. These parasites can

be particularly problematic in regions with inadequate water treatment facilities [73].

The symptoms caused by these pathogens range from mild to severe, with diarrhea, vomiting, and abdominal cramps being the most common [73]. In some cases, these symptoms can be life- threatening, particularly in vulnerable populations such as the elderly, children, and individuals

with compromised immune systems. Additionally, these pathogens can lead to foodborne illnesses, which pose significant public health risks, including increased morbidity and mortality rates [73].

2.3.2 Salmonella

Salmonella is a gram-negative, non-spore-forming, and facultative anaerobic rod and is a major cause of foodborne outbreak infections in humans worldwide. They are widely distributed in nature, with humans and animals being their primary reservoirs, and can be primarily found in the intestinal tract of animals [74]. They are usually contracted from improperly cooked meat (poultry, pork, and beef), infected eggs and milk, reptiles, pet rodents, and tainted fruits and vegetables [75]. Most persons infected with *Salmonella* develop diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infection [35]. Salmonellosis is an infection caused by *Salmonella spp*., and humans often become infected after consuming contaminated food and water [76]. Salmonellosis is grossly underreported because it is generally self- limiting gastroenteritis which may be misdiagnosed as intestinal influenza by a patient or the physician [77]. A report by Busby and Roberts in 1995 [78] showed that over five years, from 1983 to 1987, beef was the major contributor to foodborne diseases from *Salmonella* in the United States. However, Ntaate [79] reported that the incidence rates of *Salmonella* in raw beef are generally low (5%). Moreover, Shilangale [80] reported a prevalence of 0.85 % for *Salmonella* in raw beef samples from abattoirs in Namibia. However, Mann [81] and Ntaate [79] stated that the levels could be relative and reasonably high

depending on the health or handling of the animals during slaughtering. More recently, Shiningeni et al. [11], *Salmonella* was only detected in the cold, roasted beef sample purchased in the afternoon from Havana, 40% (8 of 20) and in 1 cold, roasted beef sample purchased in the afternoon from Wanaheda 11% (1 of 9).

According to a study on the prevention of *Salmonella* infection by using the aggregation characteristics of lactic acid bacteria, *Salmonella* is a food pathogen that contributes to food poisoning. *Salmonella* is a gram-negative bacillus that causes food poisoning and is the culprit responsible for paratyphoid fever, hematosepsis, and gastroenteritis. These bacteria frequently resist antibiotics such as tetracycline, trimethoprim-sulfamethoxazole, and streptomycin. Contaminated food and water are two of its entry points [82].

Another report on *Salmonella* [83] states that salmonellosis remains a serious public health issue worldwide. The expense of surveillance, inquiry, treatment and illness prevention also contributesto its detrimental economic effects. Salmonellosis prevalence is difficult to estimate because sporadic cases are frequently underreported, and only significant outbreaks are typically researched. Only 1 to 10% of cases of salmonellosis are documented in several South and Central American, Asian, and African nations [84].

In China, *Salmonella* continues to be the most common bacterial food-borne disease. Although meat products are one of the leading causes of human salmonellosis, there is a dearth of detailed, quantitative information about the *Salmonella* contamination of these foods. To better understandthe prevalence, bacterial load, and antimicrobial resistance profiles of different *Salmonellaserovars* in retail meat throughout all of China, this study set out to look at these issues [85].

Meat products continue to be one of the most influential food groups that

can act as causes of foodborne illness, with poultry meat, eggs, pork, and beef being more prominent in this regard [86]. With rates of 38.2 %-51.6% cases per 100,000 people reported in 2005, *Salmonella* and *Campylobacter* are the most frequently reported causes of food-borne diseases in Europe[86]. While in the US, according to the Centres for Disease Control and Prevention [87], around 1 in 6

Americans (or 48 million people) get sick each year, 128,000 are hospitalized, and 3,000 passes away from food-borne illnesses.

With all the reviewed articles, the similarities lie where salmonellosis is reported to be a gram- negative *Bacillus* that causes food poisoning and that most cases of salmonellosis are underreported, only those that are deemed to be significant are reported and recorded; thus, the conducting of research on food pathogens such as salmonella is vital for the documenting and recording for *Salmonella* cases in food articles.

2.3.3 Shigella

Shigella is a genus of gram-negative, non-spore-forming, non-motile, non-encapsulated, non- lactose fermenting, facultative anaerobes and rod-shaped bacteria closely related to *E. coli* and *Salmonella* found in most surface-waters, sewage, and food. Shigellosis, or bacillary dysentery, as it is commonly known, is caused by bacteria of this genus¸ which include *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* [88]. Four major *Shigella* species cause diarrhea disease. The most common species in the U.S. and other developed countries is *S. sonnei*followed by *S. flexneri* [88]. Two other *Shigella* species, *S. dysenteriae,* and *S. boydii*, are found very rarely in developed countries and have a generally low infection rate overall; however, *S. dysenteriae* causes the most life-threatening of all of these infections due to the production of Shiga toxin, which can lead to hemolytic uremic syndrome (HUS). *Shigella* is transmitted through the fecal matter of people or animals and is usually transmitted to humans by

eating foods contaminated with fecal matter through cross-contamination. The highest Shigella value of 3.3 logCfu/g was observed in the cold, roasted beef sample purchased in the afternoon from Havana. It was identified as *S. flexineri* [11].

On a recent insight into *Shigella*, the pathogen is a primary cause of diarrhea, a significant globalhealth issue. *Shigella* disease is more common than previously thought. According to improved epidemiological data and new-generation diagnostics, its impact is not limited to weak immune people in low- and middle-income nations, but include upper-income nations [89].

According to recent estimates, *Shigella* causes over 125 million episodes of diarrhea every year [90], resulting in about 160 000 deaths, with young children accounting for one-third of these [90].In children in South Asia and sub-Saharan Africa, enterotoxigenic *E. coli* and *Shigella* were foundto be the leading causes of bacterial diarrhea [91]. The Global Enteric Multicenter Study (GEMS),which was conducted, also found that *Shigella* was the most common etiology in children between the ages of 2 and 5 who had diarrhea[92].

Another article on *Shigella* [93] states that a highly effective method of transmission during some epidemics has been identified as *Shigella* transmission by flies growing on feces. Both contaminated drinking water and surface waterways can contain *Shigella*. Four main serological categories can be used to categorize *Shigella*. Group D, *S. sonnei*, which only has one serotype, comprises Group A, *S. dysenteriae*, Group B, *S. flexneri*, Group C, *S. boydii*, and Group D.

The majority of instances of dysentery in the industrialized world are caused by *S. sonnei*. *Shigella* can form and thrive in biofilms, although little is

known about it, indicating the need for more study. Most food outbreaks have been linked to contaminated salads from either food handlers or along the food chain within the country of origin. With the help of molecular techniques, detecting *Shigella* in fecal and food samples has improved and facilitated the *Shigella* outbreak investigation[94].

2.3.4 Escherichia coli

According to Sousa [95] and Newell [96], *E. coli* is a gram-negative, flagellated, rod-shaped, and lactose-fermenting microbe and part of the normal microflora of the intestinal tract of humans and warm-blooded animals. *E. coli* is the most abundant facultative anaerobe of the human colonic flora and typically colonizes the gastrointestinal tract within a few hours after birth [97] . Usually,commensal *E. coli* interact with the host in a mutually beneficial way; however, some strains of *E.coli* acquire virulence attributes that can cause a broad spectrum of diseases. STEC (Shiga toxin-producing *E. coli*) is a type of pathogenic *E. coli* that, as the name implies, produces a potent toxin called Shiga toxin (Stx), also known as verotoxin or verocytotoxin-producing *E. coli* (VTEC) [98]. Pathogenic *E. coli* are classified into specific groups based on the mechanisms they cause disease and clinical symptoms. These categories include Enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and diffusely adhering *E. coli* (DAEC) [99]. The STEC strains that cause hemorrhagic colitis (bloody diarrhea) belong to the EHEC group of pathogenic

E. coli (Yoon & Hovde, 2008). In developed countries, EHEC is the most severe pathogenic *E. coli*; however, in developing countries, EPEC is a primary disease-causing agent in children [100]. Strains of *E. coli* can be characterized serologically based on the detection of specific O (somatic), H (flagella), and K (capsule) antigens. For most *E. coli* strains, the O and H

antigens are sufficientto identify the strain. For example, *E. coli* O157:H7 is the leading cause of STEC infections internationally [101] [100] [102].

Due to their resemblance to the toxins produced by *S. dysenteriae*, STEC produces toxins known as Shiga-toxins. With a preferred temperature of 37 °C, STEC may grow from 7 °C to 50 °C. SomeSTEC can flourish in diets with low water activity and an acidic pH as low as 4.4. Consuming

fruits and vegetables, such as sprouts, spinach, lettuce, coleslaw, salad, undercooked hamburgers, dried cured salami, unpasteurized fresh-pressed apple cider, yogurt, and raw milk cheese are a fewexamples of items that are linked to a rising number of *E. coli* O157:H7 outbreaks. Contaminationmay be caused by contact with animal faeces from domestic or wild animals at some point duringcultivation or handling [103].

E. coli 0157:H7 may be acquired through the consumption of meat that has not been sufficiently cooked, and person-to-person transmission can occur through the fecal-oral route. *E. coli* 0157: H7 can be found in the diarrhea stool of infected persons. As such, their presence in ready-to-eat meats (fully cooked or semi-cooked) can indicate poor hygiene and sanitation or inadequate heat treatment (indicator organisms). The pathogens can be spread if personal hygiene and hand washing procedures are inadequate [70]. According to Shiningeni [11] out of the 96 ready-to-eat meat samples, unsatisfactory levels (explained in Table 3) of *E. coli* were 35% (34 of 96) observed in Havana 75% (15 of 20), Wanaheda 56% (5 of 9), Single Quarters 47% (7 of 15), Okuryangava38% (3 of 8), Dorado Park 20% (3 of 20) and Prosperita 5% (1 of 20).

2.3.5 Staphylococcus aureus

According to [104], *Staphylococcus aureus* is an incredibly adaptable organism that causes toxic shock syndrome, hospital and community infections, and staphylococcal food poisoning. The most potent food-borne bacterial pathogen ever to evolve is thought to be *S. aureus*. The clinical

symptoms of staphylococcal food poisoning are caused by tens of genes in the *S. aureus* metagenome that encode staphylococcal enterotoxins. Food products may contain *S. aureus*, making them a possible transmission source.

Of the many common staphylococcal bacteria, *S. aureus* is the most dangerous. It can be spread through direct contact with an infected individual, contact with contaminated objects, and inhalation of airborne droplets dispersed during sneezing or coughing. Skin infections are common, but the bacteria can spread through the bloodstream and infect distant organs [105].

According to molecular typing investigations, these enterotoxigenic isolates are genetically related to isolates implicated in human infections. Contamination of Foods, particularly those derived from animal, may occur through contact with livestock or individual working in the food and animal husbandry industries. Regardless of the source of contamination, staphylococcal food poisoning (SFP) can be caused by enterotoxigenic *S. aureus* isolates found in foods under favorable environmental circumstances for growth and enterotoxin synthesis [106].

The prevalence of *S. aureus* foodborne illness is revealed through advances in disease burden assessment. Recent studies have produced important new information that will help us better understand the pathobiology of *S. aureus* foodborne sickness and the proteomics and genomes of this foodborne pathogen. Recent discoveries, in particular, provide new insight into the functions of recently discovered enterotoxins and methicillin-resistant *S. aureus*. These discoveries point the way to better preventative and control tactics [107].

Foodborne outbreaks can result from contaminated ready-to-eat foods by

enteric pathogens such as *E. coli, Salmonella*, and *S. aureus* bacteria. *E. coli*, *Salmonella*, and *S. aureus* prevalence in ready-to-eat foods were, respectively, 33.8 percent (95% Confidence Interval: 19.9, 51.2%), 26.0 percent (95% CI: 13.8, 43.6 %), and 46.3 percent (95% CI: 24.8, 69.4 %) of which *S. aureus* wasthe highest. The results demonstrate that there is still a risk to the public's health from pathogenic

bacteria being present in ready-to-eat meals. Therefore, efficient food hygiene and safety measures are required to safeguard consumer and public health [108].

2.5 Avenues of food contaminations

As outlined in the World Health Organization (WHO) or European (EURO) Food Safety Programme laboratory manual on food microbiology [109], when food is produced and processed, there is numerous potentials for contamination because we live in a microbiological world. Healthy animals bred for food contain several foodborne bacteria, usually in their intestines. Small amounts of intestinal contents that touch with meat and poultry carcasses after slaughter can taint the meat. Similarly, they may become infected if fresh fruits and vegetables are washed or irrigated with water tainted with sewage or animal manure. Some strains of *Salmonella* can infect a hen's ovary,allowing the disease to contaminate an egg inside before the shell has even formed [110].

Oysters and filter-feeding shellfish can concentrate *Vibrio* bacteria found naturally in seawater and other microbes in human sewage dumped into the sea [111]. Other foodborne microbes can be introduced later in food processing by infected humans handling the food or by cross-contamination from another raw agricultural product. *Shigella* bacteria, hepatitis A virus, and Norwalk-like viruses, for example, can be introduced by the unwashed hands of infected food handlers. Microbes can be transferred from one food to another in the kitchen using the same knife,

cutting board, or other utensil to prepare without washing the surface or utensil [112].

