MICROBIAL ANALYSIS OF STREET VENDED READY-TO-EAT MEAT AROUND THOHOYANDOU AREA, VHEMBE DISTRICT, LIMPOPO PROVINCE, RSA.

BY

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DECLARATION

I, RAEDANI TSHIMANGADZO JEANETTE (student number: 15017846), declare that this dissertation submitted to the University of Venda for the BSc Master's degree in Biochemistry and Microbiology, Faculty of science, Engineering and Agriculture and the work contained here is my original work and this work has not been submitted in any other university and all the references have been acknowledged.

Signature:  Date: 28 May 2023

DEDICATION

I dedicate this research to The One Above Us All, The Lord God Almighty for His favour and grace that is manifested in me daily, to my parents (Raedani and Nethononda family) for supporting me in my field of endeavour, to my Pastor Bishop TI Dagada for the spiritual support, to my dearest friend Calvin Nethononda for the company and being there during the time of need.

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STUDY SUMMARY

Background: Street-vended meats are meats that are prepared and sold by vendors on the street. Different types of street vended meats are chicken, pork, beef etc. Street vendors are an integral part of urban economies around the world, offering easy access to a wide range of goods and services in public spaces. Despite these benefits, meat has been well-known as a potential channel for spreading food-borne diseases due to its high-water activity, high protein content, and approximately neutral pH, which create favourable conditions for the multiplication and survival of pathogenic bacteria. Street foods are frequently associated with food-borne illnesses due to their exposure to contamination hence this reduces the quality of these meat. Meat sold by the street vendors could be the source of infectious pathogens and previous studies showed that there is high number of pathogenic bacteria found on meat. The aim of the study was to determine the microbial quality and safety of street vended ready-to-eat meat sold around Thohoyandou Area.

Method: A total of 168 samples of street-vended meats consisting of chicken (n=84) and beef (n=84), were collected from the local street vendors around Thohoyandou area. Samples were selected using simple random sampling and purchased meat samples were transferred from vendor packaging into sterile lunch boxes at the point of purchase. The packed samples were placed in a cooler box and immediately transported to the Department of Food Microbiology laboratory, University of Venda for further analysis. Ten grams (10g) of chicken or beef samples were then transferred into a zip lock bags containing 90 ml of peptone buffered water and then cultured in different plate's containing the selective media: MSA (Oxoid Ltd) was used to culture *Staphylococcus aureus*, Eosin Methylene Blue (EMB) for *E. coli* 0157, xylose lysine deoxycholate (XLD) agar, (Oxoid Ltd) for Salmonella, and Sorbitol McConkey (Oxoid Ltd) for *Shigella*, and then plates were incubated for 24 hours at 37^{o}C. The presumptive colonies were then sub-cultured on Nutrient agar for purification and the plates were incubated at 37^{o}C for 24 hours. The microorganisms were identified using Gram staining and biochemical tests (Catalase, API 20E and Klingler iron Agar Test, and Vitek 2 system). The antibiotic susceptibility was done to determine susceptibility of the microorganisms using antimicrobial Agent such as Ampicillin, Chloramphenicol, Penicillin, Neomycin, Tetracycline, Streptomycin and Amoxicillin). The molecular

characterization was done to determine different pathotypes of *E. coli* using multiplex PCR.

Results: Out of 168 samples tested, 32 (19%) were found to be positive for *Staphylococcus spp* with highest percentage found in cooked chicken meat. The most prevalent staphylococcus species identified in this study were *S. xylosus* (13.2%) and *S. saprophyticus (10.5%).* The prevalence of *E. coli* was found to be 29 (19.3 %) in which highest percentage was found in fried chicken.

The antibiotic susceptibility profile of *E. coli* isolated showed that 100% were Resistant to Ampicillin (AMP), Tetracycline (T) and penicillin (PG) and 100% were susceptible to Neomycin (N). *Staphylococcus spp*. isolates showed 100% resistance to Ampicillin (AMP) and 100% susceptible to Neomycin(N).

The virulence genes ranged from 13,33% to 86,67% with *asta,* stx1, and *eae* being the most prevalent. The pathotypes that were detected in this study were EPEC, EHEC, ETEC, EAEC, and EIEC and majority of the isolates were positive for mixed pathotypes (contamination).

Conclusion: This current study demonstrated that the microbial quality and safety of street vended meat is inadequate and therefore not acceptable for safe consumption. Therefore, it is essential to monitor the presence of microbes in meat and the detection of these organisms in all beef and chicken meats investigated serves as a warning of foodborne diseases that could be associated with poor personal hygiene, and poor food preparation.

Keywords: *E. coli,* Meat, microbial analyses, *Staphylococcus aureus*, *Salmonella*, *Shigella* and Street vendors.

LIST OF ABBREVIATIONS

^{o}C	Degrees Celsius
%	Percentage
bp	Base pair
M	Concentration
g	Gram
km	Kilometre
µg	Microgram
µl	Microlitre
ml	millilitre
mm	millimetre
rpm	Revolution per minute
spp.	Species
API20E	Aerobic plate count/Antigen processing cells
AMP	Ampicillin
C	Chloramphenoicol
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribose Nucleotide Triphosphate
EAEC	*Entero-aggregative E. coli*
E. coli	*Escherichia coli*
EHEC	*Entero-haemorrhagic E. coli*
EIEC	*Entero-invasive E. coli*
ETEC	*Entero-toxigenic E. coli*
GEL	Gelatinase

H2S	Hydrogen sulphite
MAC	MacConkey
MDR	Multi-drug resistance
MHA	Mueller Hinton Agar
m-PCR	Multiplex-polymerase Chain Reaction
PCR	Polymerase Chain Reaction
S	Streptomycin
Stx 1	Shiga toxin 1
Stx 2	Shiga toxin 2
STR	Streptomycin
S. typhi	*Salmonella typhimuruim*
S. paratyphi	*Salmonella paratyphimurium*
XLD	XyloseLysine Deoxycholate
FBD	Food-borne Diseases
SVFs	Street Vended Foods
CFU	Colony Forming Units
MAX	Maximum
MIN	Minimum

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INTRODUCTION

1.1 BACKGROUND

Street-vended meat is meat that is prepare and sold by vendors on the street and in other public places for immediate consumption or consumption at a later time without further processing (Imathlu, 2017). In South Africa meat is a staple commodity, consumed at a rate of 41.0 kg per capita per year (Jaja *et al*., 2018). Different types of street vended meats are chicken, pork, and beef. The nutritional composition of meat includes protein, fat, vitamins, and minerals such as iron, zinc, and phosphorus. Despite these benefits, meat has been well-known as a potential channel for spreading food-borne diseases due to its high-water activity, high protein content, and approximately neutral pH, which create favourable conditions for the multiplication and survival of harmful bacteria (Ahmed *et al*., 2014). Street vendors are an integral part of urban economies around the world, offering easy access to a wide range of goods and services in public spaces. Street vended meats are the most preferable in developing countries being appreciated by consumers for their affordability, accessibility, variety, and unique organoleptic properties (Alimi, 2016).