Ready - to - eat food is susceptible to re-contamination when exposed into contact with raw foodsor their drippings which may that contain pathogens. The manner in which contaminated food is handled can influence the likelihood of an outbreak [112]. To lead to illness, many bacterial microbes require to multiply to a more significant number in food. As a result, lightly contaminated

food left out overnight can become highly infectious the next day [113]. Most microbial pathogens are mesophilic [109], except for *L. monocytogenes* and *Yersinia enterocolitica* [114].

Clostridium is a spore-forming species that requires temperatures above boiling to be fully eradicated. This is why, during the canning process, canned foods must be subjected to high temperatures under pressure. These high temperatures are necessary to ensure the destruction of the spores, which can otherwise survive boiling temperatures. It's important to note that while boiling can kill parasites, viruses, and most bacteria, it is not always effective against spores produced by certain bacteria like *Clostridium*. This is why proper food preservation techniques, such as canning and pickling, are important for ensuring food safety [115].

2.6 Prevention of food-borne diseases

2.6.1 Hygiene and surface cleaning

The significant factor in preventing foodborne illness is cleanliness. Vendors and consumers mustalways ensure that the food is handled safely before and after purchase. Everything, i.e., utensils that touch the food, should be clean. The United States Department of Agriculture (USDA) [116] states that the most important practice we can take to help prevent foodborne illness is to wash hands with soap and warm water frequently for about 20 seconds, before and after handling food, after using the bathroom, after changing a diaper, after handling pets, after tending to a sick person, after blowing your nose, coughing or sneezing and also after handling uncooked

eggs or raw meat. According to the NSW food authority [26], any food handler with symptoms or a diagnosis of an illness (such as vomiting, diarrhea, or fever) must not handle food. Food handlers must not eat, sneeze, blow, cough, spit or smoke around food or food surfaces.

Moreover, cuts and burns on hands should be covered during food preparation because infected cuts and burns can be a source of *Salmonella* species and other germs. Similarly, a waterproof covering should cover bandages and dressings on exposed body parts. Cutting boards must be kept clean and washed in hot, soapy water after each use. Lastly, under the food standards code, a food handler must take all reasonable measures not to handle food or food surfaces in such a way that is likely to compromise the safety and suitability of food [117].

2.6.2 Cooking and reheating the food thoroughly

Cooking kills' bacteria but not its spores, so food left warming can grow new germs that may causediarrhea. Cooking and reheating are the most effective ways to eliminate bacterial hazards in food. Most foodborne bacteria and viruses can be killed when food is cooked or reheated long enough at a sufficiently high temperature. The core temperature of food should reach at least 75℃ [118]. To ensure the food is thoroughly cooked, measuring the core temperature of food is the most accurate way to judge whether the food is ready for consumption. Suppose it is not feasible to measure the core temperature of the food with a food thermometer, a visual inspection can be an effective alternative, i.e., the inner part of red meat should not be red or pink for poultry meat, thejuice of the meat should run clear, and fish should be cooked until they turn opaque and can easilybe flaked and boned. If raw meat is not well refrigerated, even if well cooked, it can still become contaminated and cause sickness [116]. Most bacteria found in food can easily be killed by cooking, but *Salmonella* can be killed at temperatures

above 65 °C [116]. Similarly, any foods likely to be contaminated with pathogens should be heated to 71°C because most pathogens can be killed very quickly [119].

2.6.3 Avoiding cross-contamination

A significant food safety hazard, such as cross-contamination, can happen at every step of the food supply chain. Without adequate knowledge of proper food safety practices, cross-contamination can happen at the slightest touch. The movement of food safety hazards from one place to another should be avoided at all costs because it makes food unsafe for consumption [120]. There are steps implemented to help with these contaminations. If there is proper food safety training, it will help in minimizing cross-contamination. Proper food safety includes knowledge of food storage; foods should be arranged appropriately to avoid raw materials coming into contact with minimally processed raw materials or ready-to-eat foods. Utensils such as knives and chopping boards must be washed or separated when dealing with different types of food [120]. The cutting board and utensils can be washed with hot water and sanitized with a kitchen sanitizer to detract the pathogens [121].

2.6.4 Keeping food at proper temperatures

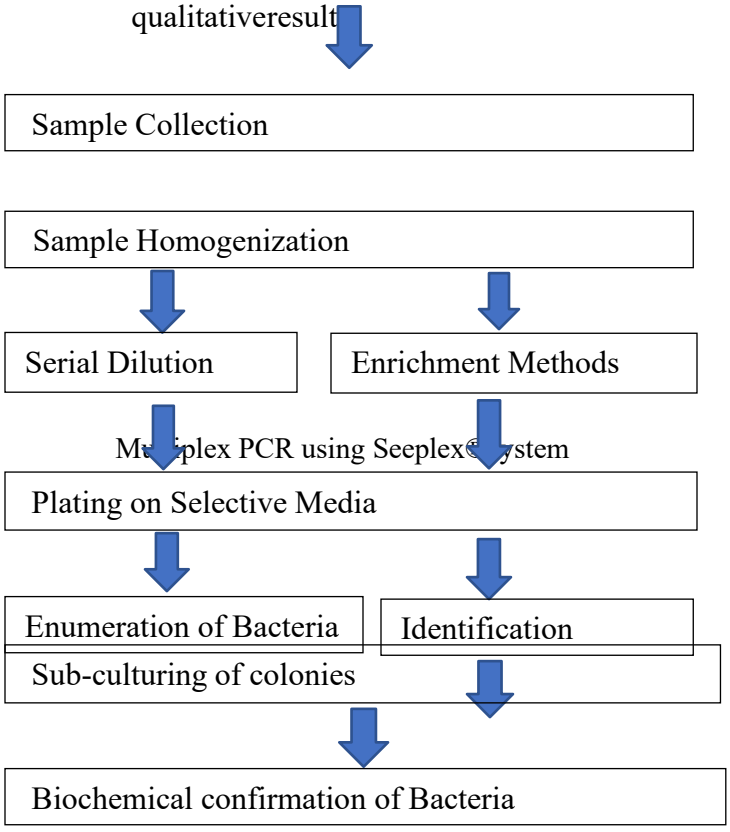
Temperatures between 4 °C and 60 °C are considered the “danger zone” for food. If the food stays in the danger zone for a prolonged period, harmful bacteria can grow to levels that could cause illness [116]. Food should not be left out of the refrigerator for over 2 hours, where as if the temperature is above 32 °C, not more than one hour. Hot cooked food should be kept at or above 60° C if not served immediately or in chaffing dishes with a warm setting of the oven between

65.5 ° C and 93.3 ° C [122]. Care must be taken to ensure that the food is stored at the proper temperature. If not, quality and safety will suffer. Food that was received frozen should be stored at temperatures that will keep it frozen. While most fresh, potentially hazardous food should be stored at five degrees Celsius or lower [123].

CHAPTER 3: MATERIALS AND METHODS

3.1 Research Design

The study utilized a mixed methods research design incorporating both culture-dependent and culture-independent techniques, specifically employing Multiplex PCR. Within the culture- dependent techniques, qualitative results were obtained through the detection, isolation, and biochemical confirmation of the isolate-related experiments, whereas quantitative results were obtained through the enumeration of microorganisms per sample. Conversely, culture-independent techniques, including nucleic acid isolation, PCR amplification, and detection, yielded qualitativeresult



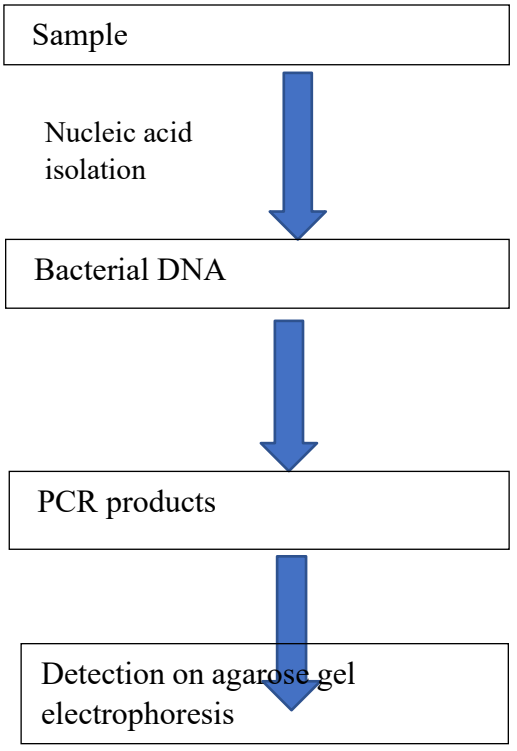


Figure 1 and Figure 2. Schematic flow chart of the culture- dependent and culture-independent methods used in this study, respectively.

3.2 Data collection

The investigation was structured based on the findings derived from a prior study conducted by Shiningeni [11]. Subsequently, the locality chosen for this research was Havana informal settlement in Windhoek, Namibia, because from the previous results of Havana out of all locationsraised more suspicions for further experimental conformity. The selection of specific ready-to-eat meat samples for collection was determined through a preliminary observation to identify the commonly consumed and most sold ready-to-eat meats.

3.3 Sample size

Based on the sample size (96) from a previous study by Shiningeni [11] on the prevalence of pathogenic bacteria in the street-vended ready-to-eat meats in Windhoek, the following equation

was used to approximate the sample size (n) of this study; $n = \frac{N \times X}{(X+N-1)}$, for a 95% confidence interval and a margin error of 0.05 [124]. When using a previous study to determine the sample

size for a new study the formula $n = \frac{N \times X}{(X+N-1)}$, helps estimate the number of samples needed to

achieve a specific number of successes, accounting for the total population size and the successrate observed previously.

Where, n = sample size, N = number of samples from previous study, X = number of successesobserved in the previous study.

Therefore, $n = \frac{96 \times 207}{(207 + 96 - 1)} = 65.8$. A sample size of ≈ 66 was required. However, a sample size of 50 was used for this study due to material limitations.

3.4 Sample Collection and storage

Ready-to-eat meat samples were collected from the street vendors situated in the Havana informalsettlement of Windhoek. The collection of ready-to-eat meat samples took place over a three- month period, spanning from April 2022 to June 2022. The purchased samples consisted of polonysausages (cut slices and the small packs), Russian sausages (cut pieces), chicken, fish, beef, and dried meat (Biltong and Droëwors). Each purchased sample was carefully transferred into sterile sample bags and placed within collection box at the point of acquisition. Collected samples werepromptly transported to the Food Hygiene section of the Central Veterinary Laboratory of the Ministry of Agriculture, Water, and Land Reform for microbiological analysis.

3.5 Microbiological Analysis

3.5.1 Enumeration of bacteria in each sample

The enumeration methods were performed according to the International Standards Organisation (ISO) 4833-1:2013 [125]. For each sample, a mass of 25 g was weighed and aseptically transferredinto a sterile stomacher bag. Subsequently, 225 ml of sterile Buffered Peptone Water (BPW) (Merck, Darmstadt, Germany) was added and blended for two minutes at 120 rpm using stomachermachine. From the homogenized mixture of the sample and BPW, a volume of 10 ml was transferred into 90 ml of Butterfield's Phosphate Buffer (BPB) (Merck, Darmstadt, Germany). Ten-fold serial dilutions (10^{-1} – 10^{-4}) were prepared in 2% sterile Butterfield's Phosphate

Buffer (BPB) (Merck, Darmstadt, Germany). A suspension of 1 ml of the sample was inoculated on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) plates for Aerobic Plate Count (APC) and incubated at 37 °C for 3 days. Furthermore, 0.1 of the sample suspensions were inoculated on the surface of Xylose Lysine Desoxycholate Agar (XLD) (Merck, Wadeville, South Africa). XLD is

a selective growth medium for *Salmonella.* Simultaneously, the sample was inoculated *on Salmonella-Shigella* (SSA) (Biolab, Merck, South Africa) medium for the selective growth of *Shigella* and *Salmonella*. On Baired Parker Agar (BPA) (Scharlau, Chemie SA, Spain) S. *aureus* was selectively grown, and lastly on MacConkey (Oxoid, USA), a selective medium tailored for

E. coli growth. The plates for APC were incubated at 30±1 °C for 30-72 hours. While the plates for *S. aureus*, *Salmonella*, *Shigella,* and *E. coli* were incubated at 37±1 °C for 18-24 hours. Results were expressed in Log cfu/g (Colony Forming Units/g). Presumptive colonies were picked, purified, and sub-cultured on respective agars using the streaking method. Presumptive colonies of *S. aureus* on BPA appear black with clear zones.

3.5.2 Isolation and Confirmation of *Salmonella* bacteria

The conventional methods for the isolation of *Salmonella* were performed according to the International Standards Organisation (ISO) 6579-3 [126] for Microbiology of the food chain and the horizontal method for the detection, enumeration, and serotyping of *Salmonella*. There are four stages to the procedures consists of four stages; pre-enrichment in non-selective medium, enrichment in/on selective media, plating out on selective solid media, and lastly, the confirmation stage. The pre-enrichment stage for all types of ready-to-eat meat samples was done using Buffered Peptone Water (BPW)

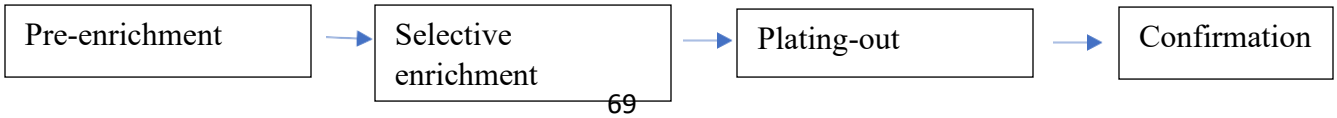


Figure 3. Diagram of four stages to the procedures for detection of *Salmonella* in food.

3.5.2.1 Gram staining

Gram staining was performed according to the procedures outlined by Quinn et al (2022) [127]. Gram-negative (-) isolates were selected for *Salmonella* identification.

3.5.2.2 Biochemical tests

In accordance with ISO 6579 standards, the approach includes three confirmation methods: TSI, Urea, and Lysine. Notably, just two tests were used, with the Lysine Iron agar test excluded.

In the process of Triple Sugar Iron Agar (TSI agar) testing, a pure culture of a suspected *Salmonella* was inoculated onto Triple Sugar Iron (TSI) agar by streaking the surface and stabbing the butt and incubated at 37±1°C for 18 to 24 hours in loosely capped tubes to maintain aerobic conditions and prevent excessive hydrogen sulphide production. TSI results were interpreted based on color changes: a yellow butt indicated glucose fermentation, a red or unchanged butt indicated no glucose fermentation, and black indicated hydrogen sulphide gas (H_2S) formation. Yellow slant signified lactose and sucrose fermentation, while no color change or a red slant indicated no fermentation. Typical *Salmonella* cultures usually exhibited alkaline (red) slants, acid (yellow) butts with gas formation, and in approximately 90% of cases, hydrogen sulphide formation. Lactose-positive *Salmonella* resulted in a yellow TSI agar slant.

Urea agar test; the urea agar was inoculated by streaking the agar slope surface with a pure culture of typical suspicious *Salmonella* from the

Nutrient agar. Urea agar slants were incubated at 37

±1°C for approximately 18 to 24 hours, followed by results interpretation. The positive reaction shows the splitting of urea, which liberated ammonia, with changes in colour from phenol red to rose pink and later to deep cerise (moderate red). The reaction is often apparent after 2 to 4 hours. When the colour of the Urea agar remains unchanged, the reaction is negative.

Gram-negative, TSI-positive, and Urea negative colonies were selected for further identification. A positive control of *Salmonella typhimurium* American Type Culture Collection (ATCC) 14028 and a negative control of *S. aureus* ATCC 25923 were used for each test.

3.5.3 Isolation and Confirmation of *Shigella* bacteria

3.5.3.1 Non-selective pre-enrichment

Procedures in 3.5.1 were followed. After presumptive *Shigella* colonies were isolated and sub cultured on nutrient agar (Merck, Wadeville, South Africa) and incubated at 37±1 °C for 18 to 24 hours. Two or more typical *Shigella* colonies from nutrient agar (Merck, Wadeville, South Africa) were further inoculated onto nutrient agar using a sterile inoculating wire loop to obtain pure cultures. The selected colonies which were preserved on Nutrient agar were used to perform biochemical and serological confirmation tests.

3.5.3.2 Biochemical confirmation

Procedures in 3.5.1 and 3.5.2.5 were followed. Table 4 shows how the changes in the medium were interpreted [128].

Table 4: Triple Sugar Iron (TSI) agar results interpretation.

Area of Slope	Appearance	Indication
Butt	Yellow Red or Unchanged Black Bubble or cracks	Glucose fermented: Positive Glucose not fermented: negative Formation of Hydrogen sulphide: Positive Gas formation
Slant Surface	Yellow Red or Unchanged	Lactose and/or sucrose utilized: Positive Lactose and/or sucrose not utilized: Negative

3.5.4 Confirmation of *Staphylococcus aureus*

Typical presumptive colonies of coagulase-positive staphylococci are black or grey, shining, and convex [129]. They can be surrounded by a clear zone, partially opaque due to the egg yolk added to the BPA media during preparation. Identified colonies of *S. aureus* were isolated and inoculated onto nutrient agar and incubated at 37±1°C for 18-24 hours; this procedure was repeated with theisolated colonies of *S. aureus* from nutrient agar to get more purified colonies. Identifying isolatesthrough gram stain to indicate a gram reaction was done using the same procedures in 3.5.2.4. An optical microscope was used to view the slides. The isolated colonies that retained the crystal violet-iodine complex and stained purple-blue (gram-positive) [127] were identified as *S. aureus* colonies. Positive control *S. aureus* ATCC 25923 was used.

3.5.5 Isolation and Confirmation of enteropathogenic *E. coli*

strains

Aseptically, a sample of 25 g was weighed out and added to 225 ml of BPW, homogenized and incubated for 18-24 hours at 37±1°C. For Selective enrichment 1ml of the culture into a tube

containing 10 ml of Lauryl Tryptose (LT) broth with Durham tubes (Scharlau Chemie S.A, Spain) and examined for gas production after 24 hours of incubation at 37 ºC. For the samples that exhibited gas production, causing displacement of the medium in the Durham tubes, a loopful of each suspension was transferred to a tube of *E. coli* Broth (EC) (Scharlau Chemie S.A, Spain) andincubated for an additional 24 hours at 37±1 ºC. The gassing EC-tubes were gently agitated, and aloopful of broth was streaked onto the MacConkey agar (Oxoid) plate and incubated at 37±1ºC for18 to 24 hours. After incubation, the plates were examined for suspicious *E. coli* colonies, i.e., bright pink, indicating the ability to ferment lactose. One (1) to five (5) colonies were identified and transferred from the MacConkey agar plate to the nutrient agar plate and incubated at 37±1ºC for 18 to 24 hours. One colony was identified out of the five colonies from nutrient agar for further testing. Identification of isolates through gram staining was made to indicate a gram reaction. A purple colour indicated a positive gram reaction, and a gram-negative (-) reaction indicated a red or pink colour [127]. Gram-negative (-) isolates were selected for *E. coli* identification. A positive control of *E. coli* ATCC 8739 was used.

3.5.6 Identification and Confirmation by Multiplex Polymerase ChainReaction (PCR)

A supernatant of each RTE meat sample was homogenized, collected in a 1.5 ml micro centrifugetube, and kept at -80 °C before analysis. GenElute^{TM} Blood Genomic DNA kit (Sigma-Aldrich, USA) was used for DNA

isolation and purification using the manufacturer's instructions. At the same time, the Seeplex® Diarrhea-B1/B2 Ace detection kit (Seegene) [130] was used for the DNAamplification.

The Seeplex Diarrhea-B1 assay permitted the simultaneous amplification of the target DNA of *Salmonella spp*. (395 base pairs) (*S. enterica* and *S. bongori*), *Shigella spp.* (330 base pairs) (*Sh. flexneri, Sh. boydii, Sh. Sonnei* and *Sh. dysenteriae*), *Vibrio spp.* (651 base pairs) (*V. cholerae, V. parahaemolyticus, V. vulnificus*), *Clostridioides difficile* toxin B (476 base pairs), *Campylobacterspp*. (227 base pairs) (*C. jejuni* and *C. coli*).

Similarly, the Seeplex Diarrhea-B2 assay enabled the simultaneous amplification of target DNA of *Clostridium perfringens* (700 base pairs), *Yersinia enterocolitica* (580 base pairs), *Aeromonas spp*. (217 base pairs) (*A. salmonicida, A. sobria, A. bivalvium,* and *A. hydrophila*), *E. coli* O157 (476 base pairs), *E. coli* H7 (370 base pairs), and VTEC (291 base pairs). The simultaneous amplification of the 476 base pairs and 370 base pairs amplicons by Seeplex Diarrhea-B2 assay confirmed *E. coli* O157:H7 detection. An internal control (1000 base pairs) was included in each multiplex assay.

3.5.6.1 Genomic DNA (deoxyribonucleic acid) isolation and purification

DNA isolation and purification were done as per the manufacturer's instructions.

3.5.6.2 DNA amplification and gel electrophoresis

The Polymerase Chain Reaction (PCR) DNA amplification technique is highly sensitive and specific. The Seeplex® Diarrhea-B1/B2 Ace detection kit (Seegene) was used based on three major processes; nucleic acid isolation, PCR amplification of target DNA utilizing Dual priming

oligonucleotides (DPO^{TM}) primers, and finally, detection on agarose gel electrophoresis. A 100- base pair DNA ladder was used to determine the amplicon size.

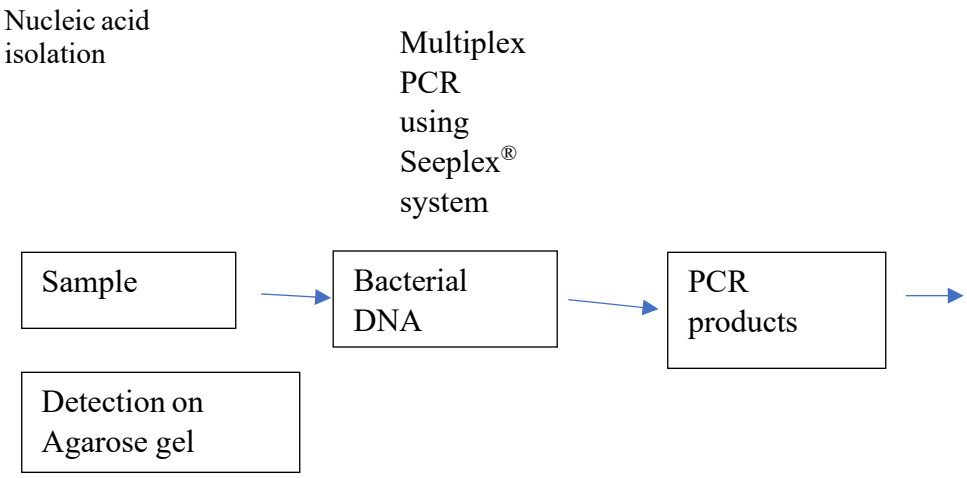


Figure 4. Overview of Seeplex® Diarrhea –B Ace detection

For the nucleic acid isolation (outlined in 3.5.6.1), a GenElute™ Blood Genomic DNA kit was utilized. A total volume of PCR master mix (900 µl) was prepared based on the total number of reactions (samples (50) + Controls (2)). A reaction mixture of 17 µl was dispersed into 0.2 ml PCR tubes, and 6 µl of the sample nucleic acid content was added to the tubes. However, only 3 µl of the PCR controls (negative and positive) was added to the tubes. The tubes were placed in a preheated (94 °C) thermal cycler. The PCR reaction was set according to the program presented in table 5. PCR product (5 µl) and DV/DB marker (5 µl) were loaded on a 2% Agarose gel (Merck,Germany) containing ethidium bromide (EtBr).

Table 5: PCR reaction setting program

Segment	No. of cycles	Temperature	Duration

1	1	94 °C	15 minutes
2	40	94 °C	0.5 minutes
		60 °C	1.5 minutes
		72 °C	1.5 minutes
3	1	72 °C	10 minutes

3.6 Data analysis

Measurements of colony-forming units were done in duplicates, and average means were calculated with standard deviation (±SD) and expressed as mean value ± standard deviation (SD).A Kolmogorov-Smirnov test was done to test for normal distribution. Differences between the prevalence of pathogens in Havana were analysed using the one-way ANOVA and Bonferroni correction multiple comparisons test to analyse the differences at a 95% confidence level. Pearson Correlation test was used to assess the relationship between the preparation methods, duration of sample collection, serving temperatures, and prevalence of pathogenic bacteria in the meat using the International Business Machines Corporations Statistical package for Social Sciences (IBM SPSS Statistics) version 29. Lastly, the microbiological quality guidelines for ready-to-eat (RTE) foods by the International Commission on Microbiological Specifications for Foods [131], servedas the basis for the evaluation of the microbiological quality of street-vended ready-to-eat meats. The Seeplex Multiplex PCR results were analysed according to the Seeplex® Diarrhea ACE Detection manual [132].

3.7 Research Ethics

Ethical clearance was sought from UNAM Research and Publication Centre. In order to maintain privacy and confidentiality pertaining to information collected from participants, data were collected anonymously, and the samples were given codes/sample IDs, not the vendor's names. Even the researchers evaluating the data could not determine the

participants' identities. The results have been intended for distribution among relevant authorities, such as the Ministry of Health and Social Services and the Municipality of Windhoek. The aim is to utilize these findings to increase awareness among street-food vendors regarding food hygiene and sanitation practices.

Nevertheless, the specific vendors whose street-vended meats yielded unsatisfactory outcomes will remain anonymous.