Microbial contamination of ready- to-eat meats may be attributed to unsanitary preparation places, inadequate clean utensils, cross contamination from raw meat, poor personal hygiene and hygienic practices of meat handlers and vendors (Shiningen *et al*., 2019). Vendors often use stands and carts that are of crude and inefficient construction, and running water is not easily accessible. Hand and dish washing is performed in the same bucket, sometimes without soap. Wastewater is usually discarded in streets and garbage is discarded nearby, providing attraction, and harbourage for insects and rodents (Kotzekidou, 2013). In many cases, toilets are not available, thus forcing the vendors to eliminate their body wastes in nearby areas and to return to their vending sites without washing their hands (Fischer *et al*., 1986).

The contamination of the food with pathogens and antimicrobial resistant bacteria has emerged as an important health concern (Aslam *et al*., 2021). Most bacteria in meat can be eradicated when food is cooked correctly, and many people prefer to eat their meat half cooked. Infections caused by the beef and chicken products, have increased the public's concern for the safety of humans (Heredia *et al*., 2018). Chicken and beef

harbour different bacteria that cause infections such as diarrhoea in humans and those bacteria are also resistant to antibiotics . Resistant bacteria are transferred to humans through several routes such as direct contact with live animals, carcass at poultry farms, slaughterhouses, human consumption of meat or food with resistant bacteria, environmental contamination of soil, water, air and animal excreta (Rousham *et al*., 2018).

The pathogenic bacteria that are found in beef and chicken reported in Africa include *Enterobacteriaceae*, *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *shigella*, *Campylobacter jejuni* and *Listeria monocytogenes* (Mogomotsi & Chinsembu, 2012). In addition, pathogenic bacteria are responsible for spoilage which may lead to large economic losses (Abebe *et al*., 2020).

1.2 STUDY RATIONALE

Meat sold by the street vendors could be the source of infectious pathogens and previous studies have shown that there is high number of bacteria found in meat (Ukut *et al*., 2010). Street vending of ready-to-eat meats is very controversial from a health standpoint, and they are one of the leading vehicles for microbial pathogens since they have been implicated in foodborne disease outbreaks in humans (Shiningeni *et al*., 2019). According to Center for Disease Control and Prevention (CDC) and National Health Career Association (NHA) reported that foodborne diseases are among the most important health problems and pose a great threat to the health of consumers with the greatest burden in both developed and developing countries (Shiningeni *et al*., 2019). Various death cases resulting from contaminated meat outbreaks have been reported in Asian African countries such as Ghana, and bacteria such as *Salmonella*, *Shigella* and *E. coli* have been identified as the cause of foodborne outbreaks (Waltner, 2019).

According to Statistics South Africa (2011), 5% of deaths in 2010 were caused by food-borne diseases. The most serious safety issues resulting in immediate consumers health problems are associated with bacterial pathogens (Edoaurd, 2017). The main meat contaminating pathogenic bacteria are *Staphylococcus aureus, Listeria monocytogenes, Salmonella, Shigella, E. coli* O157:H7 and *Bacillus cereus*. Illness caused by these pathogens include urinary tract infection, mastitis, meningitis,

diarrhoea and phlebitis (Behling *et al*., 2010). The prevalence of *Campylobacter* on chicken carcasses is very high and cross contamination can easily occur during food preparation (Mateus *et al*. (2013). Listeriosis can cause stillbirths, miscarriages, meningitis, or sepsis in immunocompromised hosts. Case-fatality rates as high as 40% have been reported during outbreaks (Mateus *et al*. (2013).

In Ethiopia, the widespread habit of meat consumption is a potential cause for foodborne illness (Kassa *et al*., 2017). A study done in Bahir Dar concluded that street vended meat is unsafe for consumption particularly, if stored for long periods (Kassa *et al*., 2017). Several studies in Kenya and other developing countries revealed the presence of pathogens in meat. However, they concluded that the street of Nairobi constitutes a potential health hazard to consumers because of high pathogenic bacterial in Chicken (Birgen *et al*., 2020).

In Thohoyandou, chicken is commonly sold as fire grilled ready-to-eat chicken, popularly known as dust chicken. Therefore, this meat is widely consumed by people at home, on the streets and in the groceries. However, there is no information on microbiological quality and safety of street vended meat in Thohoyandou Area (Mafune *et al*., 2016). Another study showed that street vended meat (chicken, beef and goat meat) is contaminated with pathogenic bacteria (Ananias *et al*., 2017).

The detection of these organisms in beef and chicken meats will serve as a warning of foodborne diseases that could be associated with poor personal hygiene and poor food preparation. Studies need to be conducted to determine the occurrence of *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, *Campylobacter jejuni* and *Listeria monocytogenes* in ready-to-eat meats. The most serious safety issues resulting in immediate consumers health problems is associated with bacterial pathogens. It is therefore necessary to determine the level of contamination in Thohoyandou in order to safeguard human health since food is a major determinant of health status of individuals within the society. Therefore, this study will determine the microbiological quality and safety of commonly consumed street vended meat (chicken and beef) around Thohoyandou in the Vhembe district.

1.3 OBJECTIVES

1.3.1 Main objective

- To determine the microbial quality and safety of street vended ready-to-eat meat sold around Thohoyandou Area.

1.3.2 Specific objectives

- To isolate and determine the prevalence of selected pathogenic bacteria (*E. coli*, *Staphylococcus aureus, Shigella,* and *Salmonella spp*.) from meat using standard culture methods.
- To determine the antimicrobial susceptibility profiles of *E. coli*, *Staphylococcus aureus, Shigella* and *Salmonella spp*. using Kirby-Bauer disk diffusion method.
- To determine the pathotypes of the selected enteric bacteria isolated from meat using Multiplex PCR.

1.4 HYPOTHESIS

Meat sold by the street vendors may not be contaminated with pathogenic microorganisms.

1.5 PROBLEM STATEMENT

The Vhembe District municipality environmental health services in 2017 indicated that Thulamela is challenged by food borne diseases linked to street food and this has led several deaths. Cooked and fried meat (chicken and beef) is commonly sold by the street vendors in informal markets in Thohoyandou area. This meat could have higher potential for food poisoning and causing foodborne illnesses which is a great threat to humans who consume it (Mukwevho *et al*., 2018).. There is a limited data on safety and quality of meat sold by vendors in Thohoyandou area.

1.6 RESEARCH QUESTIONS

a) What is the level of microbial contamination of ready to eat meats (chicken and beef) sold by the street vendors?

b) Which pathotypes of *E. coli* are prevalent in street-vended chicken and beef?

c) What is the level of antibiotic resistance among the pathogens isolated from chicken and beef?