CHAPTER 4: RESULTS

4.1 Aerobic Plate Count in ready-to-eat meat samples.

The data on aerobic plate counts on the sampled RTE meats is presented in Table 7. Majority of the samples that were analysed for APC formed visible colonies when introduced to Plate Count Agar (PCA) for enumeration. The prevalence of contamination was found to be 100% in chicken, fish, and biltong samples, with an average microbial load of 3.03 ± 0.32, 3.93±1.04 and 5.13±0.30log cfu/g respectively. Polony sausages in cut slices showed a prevalence of 90%, with a mean aerobic plate count of 3.53 ± 1.56 log cfu/g. Beef samples exhibited an 80% contamination rate, with an average microbial load of 3.27 ± 0.80 log cfu/g. Russian sausages in cut pieces had a prevalence of 60%, with a mean aerobic plate count of 3.46 ± 1.09 log cfu/g. Polony sausages in small packs showed a contamination rate of 50%, with an average microbial load of 3.52 ± 1.63 log cfu/g.The mean aerobic plate count ranged from 3.03 ± 0.32 log cfu/g for chicken to 5.13 ± 0.30 log cfu/g for biltong/droëwors. The lowest and highest aerobic plate count was observed in the same type of meat (polony cut), the lowest was 1.70 log cfu/g, and the highest was 6.47 log cfu/g.

Furthermore, 18% (9 of 50) of RTE samples did not meet the required standards for category A, as elaborated in Table 3. Among these, the levels of unsatisfactory results were notably elevated in the dried or biltong

samples, comprising 80% (4 out of 5). This was followed by the cut slices of polony sausage and fish, both displaying 20% unsatisfactory results (2 of 10 and 1 of 5

respectively), the small packs of polony sausage and cut pieces of Russian sausage had 10% (1 of 10 each). However, the aerobic plate count was satisfactory for the chicken meat samples.

The Bonferroni correction post hoc test showed a significant difference in mean aerobic plate counts between the biltong and all other RTE meat samples ($P < 0.05$). Pearson's correlation test showed significant positive correlations for the preparation methods of the RTE meats with the serving temperature of ready-to-eat meats ($r= 0.579$, $p=0.001$). The population data were normallydistributed at a 95% confidence interval.

Table 6. Aerobic Plate Counts in various sampled meat products

Type of RTE meats	No. of samples examined	% Positive Prevalence	% Unsatisfactory	Mean ± SD (log cfu/g)
Cut Polony	10	90	20	3.53±1.56
Small Polony pack	10	50	10	3.52±1.63
Cut Russian	10	60	10	3.46±1.09
Chicken	5	100	0	3.03±0.32
Fish	5	100	20	3.93±1.04
Beef	5	80	0	3.27±0.80
Biltong/Droëwors	5	100	80	5.13±0.30

4.2 Identification and prevalence of bacterial human diarrhea foodpathogens

4.2.1 Prevalence of *E. coli* in ready-to-eat meat samples.

Of the 50 ready-to-eat meat samples assessed for microbial growth on MacConkey agar (Table 8),23 (46%) showed the presence of *E. coli* (46%). Of the seven (7) different types of RTE meat samples that were assessed for *E. coli*, 6 (percentage) samples exhibited positive results. Specifically, the polony sausage (sliced) exhibited a 50% positivity rate (5 out of 10 samples), the Russian sausage (cut) 60%, chicken samples showed a 20% positivity rate (1 out of 5), fish sampleswere all positive (5 out of 5 samples), beef samples were 40% (2 out of 5 samples), and finally, biltong samples recorded an 80% positivity rate (4 out of 5 samples).

After confirmations tests were employed *E. coli* was confirmed to be present in only 20 (40%) ofthe samples, whereby it was detected in all meat types excluding the polony pack samples.

However, all the samples that tested positive for *E. coli* were unsatisfactory (explained in Table 3). The mean log cfu/g for *E. coli* in the samples ranged from 0 (small pack of polony sausages) to3.20±0.30 log cfu/g (Biltong). The lowest *E. coli* count was observed in the cold polony sausage (sliced) samples with 2.36 log cfu/g, with the highest count observed in the hot grilled beef samplewith a count of 3.43 log cfu/g.

There was no significant difference in the *E. coli* means between the

different types of meat samples ($p > 0.05$).

Table 7. Prevalence of *E. coli* in sampled meat products

Type of RTE meats	No. of samples examined	% Positive Prevalence	% Unsatisfactory	Mean ± SD (log cfu/g)
Cut Polony	10	50	50	3.04±0.44
Small Polony pack	10	0	0	0
Cut Russian	10	60	60	2.90±0.23
Chicken	5	20	20	3.15
Fish	5	100	100	3.01±0.21
Beef	5	40	40	3.20±0.33
Biltong/Droëwors	5	80	80	3.08±0.09

4.2.2 Prevalence of *S. aureus* in ready-to-eat meat samples

The prevalence and detection of *Staphylococcus aureus* in the RTE meat samples was 36% (18 of50 samples) (Table 9), however only 14% (7 of 50 samples) were confirmed to be *S. aureus*. Outof the ten samples of polony sausage (sliced), only five (percentage)o samples responded to the growth on BPA (50%), Russian sausage (cut) had 60% (6 of 10 samples), both the fish and beef samples were 40 % (2 of 5 samples each), and lastly the biltong with 60 % (3 of 5 samples). Therewas no *S. aureus* growth for the polony sausage (small packs) and chicken samples. Among the samples that tested positive for *S. aureus*, 8% (4 out of 50) were deemed unsatisfactory, and 4% (2 out of 50) were identified as potentially hazardous, as indicated in Table 3. The fish and beef samples exhibited an unsatisfactory rate of

20% (1 out of 5 samples) each. While the biltong/droëwors samples showed a 40% unsatisfactory level (2 out of 5 samples). Furthermore,

among the polony sausage (cut) samples, 20% (2 out of 10 samples) were identified as potentially hazardous. The Russians sausage and chicken samples were all satisfactory. The mean log cfu/g for *S. aureus* ranged from 0 (Polony sausage small packs and Chicken) to 3.26 ± 0.54 log cfu/g (Biltong). The lowest and highest *S. aureus* count was recorded for the cold polony sausage (cut) samples with 1.99 log cfu/g and 5.04 log cfu/g, respectively. There was no significant difference in the *S. aureus* means between the different types of meat samples (P >0.05). The data were normally distributed at a 95 % confidence level. Pearson's correlation test showed no significant correlations for the prevalence of *S. aureus* with the serving temperature, meat preparation method, and time of collection (P > 0.05).

Table 8. Prevalence of *S. aureus* in sampled meat products

Type of RTE meats	No. of samples Examined	% Positive Prevalence	% Unsatisfactory	Mean ± SD (log cfu/g)
Cut Polony	10	50	10	3.24±1.42
Small Polony pack	10	0	0	0
Cut Russian	10	60	0	2.45±0.30
Chicken	5	0	0	0
Fish	5	40	40	3.01±0.22
Beef	5	40	20	3.00±0.25
Biltong/Droëwors	5	60	40	3.26±0.54

4.2.3 Prevalence of *Shigella* in ready-to-eat meat samples

The prevalence and detection of *Shigella* in ready-to-eat meat samples was 46 % (23 out of 50 samples) but only 38% (9 out of 50) samples were confirmed to really to be *Shigella*. All the different types of street-vended ready-to-eat meat samples tested positive for *Shigella*. Either onesample per type or all samples per type tested positive (Table 10). Since all samples produced positive results, it has been deduced that all samples were unsatisfactory (explained in table 3). The unsatisfactory levels were 100 % for the fish and biltong samples (5 of 5 samples), 60% (3 of 5 samples) chicken and beef, 40% (4 of 10 samples) small polony sausage packs, 20 % (2 of 10 samples) Russian sausage (cut), and 10% for polony sausage (cut) (1 of 10 samples). The mean *Shigella* count of the street-vended RTE meats ranged from 2.61±0.56 log cfu/g (chicken) to 3.76±0.56 log cfu/g (Russian sausages) (Table 10). The lowest *Shigella* value of 1.80 log cfu/g was observed in the cold and dried meat samples (biltong), with the highest record of 4.16 log cfu/g in grilled Russian sausage samples. There was a significant difference between the *Shigella* means of RTE meat samples (small polony sausage packs and fish) $P < 0.05$. The rest of the sample *Shigella* means had no significant difference $P > 0.05$. Pearson's correlation test showed significant positive correlations between the preparation method and the serving temperature ($r = 0.545$, $P = 0.007$). The data were normally distributed.

Table 9. Prevalence of *Shigella* in sampled meat products

Type of RTE meats	No. of samples examined	% Positive Prevalence	% Unsatisfactory	Mean ± SD (log cfu/g)
Cut Polony	10	10	10	2.83±0.03
Small Polony pack	10	40	40	2.67±0.22
Cut Russian	10	20	20	3.76±0.56
Chicken	5	60	60	2.61±0.56
Fish	5	100	100	3.19±0.08
Beef	5	60	60	2.85±0.54
Biltong/Droëwors	5	100	100	2.93±0.75

4.2.4 Prevalence of *Salmonella* in ready-to-eat meat samples

No *Salmonella* was detected in the street-vended ready-to-eat meat samples before enrichment. Only after the enrichment in selective media, *Salmonella* was detected in 24% (12 out of 50 samples). The prevalence of unsatisfactory *Salmonella* contamination levels within the respective meat samples was 60% (3 out of 5 samples) for beef, 40% (2 out of 5 samples) for biltong, 30% (3 out of 10 samples) for Russian sausages, 20% (2 out of 10 samples) for polony sausages (cut), 20% (1 out of 5 samples) for chicken, and 10% (1 out of 10 samples) for polony sausage small packs. Most samples that tested positive for *Salmonella* were predominantly acquired in a cold state, except for the beef samples, which had undergone either stewing or grilling cooking methods prior to testing. Pearson's

correlation test showed significant positive correlations for the method

of sample preparation with the duration of sample collection and the serving temperature (r = 0.750, $P = 0.05$) (r = 0.774, $P = 0.003$), respectively.

Table 10. Prevalence of *Salmonella* in sampled meat products after enrichment of media

Type of RTE meats	No. of samples examined	% Positive Prevalence	% Unsatisfactory	Mean ± SD (log cfu/g)
Cut Polony	10	20	20	Detected
Small Polony pack	10	10	10	Detected
Cut Russian	10	30	30	Detected
Chicken	5	20	20	Detected
Fish	5	0	0	No growth
Beef	5	60	60	Detected
Biltong/Droëwors	5	40	40	Detected

4.3 The Seeplex Multiplex PCR

A panel of 50 different types of RTE meat samples was analysed against different enteric pathogens to determine their presence in the samples, utilizing the Seeplex® Diarrhea-B1/B2 ACE (Auto Capillary Electrophoresis) system and gel electrophoresis.

The Seeplex system (B1/B2) effectively detected 75% (9 out of 12) of the enteric pathogens assessed, as shown in Tables 12 and 13. Notably, *C. difficile* was the most prevalent pathogen among all the RTE meat samples

examined (percentage?). The Diarrhea-B1 ACE kit proved highly

successful in detecting all pathogens (5 out of 5) in the seven types of ready-to-eat (RTE) meat samples. Out of the 50 samples analysed, *C. difficile* toxin B was detected in 42% (21 out of 50) of the samples, *Salmonella spp*. in 34% (17 out of 50), *Shigella spp*. in 32% (16 out of 50), *Vibrio spp*. in 2% (1 out of 50) of the samples, and *Campylobacter spp*. in 12% (6 out of 50). These findings underscore the effectiveness of the Diarrhea-B1 ACE kit in successfully identifying a wide range of pathogens present in the RTE meat samples.

Conversely, the Diarrhea-B2 ACE kit identified 57% (4 out of 7) of the pathogens present in the ready-to-eat (RTE) meat samples. Specifically, the kit effectively detected pathogens such as *C. perfringens*, which was found in 38% of the samples (19 out of 50), *E. coli* O157:H7 in 32% of the samples (16 out of 50), *E. coli* O157 in 20% of the samples (10 out of 50), and *Y. enterocolitica* in 8% of the samples (4 out of 50). However, *E. coli* H7, *VTEC*, and *Aeromonas spp*. were not detected in any of the seven types of RTE meat samples analysed.

The presence of *Salmonella* was most prevalent in Russian sausage (cut) meat samples, with a detection rate of 90% (9 out of 10 samples), while it was lowest in both Polony sausage (cut) and Polony sausage (small packs), both of which were detected in only 40% (4 out of 10 samples). All Russian sausage (cut) samples tested positive for *Shigella*, and 60% (6 out of 10) of Polony sausage (cut) samples also showed *Shigella* presence. Additionally, *Vibrio spp.* was detected exclusively in Russian sausage (cut) samples. *Campylobacter* was detected in 60% (6 out of 10) of Polony sausage

samples.

C. difficile was found in all types of RTE meat samples except for fish and dried meat. The highest prevalence was observed in Polony sausage (small packs), with all 10 samples testing positive, while the lowest occurrence was noted in chicken meat samples, with 2 out of 5 samples testing positive. *E. coli* O157:H7 was detected in Polony sausage (cut), Russian sausage (cut), and dried

meat samples. *E. coli* O157 was found in four types of RTE meats, including chicken, Polony sausage (cut and small packs), and Russian sausage (cut), with the highest detection rate in chicken meat samples (5 out of 5). *C. perfringens* was detected in four types of RTE meats, with the highest prevalence observed in Polony sausage (small packs). And *Y. enterocolitica* was detected in Polony sausage (cut and small pack) and dried meat samples.

The system detected *Salmonella* and *Shigella* which was previously tested by routine diagnostic testing (culture-dependent methods). Additionally, the Seeplex system detected *Salmonella* in a few samples that were not picked up by the traditional culture-dependent methods. Specifically, there was a 10% increase in *Salmonella* detection when using molecular diagnostics compared to the routine testing. Overall, the system effectively detected 35.2% of the enteric pathogens present in the ready-to-eat (RTE) meat samples. Importantly, the Seeplex system demonstrated a high level of sensitivity, for most of the enteric pathogens tested. No further testing was performed to identifyor classify the enteric pathogen species.

Table 11. Seeplex® Diarrhea-B1 ACE system results

Type of RTE meats	***Salmonella spp.***	***Shigella spp.***	***Vibrio spp.***	***Campylobacter spp.***	***C. difficile toxin B***
	% Positive				
Cut Polony (10)	40	60	0	60	40

Small Polony Pack (10)	40	0	0	0	100
Cut Russian (10)	90	100	10	0	30

Chicken (5)	0	0	0	0	40
Fish (5)	0	0	0	0	0
Beef (5)	0	0	0	0	40
Biltong/Droëwors (5)	0	0	0	0	0

Table 12. Seeplex® Diarrhea-B2 ACE system results

Type of RTE meats	***E. coli* O157:H7**	***E. coli* O157**	***E. coli* H7**	**VTEC**	***Aeromonas spp.***	***C. perfringens***	***Y. enterocolitica***
	% Positive						
Cut Polony (10)	40	90	0	0	0	50	10
Small Polony Pack (10)	0	30	0	0	0	80	10
Cut Russian (10)	80	90	0	0	0	0	0
Chicken (5)	0	60	0	0	0	60	0
Fish (5)	0	40	0	0	0	0	0
Beef (5)	0	0	0	0	0	0	0
Biltong/Droëwors (5)	80	10	0	0	0	60	40

CHAPTER 5: DISCUSSION

5.1 Aerobic Plate Count

The present study recorded lowest and highest APC values of 1.70 log cfu/g and 6.47 log cfu/g respectively. These findings align recorded in the present study, were closely with results related to those reported by Zhang [133] in China where. Specifically, the lowest and the highest APC recorded ranged between was 1.94 log cfu/g, and the highest was 7.43 log cfu/g in five group samples with 17.6% exceeding microbial standards., Contrasting with a study in Bangladesh [21], where 44% of 110 street-vended food samples were unsatisfactory, the current study exhibited a lower rate of 18%. with a narrow margin of only 0.24 and 0.96 log cfu/g when compared to the APC value range of the current study. The street-vended food included fully cooked food withoutand with minimum handling prior to consumption.

Zhang indicated that the average APC value of tested 5 group samples ranged from 3.41 to 4.37 log cfu/g. According to the microbial standards set by the Chinese Population and Family Planning Commission, 13 of 74 samples (17.6%) were out of the limits of APC.

Comparing the current study to a previous study in Bangladesh [24], the unsatisfactory level was 44% of 110 street-vended food, which is higher when compared to the current study (18%). Shiningeni [11] also reported unsatisfactory high levels of 32% on the RTE street-vended meats, with the highest APC value of 7.74 log cfu/g in Havana. observed in cold, roasted beef purchasedin the exact location (Havana) as the current study. All RTE

street-vended foods analysed in Johannesburg, South Africa, were of acceptable quality and safety [23] differing from the findings of the current study. However, the same conclusion cannot be drawn from this study.

As observed during sample collection, the polony products are sold mainly by children or teens (11-19) and only a few adults the predominant sale of polony products by children and teens (11-19 years old), with only a few adults involved, was noted during sample collection. Approximately (80%) of Polony samples utilized for the current study were predominantly (80%) acquired from street vendors in an approximate age group of 11-24. Evidently, these vendors lacked knowledgeon food safety or how to handle food; however, the adult vendors were not an exception either. These vendors are also not fixed to a specific place; they are mobile and look for their customers Are from place to place. These could be the possible reasons that contributes to contamination and unacceptable number of microorganisms in the products.

Other observations which could have contributed to unacceptable results could be that, their products, especially the polony, were not covered with proper utensils but with simple A4 papers or newspapers tied with a thin wire or rope. The knife used for cutting is constantly exposed to theair and is neither washed before nor after serving a customer. Most of the products sold were not covered and were sold in the open, exposed to microbial contaminants and all external forces during the selling period. At the time of sample collection, there was road construction going on, and the air was polluted with dust. There were also no toilets in the vicinity, and vendors hardly had access to water. There was garbage all over the selling points and the place was infested with flies flying over the products. Only a few vendors had access to storage facilities, i.e., fridges or freezers, and most

relied on re-heating perishable products, if they failed to sell all their products within a day. Generally, the APC is used to evaluate the hygienic quality and bacterial contamination in foods which can reflect the total hygienic quality of the street food. These factorswere likely the contributing factors to the unsatisfactory high levels recorded.

5.2 Prevalence of *E. coli* in ready-to-eat meat samples

The incident of *E. coli* prevalence in this study was 46%, aligning with which is comparable to theresults of Hassanin's [134] findings of, where the incidences of *E. coli* were 46.7%, 40%, and 33.3% in of the examined ready-to-eat meat hawawshi, kofta, and shawarma samples, respectively. The presence of *E. coli* in food of animal origin and RTE meats is considered an indicator of faults during preparation, poor pre- and post-cooking handling practices, storage, or service resulting in direct or indirect faecal or environmental contamination [3] [135].

Similarly, there was no statistical significance between this study's prevalence and that from Shiningeni [11] study, where they documented a 42% prevalence of *E. coli* in ready-to-eat meats gathered from various areas in Windhoek. The presence of unsatisfactory *E. coli* levels was most notable in Havana exhibited, with a prevalence rate of 75% while the. However, in this current survey recorded a 100%, unsatisfactory levels in the same location. Despite this, the prevalence of unsatisfactory levels remains high, considering the shared location both studies indicated high. The average *E. coli* counts for both Shiningeni's study and the present study ranged from 0 to 3.55

± 1.72 log cfu/g in Shiningeni's study and 3.20 ± 0.33 log cfu/g in the present study, respectively.

A study conducted by Elhag [136] reported a 26.7% prevalence of *E. coli,* which is lower than the46% prevalence observed in this study. The findings of this research were also greater than those of a study on ready-to-eat

(RTE) street-vended food in Johannesburg, South Africa, where the prevalence of *E. coli* was recorded at 1.8%, with 8% detected exclusively in beef-based foods [137].

Another study reported a prevalence of 9% of 20 samples with a mean value of 3.75 ± 0.56 log cfu/g, slightly exceeding the 3.20 ± 0.33 log CFU/g recorded in the present study, their samples

included hot RTE meats [138]. Diaz also reported positive *E. coli* results in 5 out of 43 (11.6%) grilled chickens from street vendors in Reynosa, Tamaulipas, Mexico [139].

Conversely, the mean *E. coli* levels observed in the current survey were notably lower than those documented by Ire and Imuh [140]in a study involving randomly selected RTE foods sold in PortHarcourt City, Nigeria, where a mean range of 5.0 × 10^5 to 6.0 × 10^5 log CFU/g was recorded, alongside unsatisfactory levels of 100%. This study contrasts with Saif's [141] examination on themicrobiological quality assessment of ready-to-eat (RTE) meals, which revealed the absence of E. coli in fried chicken or chicken curry from all sampled sites.

Appropriate food preparation, including sufficient cooking, is a critical contribution to food safety. Food becomes unsatisfactory and unacceptable for consumption because bacterial counts are above the microbial limits established by FSANZ [3]. Prior to consumption, perishable, uncured meats should be cooked fully to an internal temperature of at least 70 °C to eliminate vegetative infectious pathogenic bacteria [142].

The unsatisfactory level of the biltong/droëwors is in question because these samples are subjectedto high heat, dry salting involving various spices, and acidic liquids that decrease the microbial load during processing [143]. Understanding the specific methods of preparation and storage is crucial to comprehensively assess and address potential sources of contamination during production. According to Naidoo [144], the survival of bacterial

pathogens throughout the manufacturing process has been reported, and cross-contamination between the biltong/droëwors and the surface could cause a high microbial count [145].

Poor microbial conditions of raw materials may contribute to unsatisfactory levels of street-vended food [3]. *E. coli* counts are standard methods for determining the contamination of street-vended

food, especially raw meats and ready-to-eat meats [146]. Analysis of raw street foods was not done, but chicken and pork and their entrails are known to have high microbial loads due to their positions, which favour the growth of microorganisms [142] [147]. Von Holy [23] also stated that cross-contaminations of raw meats and RTE meats might occur at vending sites during cutting andchopping.

The differences in findings between this study and other studies could be due to a number of variables. This includes variances in sample collection methods, ready-to-eat meat sources, and local conditions. Hygiene practices during manufacturing, storage circumstances, and the rate at which samples were tested could all play a role. Furthermore, vendor methods in handling the products may be a significant factor, differences in cleaning, storage, and transportation policies among vendors may have an effect on *E. coli* levels. Recognizing these many aspects helps to explain why the results may differ from those of other studies.

As observed in this study, the same knives were used for cutting onions, tomatoes, and the meat that was served to the customers. Moreover, there was no washing before or after use. Grilled sausage and polony were served on a little unclean piece of paper; the vendors lacked serving utensils. Some vendors, especially the ones selling grilled beef, use bare hands to handle their products, and they use untidy towels, which are multipurpose because they are used for wiping thehandling folk/knife and also by the customers. All these could be the potential factors causing high microbial loads as a result of cross contamination.

5.3 Prevalence of *S. aureus* in ready-to-eat meats samples.

The prevalence of *S. aureus* in the ready-to-eat (RTE) meat samples was observed to be 36%, indicating its presence in 18 out of 50 samples. Notably, 12% (6 out of 50) of the samples failed

to meet the established satisfactory standards. These findings exhibit similarities to those presented by Shiningeni [11] , who reported a notably higher prevalence of *S. aureus* at 75% in 20 RTE meat samples collected from Havana informal settlement. However, the comparison lies in the unsatisfactory levels, which were 15% in Shiningeni's study compared to the current survey's 12%.

Shiningeni's study yielded a mean bacterial count ranging from 0 to 3.46 ± 1.81 log cfu/g. The highest recorded count of *S. aureus* was 5.12 log cfu/g, identified in cold, roasted beef samples. In contrast, the current study reported a range from 0 to 3.27 ± 0.54 log cfu/g, with the highest count found in sliced cold polony sausage meat samples, measuring 5.04 log cfu/g.

Both studies indicate a significant presence of *S. aureus* in RTE meat samples. Shiningeni's study displayed higher prevalence but similar unsatisfactory rates. There were minor distinctions in mean bacterial counts. Despite the differences between the two studies, the prevalence and unsatisfactorylevels of *S. aureus* remain consistent.

In a study conducted in Zambia, the contamination levels of *S. aureus* were lower [148], with a record of 10% of 200 samples. *S. aureus* count was high as 7.0 log cfu/g, which was reported in beef meat balls. In another study, 9% of *S. aureus* was detected, and the mean value was 3.75±0.56log cfu/g [138]. Djoulde [149] reported *S. aureus* contamination levels of 19% in Cameroon, whichwas higher than the present study.

The presence of *S. aureus* indicates improper handling and possible cross-contamination which can lead to a severe public health hazard [150][151]. In addition, spices, equipment, dressings, knives, and other additives are considered as the source of contamination [152]. *S. aureus* is a normal flora of the human skin, nasal passage, and throat of most healthy people and may have entered the food chain through such sources, which suggests poor hygiene practices of the

operators. When *S. aureus* is permitted to grow in foods, it can produce a toxin that causes illness and may be destroyed by heat, but its toxin is heat stable [140]

5.4 Prevalence of *Shigella* in ready-to-eat meat samples

Shigella was identified in 46% of the analysed samples, leading to the classification of each of these samples as unsatisfactory. The highest observed *Shigella* concentration in this study was 4.16 log cfu/g, with the mean concentration ranging from 2.61 ± 0.56 to 4.16 ± 0.56 log cfu/g.

In comparison, a different study conducted in Windhoek [11] reported a detection rate of *Shigella* of 6% across the samples, indicating a lower prevalence than the current findings. Evidently, the highest level of unsatisfactory samples recorded in that study was 20%, a figure that was particularly associated with Havana informal settlement. Within their investigation, the greatest concentration of *Shigella*, measuring 3.3 log cfu/g, was identified in roasted beef samples sourced from Havana. Furthermore, the isolated strains were specifically identified as *S. flexineri* at the species level.

The outcomes of this study contrast with those of a study conducted in Turkey on *Shigella* and *Salmonella* contamination in foodstuffs, which found that out of 416 food samples analysed,; no *Shigella* was isolated from the samples of chicken, ready-to-eat salads, mince, and other dairy products [153] .This study is closely similar to a study conducted in Ethiopia, where only 0.6% of*Shigella* unsatisfactory levels were detected in the ten ready-to-

eat meat samples [154]. *Shigella* unsatisfactory levels of 3.70% (5 of 135) ground meat samples were indicated, while 80 % (4 of 5samples) and 20 % (1 of 5 samples) were positive for the other two species of *Shigella*, namely *S.sonnei* and *S. flexineri* [155].