1.7 EXPECTED OUTCOMES

The prevalence of microorganism in meat will be determined. Antibiotic susceptibility patterns will be determined among the study isolates and the different *E.coli* pathotypes will be characterized.

1.8 SIGNIFICANCE OF THE STUDY

The study will determine the presence of harmful pathogens. The findings of the study may contribute to the knowledge needed by the environmental health practitioners, to identify the gaps in food safety guidelines and regulations amongst street food vendors in order to support the development of more targeted and effective training programme. Furthermore, the findings of the study will assist in developing street vending policies to assist street food vendors to protect public health.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND

Meat is the general term for the animal parts that are eaten as sustenance (Boler *et al*., 2017). Only about 1% of skeletal muscular meat is made up of carbohydrates (stored as glycogen). Because an animal can breathe and engage in aerobic (oxygen-based) metabolism while it is living, carbohydrates are crucial for the quality of the meat. However, aerobic metabolism halts and anaerobic (meaning without air) metabolism lasts for a while after the animal is killed. Lactate is produced when glucose is broken down anaerobically (Boler *et al*., 2017).

Under typical (living) circumstances, the circulatory system would carry lactate to the liver where it would be transformed back to glycogen. The blood has been eliminated, though. Since the animal''s blood has been removed, there is no way to eliminate these waste by-products, so they accumulate in the muscle. The build-up of lactate in the muscle causes a drop in pH and is linked to the transformation of muscle (Boler *et al*., 2017).

The tissue's capacity to retain water is changed as a result of the pH drop when living muscle is transformed into edible meat. Given that flesh is primarily composed of water, this is particularly crucial. There are three ways that water is stored in muscle: bound, immobilized, or open water. The majority (nearly 95%) of the water present in beef is free or immobilized. This is crucial because open water and immobilized water are kept in place by weak to moderate forces by the proteins found in meat, and they can be removed by mechanical and physical forces like freezing and thermal processing (Boler *et al*., 2017).

The eating experiences, such as juiciness and tenderness, can be adversely impacted by water loss during these processes. The most prevalent type of meat is skeletal muscle, which is primarily composed of 75% water, 20% protein, 4% fat, and 1% vitamins and minerals (Boler *et al*., 2017).

2.1.1 Common bacteria found in ready- to - eat meats

Numerous studies revealed that various pathogens, including Staphylococcus aureus, Escherichia coli, Salmonella spp., Shigella spp., Klebsiella spp., Pseudomonas spp., Vibrio spp., Campylobacter spp., and Listeria monocytogenes, have been isolated from RTE foods in various nations (Rather *et al*., 2021). According to Stewart and Humphrey (2002), many locally produced foods were processed in poor and insufficiently sanitary conditions, which contributed to cases of food contamination and intoxication.

Poor hygiene and sanitation are frequently linked to *E. coli* and *S. aureus,* which are typically responsible for an epidemic of food-borne illnesses. *S. aureus* is capable of producing a protein toxin that causes illness in humans. Food poisoning signs typically appear between one and seven days, or 2 to 4 hours, after consuming food that contains staphylococcal enterotoxins (Mohammad, 2018). The most typical signs and symptoms include diarrhoea, stomach cramps, nausea, vomiting, and retching. In extreme instances, headaches and collapsing could happen (Mohammad, 2018).

2.1.2 Factors affecting spoilage of beef and chicken

Meat is known as one of the most perishable foods. However, it consists of chemical makeup, which favor the development of microbes (bacteria) to levels that contribute to meat spoilage (Gram *et al*., 2002). Raw meat has a lot of bacteria presents, which may undergo changes that make it unattractive and unfit for human consumption (Gram *et al*., 2002). According to other research, the presence of bacteria in beef is influenced by events that take place during processing, transportation, and market storage (Mills *et al*., 2014). There are four well-established biological determinant groups for food: intrinsic, implicit, extrinsic, and processing (Mills *et al*., 2014).

2.1.3 Sources of meat contamination

Meat is not suitable for human consumption when it is rotted. Numerous factors, including poor handling, exposure to air and high temperatures, conditions that triggers chemical reactions or microbial contamination, can lead to spoilage (Guerroro, 2009). The existence of microorganisms and the production of metabolites are the most frequent causes of meats spoilage. Meat products are inedible primarily because of

the flavor and off-odor. The discoloration, blown packages, souring, surface slime, and other meat quality changes can also cause customer rejection. Meat may also contain pathogens without displaying any warning sign of deterioration. The bacteria that cause meat to spoil can either infect the animal while it is still living (endogenous disease) or can contaminate the meat after the animal has been killed (exogenous disease) (Guerroro, 2009).

Humans are susceptible to a wide range of diseases, including anthrax, bovine tuberculosis, brucellosis, salmonellosis, listeriosis, trichinosis, and taeniasis, which can all be contracted from endogenously contaminated meat. However, it is important to eliminate contaminated meat during production. Otherwise, customers will more frequently come into contact with meat that has been exogenously spoiled by bacteria or fungi after the animal has died. Bacteremia (the prevalence of bacteria in the blood of slaughtered animals) is one source of infectious organisms (Mohammed, 2004).

Salmonella spp., Shigella spp., *E. coli*, *Proteus, S. albus* and *S. aureus*, *C. welchii, B. cereus*, and faecal streptococci are among the bacterial genera that frequently contaminate flesh during processing, cutting, packaging, transportation, selling, and handling. These bacteria are commonly carried by humans. The soil contains harmful bacteria like C. botulinum. When these microorganisms colonize a portion of meat, they start to decompose it and leave behind toxins that can lead to enteritis or food poisoning, or even botulism in rare instances, which can be fatal. After complete cooking, some of the microbial toxins and spores remain in the flesh (Feglo *et al*., 2012).

Street foods are frequently associated with diarrhoeal diseases which occur due to improper use of additives (Feglo *et al*., 2012).Vendors are often poorly educated, unlicensed, untrained in food hygiene, and they work under crude unsanitary conditions with little or no knowledge about the causes of food-borne disease. Most of foods are not well protected from flies, which may carry food borne pathogens. Safe food storage temperatures are rarely applied to street foods (Feglo *et al*., 2012).

Potential health risks are associated with contamination of food by *E. coli*, *Salmonella typhi, Pseudomonas sp., S. aureus or Proteus spp*. during preparation, post cooking and other handling stages (Tambekar *et al*., 2008). Even though, people are aware

that food-borne diseases could occur due to consumption of street foods, the majority disregards these health hazards (Tambekar *et al*., 2008). Human food-poisoning is commonly associated with bacteria originating from animal sources. In most cases, infection is contracted indirectly by eating contaminated meat and meat products (Dempster *et al*., 1973).