In Table 10, the average mean of detected *Shigella* counts ranged from 2.55±0.63 log cfu/g to 3.80±0.56 log cfu/g, with the highest mean being indicated in Russian sausage samples. The recorded highest CFU means in Russian sausage meat sample could be influenced by non- reheating of the product before serving. *Shigella* can endure acidic and salty environments, spreadto a human host, and cause illness [92]. *Shigella spp*. is more likely to contaminate fresh produce, deli meats, and raw milk [156]. Additionally, several studies showed that ground beef had been the source of an isolated *Shigella spp* [155].

The presence of *Shigella* in ready-to-eat meat products poses significant risks to consumers. The potential for *Shigella* to cause foodborne illness, primarily characterized by symptoms such as diarrhea, raises significant public health concerns.

5.5 Prevalence of *Salmonella* in ready-to-eat meat samples

The occurrence of *Salmonella* in the examined ready-to-eat (RTE) meat samples was found to be 24%, indicating its presence in 12 out of 50 samples. Detecting *Salmonella* spp. in a 25 g portion of any meat sample classifies it as potentially hazardous or unacceptable (as shown in Table 3) **[**29**]**. Notably, the highest rates of unsatisfactory samples were identified in beef samples.

When comparing these results to a similar study conducted in the same area (Havana) [11], a relatively higher prevalence was observed. In that study, out of the 20 samples analysed, only 8 (40%) contained *Salmonella,*

detected only in roasted beef samples. In both studies, the presence of *Salmonella* was only evident in the samples following the enrichment process using selective media. A similar approach was also employed in another study where an enrichment method was developed to successfully detect *Salmonella* in the analysed samples [157].

A study conducted in China, 807 retail meat samples overall were gathered between July 2011 and June 2016, representing the majority of Chinese provincial capitals. Overall, 159 samples (19.7%) were had positive results for *Salmonella*. Pork (37.3%, n = 287) had the highest contamination rate, followed by beef (16.1%, n = 161), mutton (10.9%, n = 92), dumplings (6.6%, n = 212), and
smoked pork (6.6%, n = 212)[85].

The present study, as indicated in Table 11, reports average means ranging from 0 to 3.05 cfu/log. In contrast, a separate investigation focused on the enumeration of foodborne pathogens such as *Salmonella*, observed an average mean of 8.72 cfu/log in the results of the 78 ready-to-eat samples, which is lower than the magnitudes of contamination seen in the current study's average mean
[158] .

A study conducted in Spain titled the prevalence of *L. monocytogenesis* and *Salmonella* in ready- to-eat food, pathogenic bacteria like *Salmonella* can infect food products before, during, or after production [159]. Food that is ready-to-eat (RTE) is not further processed to ensure it is safe before being consumed; thus, the risk of contracting a food-borne illness must be considered if these bacteria are present [160].

In summary, the study demonstrates a significant prevalence of *Salmonella* in ready-to-eat (RTE)
meat samples. The unsatisfactory levels of *Salmonella* contamination varied among the RTE meat types, ranging from 10% to 60%. These findings

underscore the need for stringent measures in handling, storage, and hygiene practices throughout the production and distribution of RTE meat products to effectively address the risk of *Salmonella* contamination.

5.6 The Seeplex Multiplex PCR

Fifty types of ready-to-eat meat samples were assessed for firstly analysed for the presence of enteric pathogens with culture-dependent methods and then finally assessed with the molecular methods using the Seeplex® Diarrhea-B1/B2 ACE kit (Seegene, South Korea) to compare and confirm culture -dependent the results. During the culture methods, 38% of diarrhea pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, and *Shigella*, were identified in 50 different types of street-vended ready-to-eat meats. In contrast, molecular methods (PCR) identified 35.2% of enteric pathogens in the meat samples. This difference could be due to various factors, such as the sensitivity of the testing techniques and the ability of PCR to detect specific DNA sequences even in low concentrations.

In the current study, there was a 10% increase in *Salmonella* detection when using molecular diagnostics compared to the routine culture method testing. In contrast, the analysis of discordant results revealed that *Salmonella* was detected more frequently by routine culture methods, likely due to the use of selective enrichment broth that increased the microbial load and recovery. The unique unconfirmed sample may be the result of a bacterial load that was so low that it could not be detected even by single plex PCR [161].

Multiplex PCR, which allows for the simultaneous detection and identification of numerous infectious agents in a single response, is a faster, more sensitive, specific, and cost-effective alternative to culture methods and mono-specific PCR [162]

A generic strategy for detecting enteric pathogens utilizing the Seeplex technology revealed considerable improvement in a 2012 investigation [163]. An additional 23.8% of enteric pathogenswere identified, a category that was not originally part of routine diagnostics. This improvement

in pathogen detection is exemplified in specific instances in the current study, such as *Salmonella*,where the Seeplex system detected pathogens in 10% more samples than routine testing. This highlights the Seeplex system's enhanced sensitivity, such advancements in detection methods arecritical for enhancing the accuracy and efficiency of food safety testing protocols.

Additional enteric pathogens such as, *Campylobacter spp*, *C. difficile toxin B*, *E. coli* O157:H7, and O157, *C. perfringens* and *Y. enterocolitica* were only detected in molecular testing, of which routine testing was not done. Findings reiterate that the application of molecular methodologies to the diagnosis of infectious intestinal disease (IID) is likely to reduce the diagnostic gap [164] and provide further insight into the relative frequency of different enteric pathogens. in cases of gastroenteritis for which testing was not performed as part of the routine diagnostic testing algorithm or not available.

The Seeplex system, as a whole, demonstrated a sensitivity of 35.2% for detecting enteric pathogens in RTE meat samples. While this percentage might seem modest, it is important to notethat molecular diagnostic methods often excel in sensitivity, identifying pathogens that may be missed by conventional techniques. The high sensitivity, coupled with the ability to detect a broad range of pathogens, positions the Seeplex system as a valuable tool in enhancing food safety measures.

The findings of the study carry significant implications for public health, sanitation, and the integrity of our food supply. The identified pathogens, including *C. difficile*, *Salmonella spp*., *Shigella spp*., *Vibrio spp*., and

Campylobacter spp., raise critical concerns about the safety of ready-to-eat (RTE) meat products and prompt a closer look at our existing systems. Firstly, these results shed light on potential vulnerabilities in our sanitation infrastructure. The study suggests that the prevalence of these pathogens could be indicative of shortcomings in waste disposal and

water treatment processes. Addressing these issues becomes crucial for safeguarding water sources used in food processing, emphasizing the need for upgraded sanitation facilities and waste management systems.

The presence of multiple pathogens in ready-to-eat (RTE) meat samples means a higher risk of foodborne diseases, necessitating the health system's preparation to address more cases. This includes increased surveillance, early detection, and effective treatment procedures. The report emphasizes the importance of extensive food safety testing. The Diarrhea-B1 ACE kit's ability toidentify a wide spectrum of pathogens emphasizes the significance of widespread testing throughout the food supply chain. A robust food safety system requires regular monitoring of manufacturing facilities, adherence to hygiene requirements, and constant testing for various diseases, particularly those identified as common in the study.

Differences in detection rates between culture methods (38%) and molecular methods (35.2%) canbe attributed to a variety of reasons. Culture methods, noted for their larger spectrum, may capture a diverse range of strains that the Seeplex system's more specialized molecular targets may overlook [165]. These variations may be explained by the viability requirements of culture methods and the presence of PCR inhibitors in samples [166]. The nature and abundance of infections, as well as the inherent properties of samples, may all have an impact on the efficacy of these detection approaches. Despite the absence of a specific rainy season during the sampling period, the complex interaction of factors such as target specificity, pathogen viability, and environmental dynamics highlights the

challenges in achieving uniform pathogen detection rates across different methodologies.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The findings from the study revealed that a significant number of street-vended ready-to-eat (RTE) meats sold in Havana informal settlement exhibited unsatisfactory and unacceptable levels of microbiological contamination. These street-vended RTE meats were found to be contaminated with enteric pathogens such as *E. coli*, *S. aureus*, *Salmonella*, and *Shigella*. The presence of these bacteria in street-vended foods poses a potential health risk for consumers. The high prevalence of these pathogens underscores the crucial need to enforce stringent hygienic practices within the street-vended food sector. Windhoek experiences industrialization, leading to an average influx of 15,000 individuals, with many settling in informal settlements such as Havana. The high unemployment rate prompts some individuals to turn to street vending as a means of livelihood.

The study identified high microbial loads in polony samples and Biltong/Droëwors, and several factors may contribute to this finding. Firstly, the polony was being sold by children or teenagers who may not have received proper education on food hygiene practices. Additionally, the products were not stored at the correct temperatures throughout the selling period, and the vendors lacked appropriate storage facilities. It is possible that the raw meats used in these products were sourced from non-commercial abattoirs or other suppliers where the implementation of sanitary practices and the Hazard Analysis Critical Control Point (HACCP) system was inadequate.

These findings underscore the need for improved and stricter adherence to hygienic practices during the collection of raw materials, food preparation, handling and storage and serving of food. It is also essential to establish properly developed open markets in informal settlements, equipped with accessible water and toilet facilities. Such infrastructures are vital because the primary cause

of high microbial loads in street-vended foods is attributed to poor hygienic practices during food processing and handling. Furthermore, it's crucial to develop educational programs that effectively communicate the risks associated with these factors to manufacturers, distributors, and consumers. Public health laws should be strictly enforced, and regular inspections should be conducted to ensure compliance with hygienic standards.

6.2 Recommendations

The study acknowledged the detection of certain diarrhea pathogens, but it did not provide specific information regarding the species of these pathogens. For instance, *Salmonella* and *Shigella* were identified using culture methods, but the specific species were not specified. Further studies are recommended to determine the species.

To establish a comprehensive understanding of the potential causal relationship between the isolated bacterial strains from the street-vended ready-to-eat (RTE) meats and diarrhea, it is advisable to conduct research that involves studying both affected individuals and the street food within the same area. This can be achieved by coordinating with diarrhea patients or consumers, as well as relevant clinics or hospitals, to facilitate the necessary research procedures. By doing so, it would be possible to establish a direct link between the identified bacterial strains and those responsible for causing diarrhea. This approach would provide valuable insights into the similarities and potential connections between the isolated bacterial strains and the strains associated with diarrhea cases.

The City or Municipality of Windhoek should prioritize enhancing the operating conditions and facilities for food stalls. One potential solution could involve establishing a well-designed open market that caters to the needs of residents. This market should feature clean and well-maintained

structures, ensuring access to safe and drinkable water, as well as efficient waste collection and disposal systems.

To ensure compliance with food and safety regulations, regular inspections of these facilities should be conducted. It is recommended to enforce stricter operational terms related to food hygiene and safety. Additionally, the introduction of certificates of operations can be considered,which would be granted to vendors who demonstrate their willingness to adhere to the specified terms and conditions.

By implementing these measures, the City or Municipality of Windhoek can enhance the overall quality and safety of street food operations, providing residents with improved options while mitigating potential health risks.

The Ministry of Health and Social Services, along with other pertinent stakeholders in the food industry, should place significant importance on providing training opportunities for street food vendors in order to enhance the safety of street food. It is crucial to implement policies and measures aimed at improving the knowledge, attitudes, and practices related to food safety amongvendors in Havana.

In addition to vendor training, efforts should be made to educate consumers about the risks of foodborne illnesses and the precautions they should take when consuming street food. Promoting awareness among consumers will help foster a culture of caution and encourage responsible choices.

By prioritizing vendor training, implementing appropriate policies, and

educating consumers, the overall safety of street food in Havana informal settlement can be significantly improved. These

collective efforts will contribute to a healthier and safer street food environment, reducing the incidence of foodborne illnesses and promoting better public health.

In order to comprehensively address the issue of food safety, it is essential to conduct a thorough study of the distribution and production line chain of raw materials. This study should encompass various stages, including slaughtering, cutting, deboning, storage, and the subsequent distribution to street food vendors. By examining each step of the process, it becomes easier to identify potential points of contamination or how pathogenic bacteria could enter the food chain.

Based on the findings of this study, it is possible to establish stricter microbial control measures that target specific areas of concern. This could involve implementing enhanced hygiene practices, improving storage conditions, or introducing effective monitoring systems to prevent the spread of pathogens.

Additionally, it is crucial for street food vendors to source their raw meat products from certified distributors or abattoirs that fully implement Hazard Analysis Critical Control Points (HACCP). HACCP ensures that proper food safety measures are in place throughout the production and distribution process, significantly reducing the risk of contamination.

By studying the distribution and production line chain, identifying potential sources of contamination, and enforcing strict microbial control measures, the overall safety and quality of street food can be significantly improved,

safeguarding public health and minimizing the occurrence of foodborne illnesses.

Lastly, further studies can be conducted to determine the prevalence of pathogenic microorganisms in other street-vended foods commonly sold on the streets, such as fruits and vegetables, fat cakes and breads and ingredients used on meat such as *kapana* spice, and traditional brews like

Oshikundu. Street-vended foods are viewed as the primary source of pathogenic microorganisms.However, RTE foods sold by commercialized retailors should be analyzed for microorganisms.

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Appendices

Appendix 1

Table 13. Sampling results showing prevalence of pathogens based on preparation methods, time of collection and serving temperature

Samples Description	Prevalence				Preparation method	Time of collection	Serving temperature
	APC	*E. coli*	*S. aureus*	*Shigella*			
Pc01	6.47	2.35	2.71		Normal	09:00-12:30	Cold
Pc02	1.70				Normal	09:00-12:30	Cold
Pc03	4.02		4.45		Normal	09:00-12:30	Cold
Pc04	4.02				Fried	09:00-12:30	Warm
Pc05	5.24	2.89	5.04		Normal	09:00-12:30	Cold
Pc06	2.30	3.27			Fried	09:00-12:30	Warm
Pc07	2.23	3.18	2.02		Normal	09:00-12:30	Cold
Pc08	2.69				Fried	11:00-11:30	Cold
Pc09	2.70				Fried	11:00-11:30	Cold
Pc10		3.52	1.99	2.83	Normal	11:00-11:30	Cold
Rc03	2.87	2.46	2.79		Grilled	09:20-09:34	Warm
Rc05	4.32	3.01	2.22	4.16	Grilled	09:20-09:34	Warm
Rc06	5.29	3.13	2.06	3.37	Normal	09:00-12:30	Cold
Rc07		2.97			Fried	09:00-12:30	Cold
Rc08	2.75				Grilled	09:00-12:30	Cold
Rc09		2.88	2.70		Grilled	09:00-12:30	Cold

Rc10	2.92	2.98	2.40		Grilled	09:00-12:30	Cold
Pp03	1.95				Normal	11:00-11:30	Cold
Pp05	3.14			2.93	Normal	11:30-12:15	Cold
Pp06	3.76			2.76	Normal	11:30-12:15	Cold
Pp07	2.56				Normal	11:30-12:15	Cold
Pp09				2.50	Normal	11:30-12:15	Cold
Pp10	6.17			2.48	Normal	11:30-12:15	Cold
Ch01	3.28			2.22	Fried	10:30-11:50	Cold
Ch02	2.95				Fried	10:30-11:50	Cold
Ch03	3.01				Stewed	10:30-11:50	Cold
Ch04	3.36	3.15		3.25	Stewed	10:30-11:50	Cold
Ch05	2.54			2.35	Fried	11:00-11:30	Cold
Fi01	3.16	3.00	3.17	3.10	Fried	11:30-12:15	Cold
Fi02	3.15	2.71	2.85	3.27	Fried	11:30-12:15	Cold
Fi03	4.04	3.04		3.80	Fried	11:30-12:15	Cold
Fi04	3.40	3.29		3.15	Fried	11:30-12:15	Cold
Fi05	5.66	3.03		3..15	Fried	10:30-11:50	Cold
Bi01	5.35	3.01		2.94	Dried	10:30-11:50	Cold
Bi02	5.17	3.02		3.18	Dried	10:30-11:50	Cold
Bi03	5.23	3.019	2.87	1.80	Dried	11:00-11:30	Cold
Bi04	4.60		3.04	2.85	Dried	11:00-11:30	Cold
Bi05	5.30	3.12	3.88	3.88	Dried	11:00-11:30	Cold
Be02	3.25		2.83	2.73	Grilled	11:00-11:30	Hot

Be03	3.30		3.18	2.39	Grilled	11:00-11:30	Hot
Be04	2.27				Stewed	11:00-11:30	Hot
Be05	2.24	3.43		3.44	Stewed	11:00-11:30	Hot

Appendix 2

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Apc	50	.00	6.47	2.8200	1.87207
Shigella	50	.00	5.04	1.6382	1.62532
S. aureus	50	.00	5.04	1.0554	1.50056
E. coli	50	.00	3.71	.7574	1.37123
Salmonella	50	.00	.00	.0000	.00000
Valid N (listwise)	50				

Statistics						
		Apc	*Shigella*	*S. aureus*	*E. coli*	*Salmonella*
N	Valid	50	50	50	50	50
	Missing	0	0	0	0	0
Mean		2.8200	1.6382	1.0554	.7574	.0000
Std. Error of Mean		.26475	.22986	.21221	.19392	.00000
Std. Deviation		1.87207	1.62532	1.50056	1.37123	.00000
Variance		3.505	2.642	2.252	1.880	.000
Range		6.47	5.04	5.04	3.71	.00
Minimum		.00	.00	.00	.00	.00
Maximum		6.47	5.04	5.04	3.71	.00

Appendix 3

ANOVA

APC						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16.78022	6	2.796703	2.091103	0.081925	2.39908
Within Groups	42.79775	32	1.33743			
Total	59.57797	38				
Shigella						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.860171	6	0.476695	0.776626	0.597676	2.598978
Within Groups	12.27606	20	0.613803			
Total	15.13623	26				
S. aureus						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Between Groups	2.18832	4	0.54708	0.778725	0.55856	3.179117
Within Groups	9.13293	13	0.702533			
Total	11.32125	17				
E. coli						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.26721	4	0.066803	0.707429	0.601035	3.179117
Within Groups	1.22759	13	0.09443			
Total	1.4948	17				

Correlations

Aerobic plate Count		Method of preparation of the RTE meats	Serving temperature
Method of preparation of the RTE meats	Pearson Correlation	1	.579**
	Sig. (2-tailed)		<.001
	N	39	39
Serving temperature	Pearson Correlation	.579**	1
	Sig. (2-tailed)	<.001	
	N	39	39

Shigella		Serving temperature	Method of preparation of the RTE meats
Serving temperature	Pearson Correlation	1	.545**

	Sig. (2-tailed)		.007
	N	23	23
Method of preparation of the RTE meats	Pearson Correlation	.545**	1
	Sig. (2-tailed)	.007	
	N	23	23

Salmonella		Duration of sample collection in hours	Serving temperature	Method of preparation of the RTE meats
Duration of sample collection in hours	Pearson Correlation	1	-.520	-.750**
	Sig. (2-tailed)		.083	.005
	N	12	12	12
Serving temperature	Pearson Correlation	-.520	1	.774**
	Sig. (2-tailed)	.083		.003

	N	12	12	12
Method of preparation of the RTE meats	Pearson Correlation	-.750**	.774**	1
	Sig. (2-tailed)	.005	.003	
	N	12	12	12
**. Correlation is significant at the 0.01 level (2-tailed).				

Appendix 4

Kolmogorov-Smirnov Test

E. coli

One-Sample Kolmogorov-Smirnov Test			
			VAR00001
N			22
Normal Parameters[a,b]	Mean		3.0245
	Std. Deviation		.28247
Most Extreme Differences	Absolute		.196
	Positive		.097
	Negative		-.196
Test Statistic			.196
Asymp. Sig. (2-tailed) [c]			.027
Monte Carlo Sig. (2-tailed) [d]	Sig.		.027
	95% Confidence Interval	Lower Bound	.023
		Upper Bound	.031

a. Test distribution is Normal.
b. Calculated from data.
c. Lilliefors Significance Correction.
d. Lilliefors' method based on 10000 Monte Carlo samples with starting seed 2000000.

APC

One-Sample Kolmogorov-Smirnov Test		
		VAR00002
N		39
Normal Parameters [a,b]	Mean	3.6154
	Std. Deviation	1.25213
Most Extreme Differences	Absolute	.184
	Positive	.184
	Negative	-.124
Test Statistic		.184
Asymp. Sig. (2-tailed) [c]		.002
	Sig.	.003

Monte Carlo Sig. (2-tailed) [d]	95% Confidence Interval	Lower Bound	.001
		Upper Bound	.004
a. Test distribution is Normal.			
b. Calculated from data.			
c. Lilliefors Significance Correction.			
d. Lilliefors' method based on 10000 Monte Carlo samples with starting seed 1314643744.			

S. aureus

One-Sample Kolmogorov-Smirnov Test		
		VAR00003
N		18
Normal Parameters [a,b]	Mean	2.9317
	Std. Deviation	.81606
Most Extreme Differences	Absolute	.214
	Positive	.214

	Negative		-.124
Test Statistic			.214
Asymp. Sig. (2-tailed) [c]			.029
Monte Carlo Sig. (2-tailed) [d]	Sig.		.030
	95% Confidence Interval	Lower Bound	.026
		Upper Bound	.035
a. Test distribution is Normal.			
b. Calculated from data.			
c. Lilliefors Significance Correction.			
d. Lilliefors' method based on 10000 Monte Carlo samples with starting seed 624387341.			

Shigella

One-Sample Kolmogorov-Smirnov Test	
	VAR00005
N	27

Normal Parameters[a,b]	Mean		3.0337
	Std. Deviation		.76300
Most Extreme Differences	Absolute		.119
	Positive		.119
	Negative		-.053
Test Statistic			.119
Asymp. Sig. (2-tailed) [c]			.200[d]
Monte Carlo Sig. (2-tailed) [e]	Sig.		.407
	95% Confidence Interval	Lower Bound	.394
		Upper Bound	.419
a. Test distribution is Normal.			
b. Calculated from data.			
c. Lilliefors Significance Correction.			
d. This is a lower bound of the true significance.			
e. Lilliefors' method based on 10000 Monte Carlo samples with starting seed 334431365.			