The high incidence of bacterial food poisoning in man indicates that it is necessary to prevent contamination of meat and meat products in the food industry (Dempster *et al*., 1973). Sliced cooked meats are important vehicles of bacterial food poisoning. Contamination of these foods may be associated with inadequate processing, or they may be contaminated after cooking from a source such as raw meat, the hands of personnel or dirty equipment and work surfaces. One important vehicle appears to be the blades of food-slicing machines which can spread bacteria from one slice of meat to the next. Wiping cloths are also important reservoirs of bacteria for contamination of hands, equipment, and surfaces (Dempster *et al*., 1973).

2.1.4 Global impact of food-borne diseases

People in the world fall ill after consumption of contaminated food with the highest burden in Africa, followed by South – East Asia and Europe has the lowest burden of food-borne diseases globally (Torgerson *et al*., 2015). According to the Bisholo. (2018), 420 000 people die as a result of ingestion of contaminated food. Of these deaths, 30% represents children under the age of 5 years, despite them making up only 9% of the world's population.

In Australia, a study found that most food-borne illness occurred as gastroenteritis, but the effect of non-gastrointestinal illnesses and sequelae were substantial. Bisholo, (2018) stated that salmonellosis and campylobacteriosis increased from 2000 to 2010 and this was associated with hospitalization. China reported 31.1% of food-borne cases caused by *Vibrio parahaemolyticus* and 17.9% caused by *Salmonella* and the most implicated foods are meat and seafood products. Cooked meat and seafood obtained from farmers' markets are more susceptible to contamination compared to those from supermarkets in China (Bisholo, (2018).

In the USA, it was estimated that cases of food-borne illnesses resulted in $5–17 billion in economic and productivity losses annually (Scharff, 2012). Nevertheless, the importance of food-borne diseases as a public health problem is often overlooked

because the true incidence is difficult to evaluate while the severity of the health and economic impact is often not fully understood (Hussain & Dawson, 2013).

In Africa, more than 91 million people are estimated to contract food-borne diseases, resulting in 137 000 deaths each year (Vos, 2015). Some food safety experts allege that in Africa, approximately 2000 people die from food-borne diseases each day. *Staphylococcus typhi* Has reportedly caused 19 824 illnesses and nine deaths in four African countries at the same time. In 2011, 19 people died of food-borne diseases and 7 died of suspected food-diseases in Nigeria and Ghana, respectively (Bisholo, 2018)

In South Africa, this sector is confronted with many challenges. There is inadequate supervision and proper monitoring by EHPs, lack of training on food safety principles, storage at improper temperature and preparation of meals in unhygienic surroundings. These factors increase the risk of food contamination. Prevention also needs to address both adverse environmental health practices as well as adverse behaviour for an improved environment and healthier lifestyles (Mwove, 2020).

2.1.5 Food-borne disease cases in South Africa

The listeriosis outbreak of 2017 to 2018 in South Africa was an example of how a foodborne disease (FBD) outbreak can have catastrophic consequences and underscored the need for improving food safety control and intervention in the country. Africa has the highest burden of FBD per population worldwide, most of which are attributed to diarrhoeal disease agents. Several factors contribute to the high burden of FBD in this region: unsafe water used for the cleaning and processing of food; poor food-production processes and food handling; inadequate food storage infrastructure; inadequate or poorly enforced regulatory standards; and a move to intensive animal husbandry practices as economies develop (Bisholo, 2018).

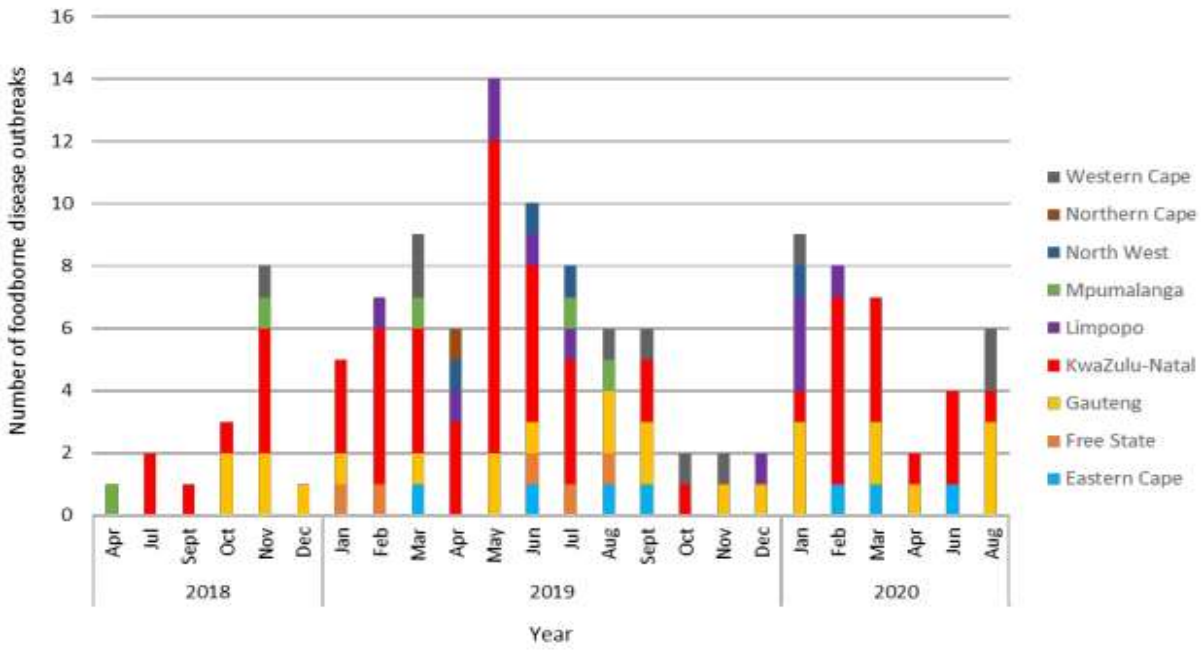

Figure 2.1. Number of foodborne disease outbreaks reported to the National Institute for Communicable Diseases (NICD) by month and province, South Africa, March 2018 – August 2020 (n=129).

More outbreaks were reported during the period April to December 2019 as compared to the same period in 2018 (56 vs 16 outbreaks respectively). Fewer outbreaks have been reported in 2020 to date (n = 36) compared to the same period in 2019 (n = 65). Information on the outbreak setting was available for 121 outbreaks (94%): 44% (54/121) occurred in households, 39% (47/121) in institutional settings (including schools, universities, day-care centres and correctional services facilities), and 7% (9/121) at large social gatherings (Adzitey *et al*., 2020).

According to Statistics South Africa (2011), 5% of deaths in 2010 were caused by food-borne diseases. Many school children have suffered food-borne disease in different provinces. In South Africa as reported by the media Cape, KwaZulu-Natal and Limpopo provinces are amongst the beneficiaries of the National School Nutrition Programme that feeds children at schools to alleviate hunger and improve the capacity to learn, but schools in these provinces had high food-borne outbreaks (Adzitey *et al*., 2020).

2.2 STAPHYLOCOCCUS SPECIES

2.2.1 Background

Staphylococcus taxonomy and morphology

S. aureus is a member of the Bacteria domain, Eubacteria kingdom, Phylum Firmicutes, Class Bacilli, Order Bailages, Family *Staphylococcaceae*, Genus *Staphylococcus*, *Species aureus* (Nandhini *et al*., 2022). *Staphylococcus* spp. is a non-motile, facultative aerobic, Gram-positive, spherical (coccus) bacteria found in grape-like (staphylo) clusters; When cultivated on blood agar plates, it produces big, spherical, golden-yellow colonies, typically with hemolysis, when seen under a microscope you don't need a microscope to observe hemolysis. *Staphylococcus* spp. uses binary fission to reproduce asexually. In *Staphylococcus* spp. autolysin is required for complete separation of the daughter cells; without it or targeted inhibition, the daughter cells remain attached and appear as clusters (Nandhini *et al*., 2022).

S. Xylosus, S. aureus, S. saphrophyticus are some of the *Staphylococcus spp*. That cause of gastroenteritis all over the world resulting from consuming foods which are contaminated. The contamination of food by *Staphylococcus spp*. mostly results from improper product handling. The consumption of food in which the bacterium grows to levels of usually greater than hundred thousand organisms per gram which are enough to accumulate toxin which results to illness (Buzby *et al*., 1996).

Staphylococci have an ability to survive under dry conditions and have been isolated from surfaces in a range of environments, such as the domestic environment, healthcare facilities and food preparation facilities. Although *Staphylococcus aureus* is one of the most frequent causes of foodborne disease it is not mostly mentioned among the most important food pathogens due to the limit of severity of the illness (Buzby *et al*., 1996).

2.2.2 Characteristics of *S. aureus*

S. aureus is primarily found on the skin and mucous membranes of warm-blooded vertebrates; however, it can be isolated from the environment. It can grow over a wide range of temperature 7 to 48.5 °C, with an optimum of 30-37 °C. When the normal balance of the skin flora is disturbed by frequent washing or antibiotic treatment, *S. aureus* may become established as a part of the resident flora (Langsrud, 2009).

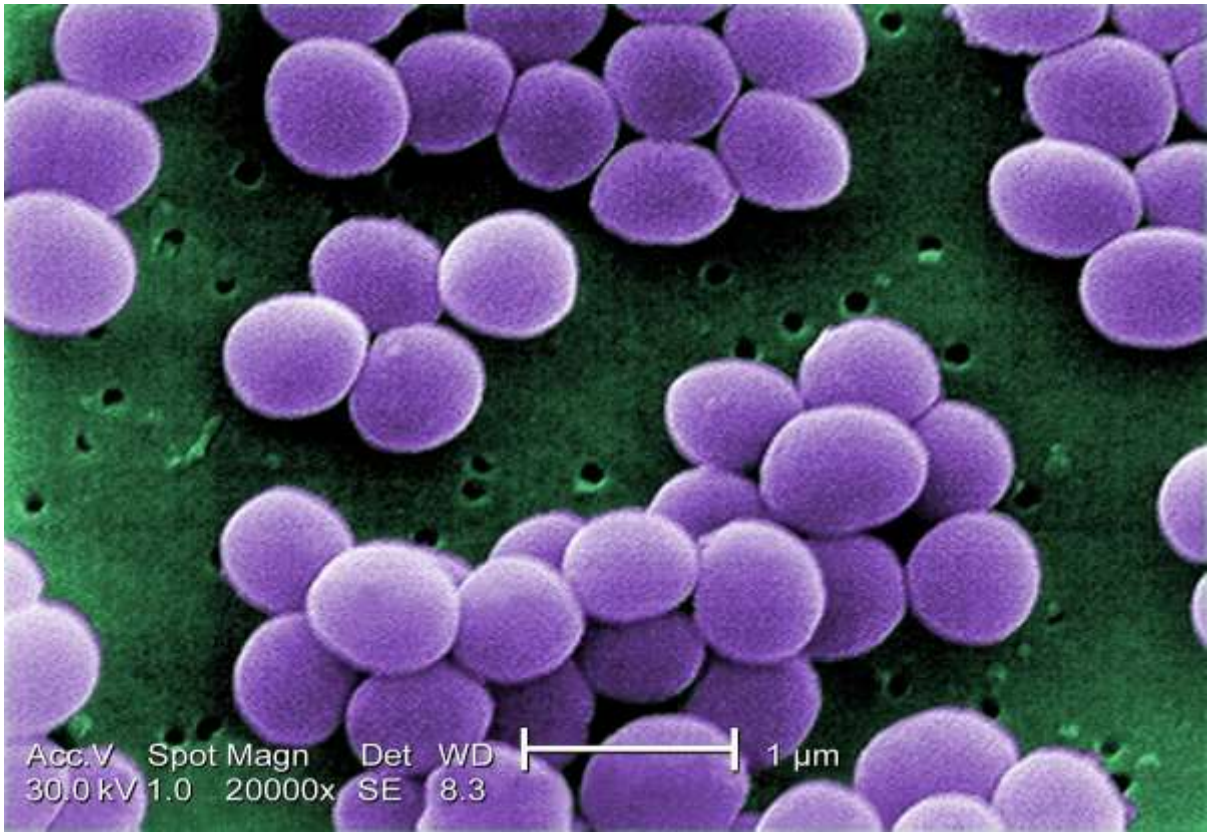

Figure 2.2. A picture showing the structure of *Staphylococcus aureus* (*staphylococcus aureus - Bing images*)

2.2.3 Epidemiology

Staphylococcal infections are found throughout the world. Nearly one-third of the adult population is asymptomatic carrier of staphylococci. Hospital infections caused by *S. aureus* are worldwide in distribution. Staphylococci are primary pathogens of humans and animals. They are present as commensals on skin, in the glands of the skin and on mucous membranes. The cocci are commonly found in the intertriginous skin folds, perineum, axillae, and vagina. Approximately, 35–50% of normal adults carry *S. aureus* in the anterior nares, 10% in the perineum, and 5–10% in the vagina (Simor *et al*., 2009).

Human cases and carriers are the important reservoir of infection. Human cases of cutaneous and respiratory infections shed large numbers of staphylococci into the environment for a prolonged period. Staphylococci colonize the skin very early in life (in neonates on the umbilical stump). Staphylococci shed by the patients and carriers contaminate handkerchiefs, bed linens, blankets, and other inanimate fomites and

persist in them for weeks. *S. aureus* found in the nose and sometimes on the skin, especially in hospital staff and patients is the main source of infection in hospitals. Domestic animals, such as cows, can also be reservoirs of staphylococcal infection (Ladhani *et al*., 1999).