Appendix 5

PCR results using the Seeplex® Diarrhea-B1/B2 ACE detection

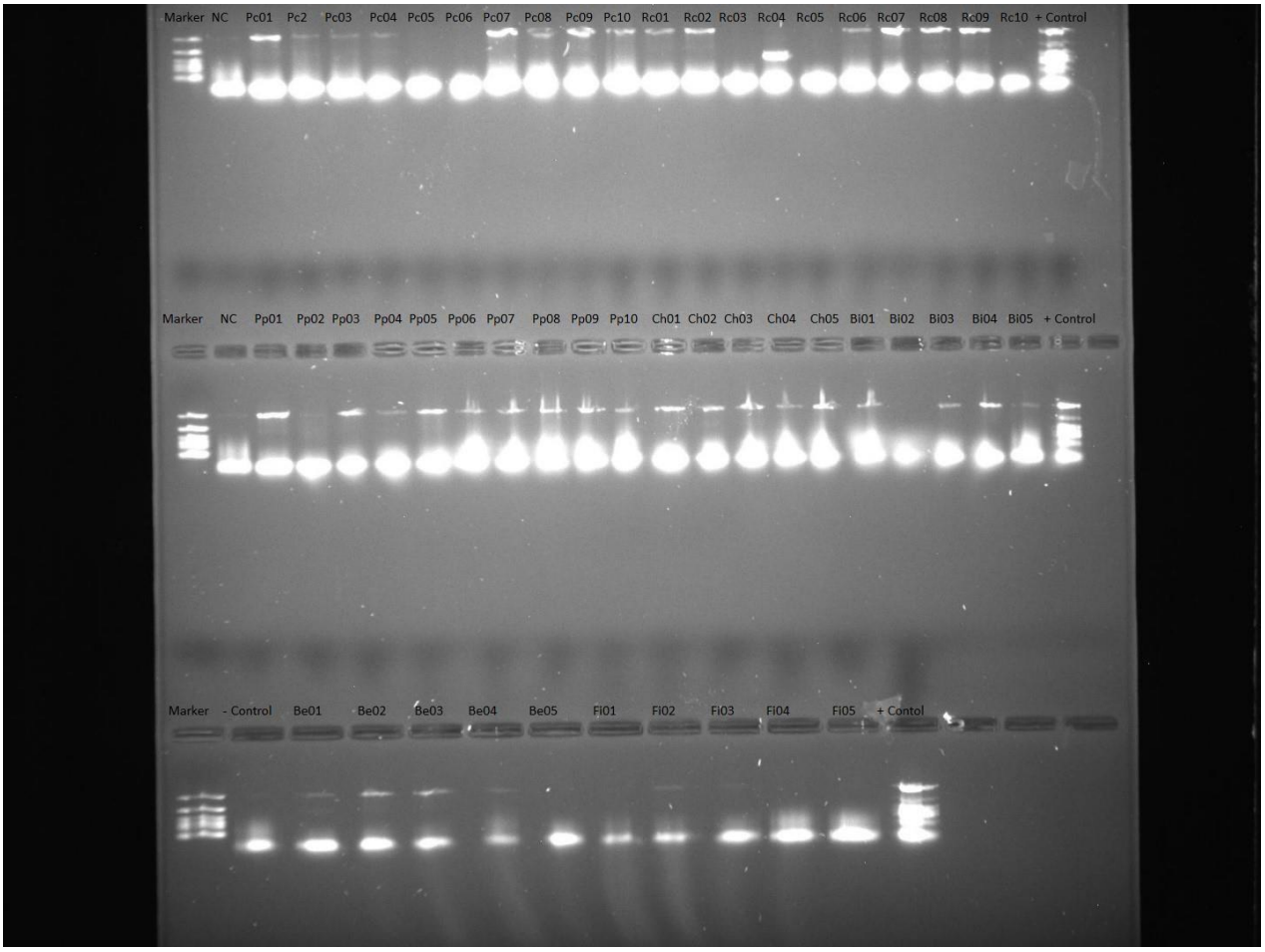


Figure 5: Seeplex® Diarrhea-B1 results on gel-electrophoresis

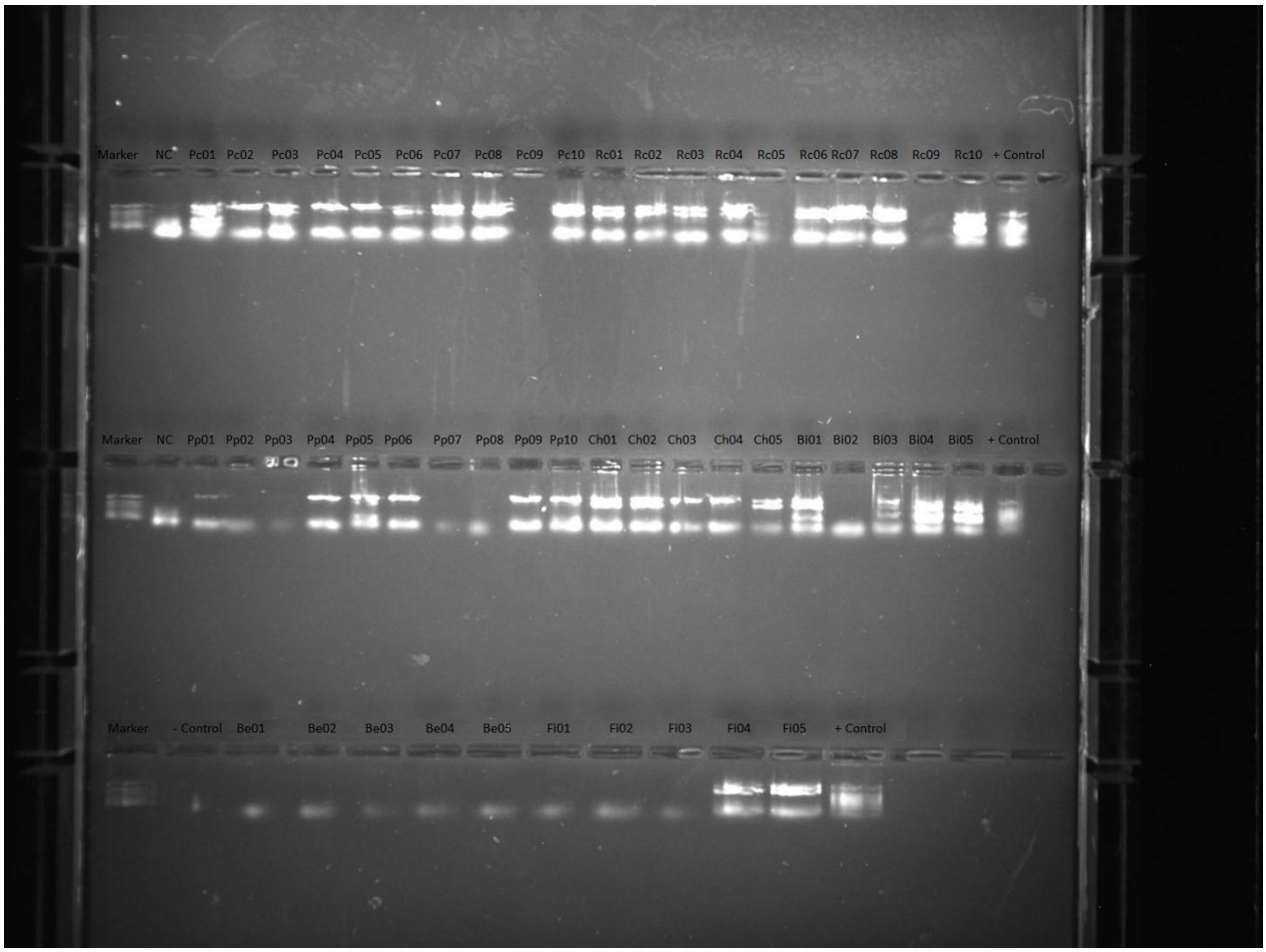
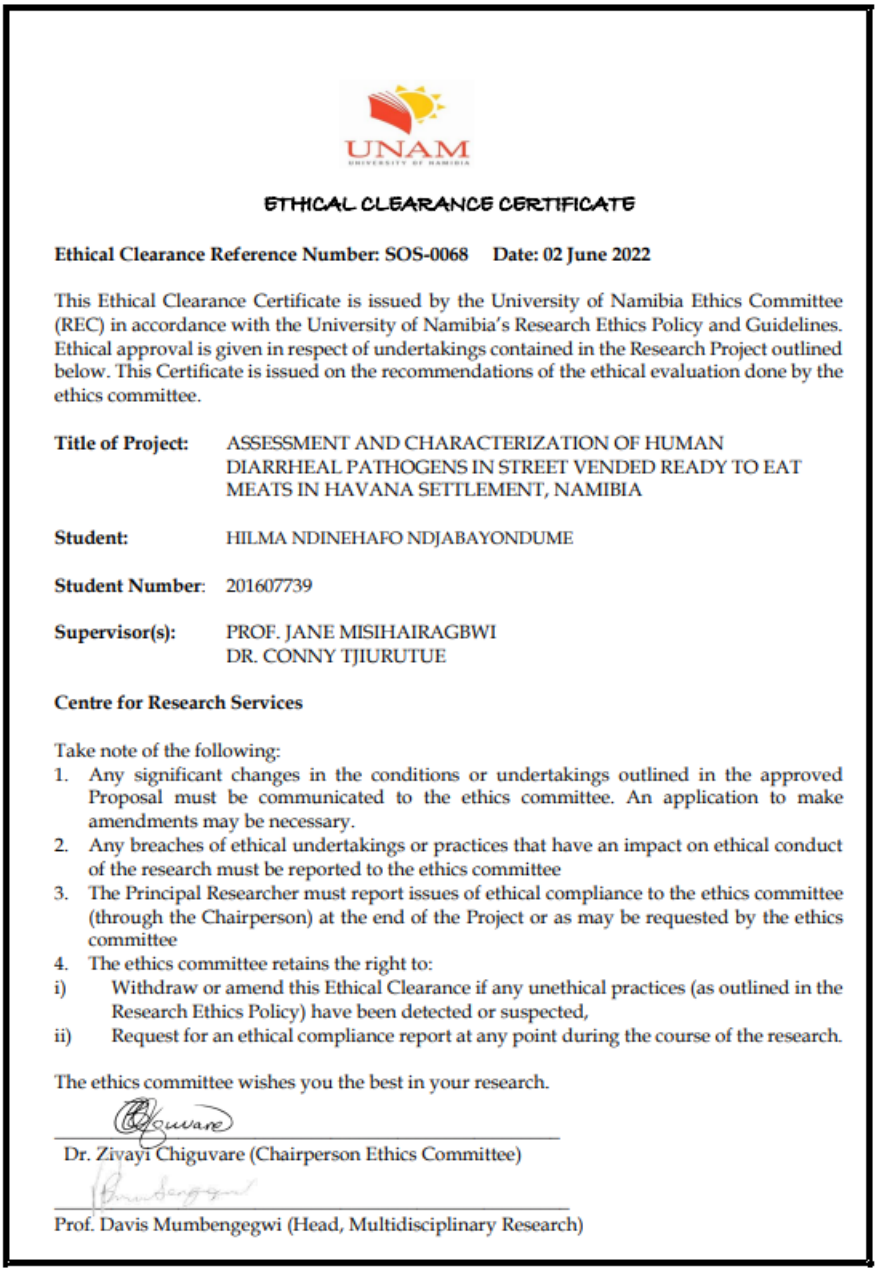


Figure 6: Seeplex® Diarrhea-B2 results on gel-electrophoresis

Appendix 6

Ethical clearance certificate Number: SOS-0068



UNAM

UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SOS-0068 Date: 02 June 2022

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

Title of Project: ASSESSMENT AND CHARACTERIZATION OF HUMAN DIARRHEAL PATHOGENS IN STREET VENDED READY TO EAT MEATS IN HAVANA SETTLEMENT, NAMIBIA

Student: HILMA NDINEHAFO NDJABAYONDUME

Student Number: 201607739

Supervisor(s): PROF. JANE MISIHAIRAGBWI
DR. CONNY TJIURUTUE

Centre for Research Services

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:

i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

Dr. Zivayi Chiguvare (Chairperson Ethics Committee)

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

Figure 7: Ethical clearance certificate by the University of Namibia Ethics Committee, 2022.

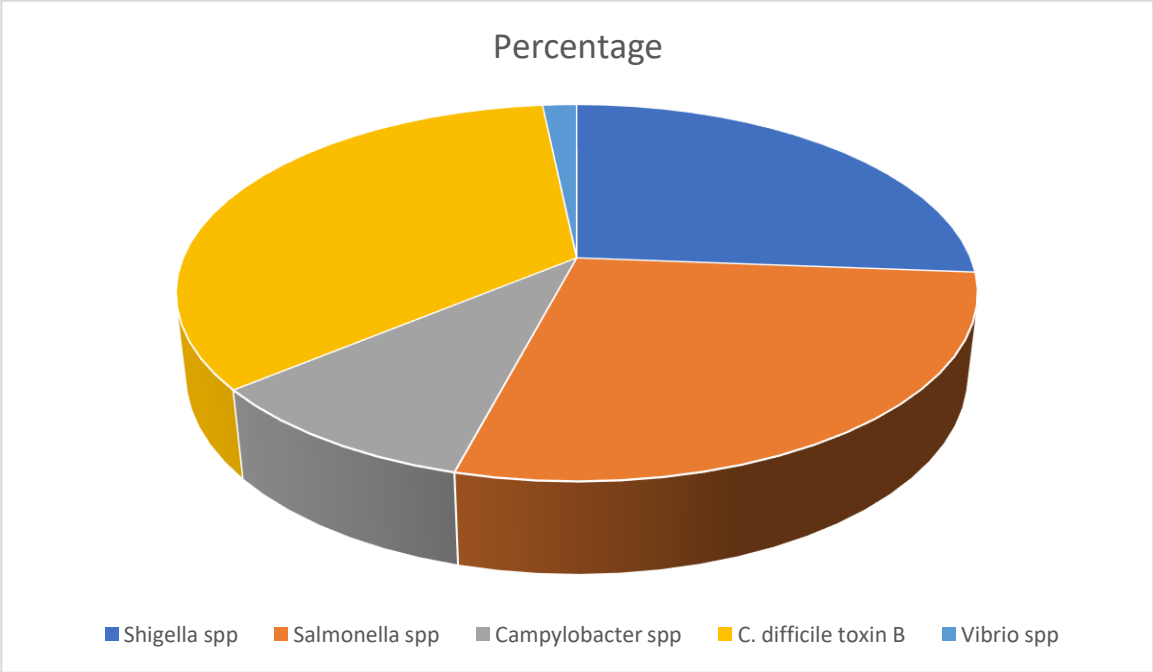


Figure 7: Seeplex® Diarrhea-B1 results

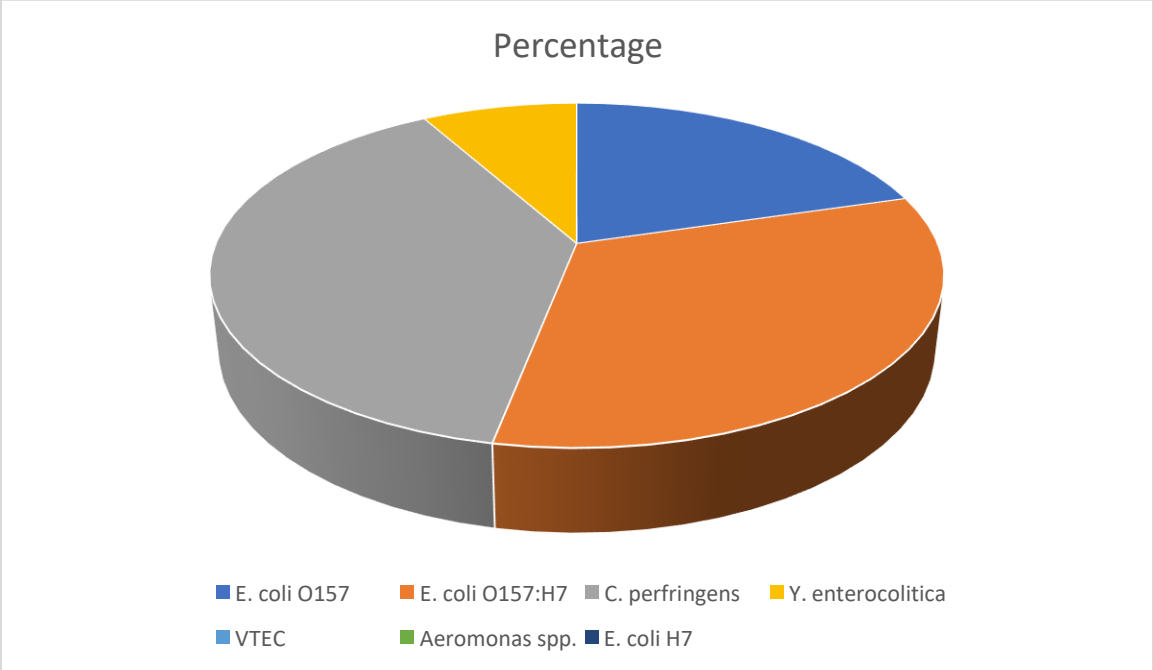


Figure 8: Seeplex® Diarrhea-B2 results