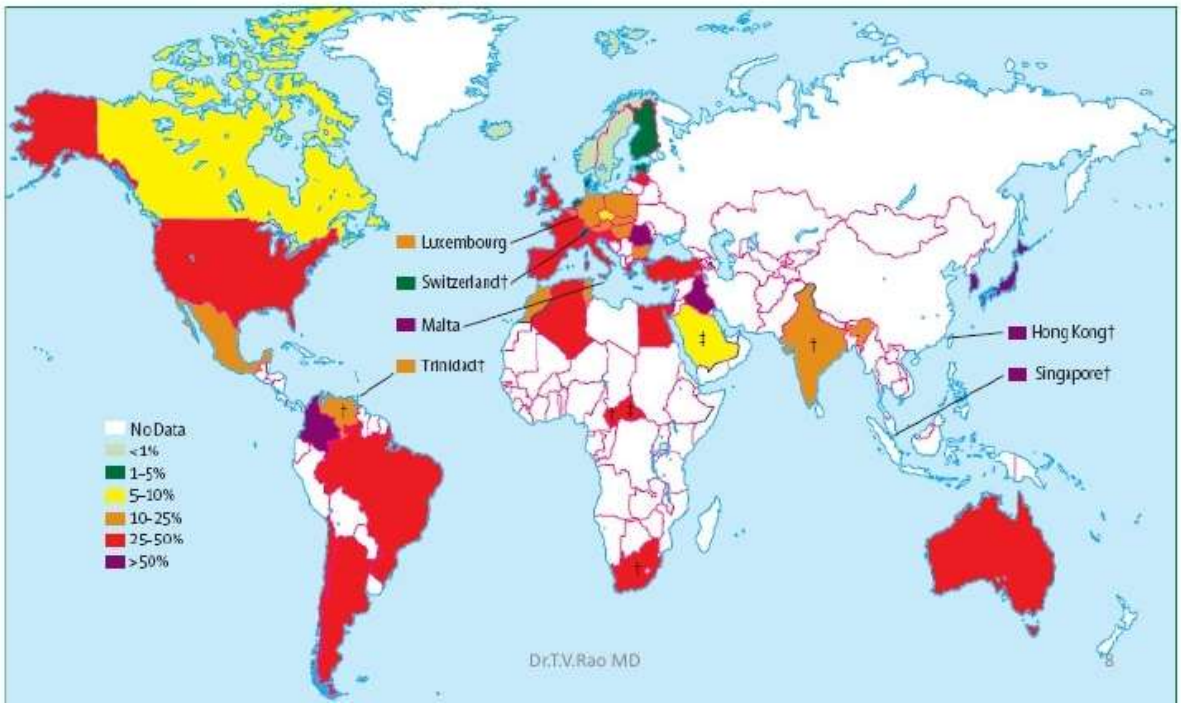

Figure 2.3. A map showing a worldwide prevalence of MRSA among *Staphylococcus aureus* isolates (vancomycin-resistant-staphylococcus-8-728.jpg (728×546) (slidesharecdn.com))

2.2.4 Food Contamination of *S. aureus*

S. aureus is likely to be a natural contaminant of raw foods of animal origin, although this can be minimised through good hygienic practices. The presence of *Staphylococcus* in well-cooked foods indicates cross-contamination from raw product or more contamination from a food handler. It is not easy to screen food handlers and exclude carriers of *S. aureus* from work unless in the case of those with obviously infected skin lesions (Adams *et al*., 2009).

2.2.5 Antimicrobial resistance and susceptibility

The issue of MRSA in food animals is an emerging concern. Studies have been published in which MRSA isolates obtained from meats were being compared with

genotype databases. Researchers have been able to determine that these strains in meat and meat products were in most cases of human origin and not zoonotic genotypes (Molla *et al*., 2012). This means that MRSA is present in the food chain but most likely due to human contamination. Attention needs to be focused on preventing the introduction of MRSA from human carriers onto the foods they handle which can result in spreading of the pathogen (Molla *et al*., 2012).

2.2.6 Pathogenesis

Staphylococcus aureus is mostly transmitted to the foods by the food handlers who have skin lesion infection especially in the meat cut and salad preparation. Infections occur due to *S. aureus* inoculation into an open wound. Initial exposure of *S. aureus* to host tissues beyond the mucosal surface or skin is thought to trigger upregulation of virulence genes (Doyle *et al*., 2012).

For the host, resident phagocytes, and epithelial cells in the skin or mucosal tissue respond to either bacterial products or tissue injury by activation of the immune system. *Staphylococcus aureus* peptidoglycan and lipoprotein are sensed by host pattern recognition molecules. Endogenous toll like receptor ligands released by necrotic tissues during infection further augment pro-inflammatory signalling leading to local immune cell activation and neutrophil and macrophage recruitment (Shiriang *et al*., 2017).

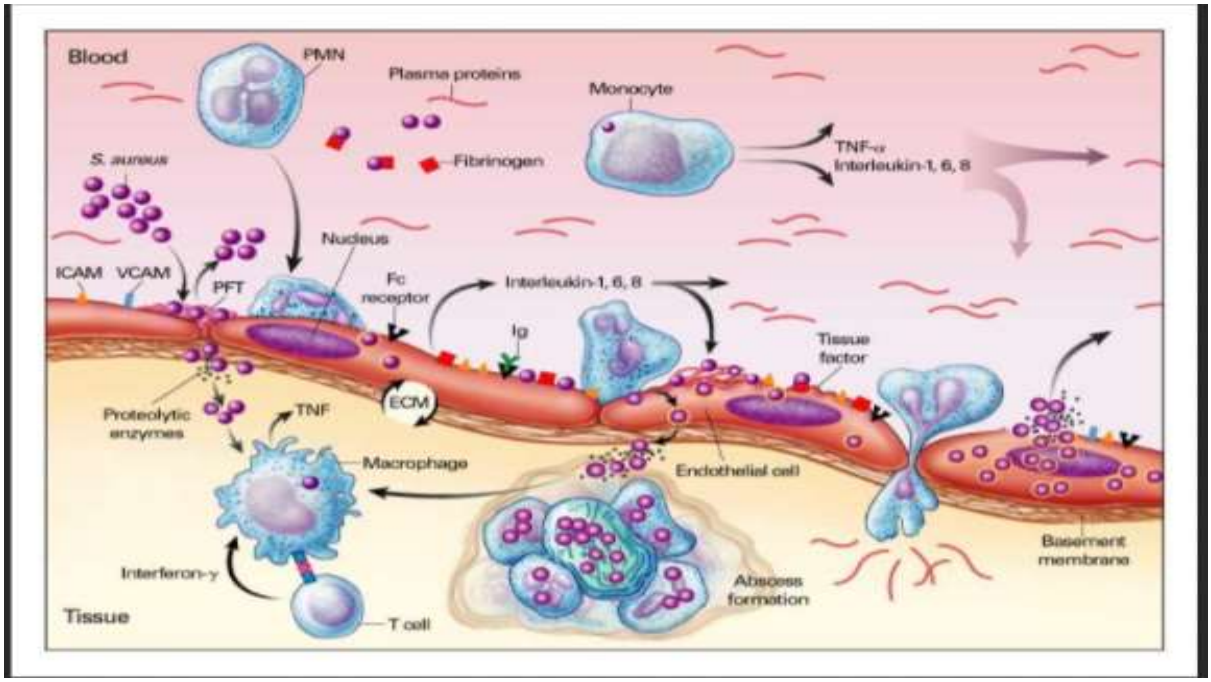

Figure 2.4. A picture showing the pathogenesis of *Staphylococcus aureaus* (*staphylococcus aureus* pathogenesis - Bing images).

2.2.7 Virulence factors

Staphylococcus aureus mostly survive well in both inside and outside of host cells. In the extracellular milieu, *S. aureus* must overcome opsonisation by complement and antibodies, which directly or indirectly leads to killing of *Staphylococcus aureus* or uptake by phagocytes through complement receptors. *Staphylococcus aureus* avoids opsonisation by expressing a capsule, clumping factor A, protein A, and complement inhibitors on its surface, all of which inactivate or prevent host opsonins from binding or targeting the bacterium for destruction (Lewis *et al*., 2018). There are numerous virulence factors that facilitate the pathogenicity of the organism. Virulence factors help bacteria to invade the host, cause disease and to evade the host defenses. They are different types of virulence factors such as, Adherence factors: Many pathogenic bacteria colonize mucosal sites by using pili to adhere to cells, Invasion factors: surface conmponent that allow the bacterium to invade hostcan be encoded on plasmids, Capsules: many bacteria are surrounded by capsules that protec them from opsonization and phygocytosis, Endotoxin: The lipopolysaccharide endotoxins on Gram-negetive bacteria cause fever, changes in blood pressure and many other toxic events, exotoxins: include several types of protein toxins and enzymes produced from

pathogenic bacteria,and Siderophores: are iron -binding factors that allows some bacteria to compete with the host for iron.

2.3 *ESCHERICHIA COLI* SPECIES

2.3.1 Background

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped and are about 2.0 micrometers (μm) long and 0.25-1.0 μm in diameter, with a cell volume of 0.6–0.7 μm. It can live on a wide variety of substrates. Strains that possess flagella are motile. The flagella have a peritrichous arrangement. Antigenic properties: There are more than 1000 antigenic types of *Escherichia coli*: O-Cell wall antigens (>150 types), H- Flagellar antigen (>50 types) and K- capsular antigen (>90 types) (Allam, 2018).

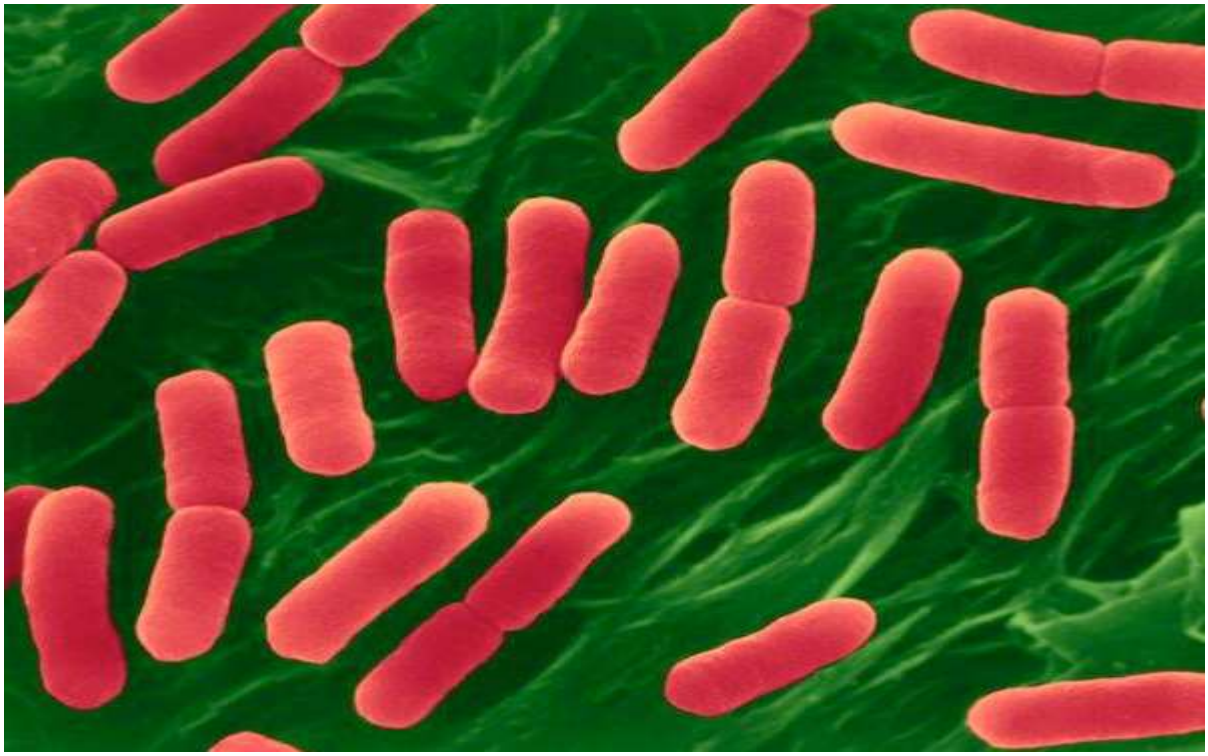

Figure 2.5. A picture showing the structure of *E. coli* (*E. coli O157 - Bing images*)

2.3.2 Biochemical characteristics of *E. coli*

E. coli ferments lactose, glucose, mannitol, maltose, and many other sugars with the production of acid and gas. They do not ferment sucrose. Some strains of *E. coli* are late lactose or nonlactose fermenters. They do not liquefy gelatin, do not produce hydrogen sulfide (H_2S), or do not utilize urea. Some variant strains of *E. coli* produce

H_2S. The indole, methyl red (MR), Voges–Proskauer (VP), and citrate utilization tests, generally referred to as the "IMViC" tests, are four important biochemical tests widely used in the classification of enterobacteria. *E. coli* is indole and MR positive (Allam, 2018).

2.3.3 Growth and survival characteristics of *E. coli*

E. coli is an aerobe and a facultative anaerobe. It grows at a temperature range of 10–40°C (optimum 37°C) and a pH of 7.2. The bacteria grow on a wide range of media including Mueller–Hinton agar, nutrient agar, blood agar, and MacConkey agar. Primary isolation can be made on nutrient agar and blood agar. Nutrient agar: *E. coli* on nutrient agar after 18 hours of incubation at 37°C produces large, circular, low convex, grayish white, moist, smooth, opaque, or partially translucent colonies (smooth or S forms). These smooth colonies are easily emulsifiable in saline. The rough or R forms produce rough colonies with an irregular dull surface (Sultana *et al*., 2019).

The smooth to rough variation (S–R variation) is associated with the loss of surface antigens and with the loss of virulence and occurs because of repeated subculturing. MacConkey medium: *E. coli* produces bright pink flat colonies due to lactose fermentation. Many strains, especially those isolated from pathologic conditions, produce beta-hemolytic colonies on blood agar. They do not grow on selective media, such as DCA (deoxycholate citrate agar) or SS (*Salmonella–Shigella*) agar, used for the culture of *salmonellae* and *shigellae.* Liquid broth culture: *E. coli* produces turbid growth with a deposit, which disperses completely on shaking (Allam, 2018).

2.3.4 Epidemiology

Large numbers of *E. coli* are present in the gastrointestinal tract. Although this organism can be opportunistic pathogen when the intestines are perforated, most *E. coli* that cause gastrointestinal and extraintestinal disease do so because they have acquired specific virulence factors encoded on plasmids or in bacteriophage DNA. The effectiveness of *E. coli* as a pathogen is illustrated by the fact the bacteria are the most common Gram-negative rods isolated from patients with sepsis. Most infections (except for neonatal meningitis and gastroenteritis) are endogenous; that is, the *E. coli* that are part of the patient's normal microbial flora can establish infection when the

patient's defences are compromised (e.g., through trauma or immune suppression) (Jenifer *et al*., 2020).

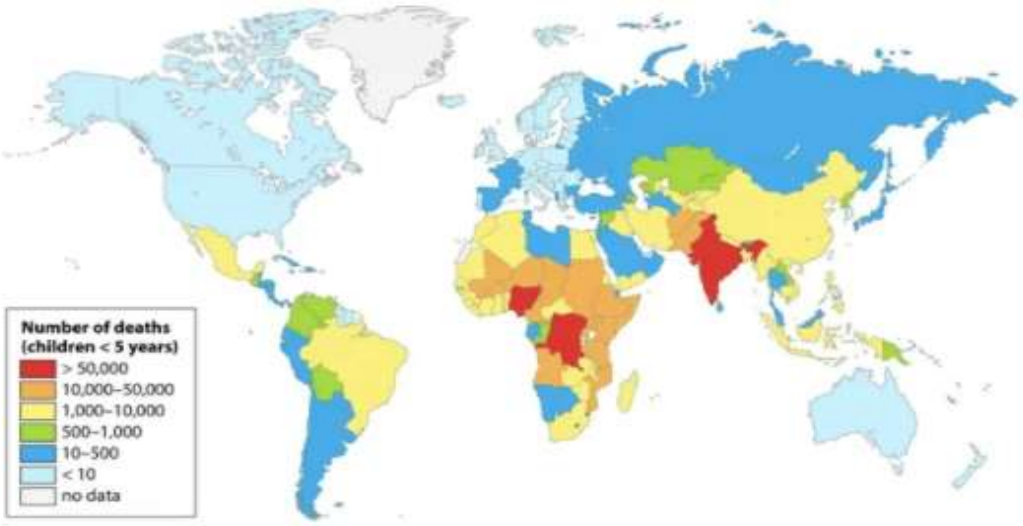

Figure 2.6. A map showing the major global outbreaks of *E. coli* by numbers from 1998 to 2007 (Global E. coli Outbreaks - Safe Food Alliance)

2.3.5 Pathogenesis and virulence factors

E. coli possesses a broad range of virulence factors. In addition to the general factors possessed by all members of the family *Enterobacteriaceae, Escherichia* strains possess specialized virulence factors that can be placed into two general categories: adhesins and exotoxins (Kaper *et al*., 1998).

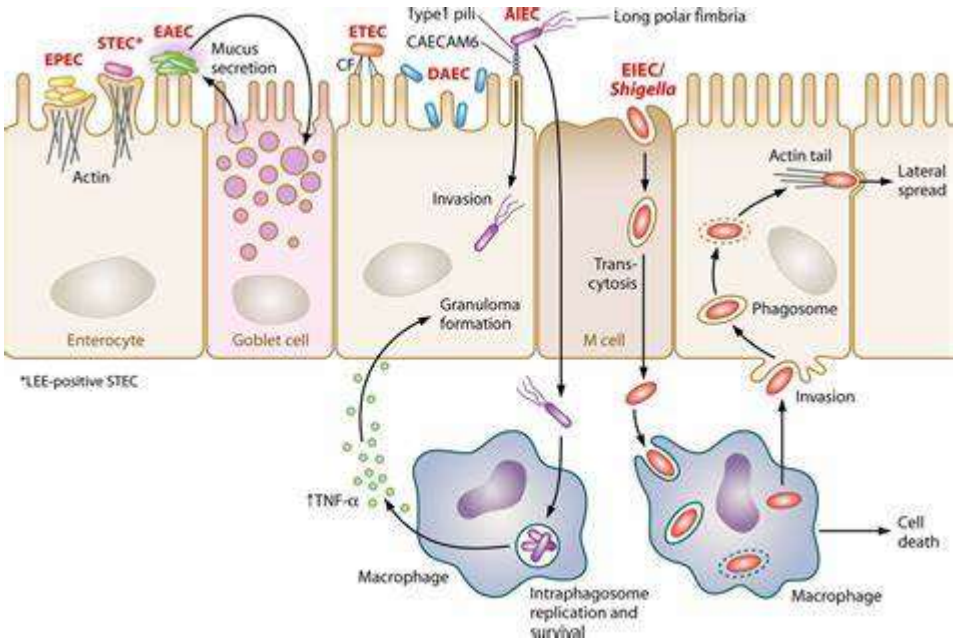

Figure 2.7. A picture showing the pathogenesis of *E. coli* (e. coli pathogenicity - Bing images)

2.3.6 Clinical manifestation

The incubation period is 1–2 days, with anorexia, vomiting and abdominal cramps in 25% of patients. The diarrhoea is explosive, voluminous, and watery, up to 10 times a day. The illness is self-limiting and usually lasts 1–5 days in well-nourished persons, but up to 3 weeks in malnourished children. Dehydration is the major complication as reported in a study in Bangladesh, where 46% of adults and 16% of children were affected (Darlison *et al*., 2019).

2.3.7 Treatment and antimicrobial resistance of *E. coli*

The mainstay of treatment is the assessment of dehydration and replacement of fluid and electrolytes. Administration of antibiotics has been shown to shorten the course of illness and duration of excretion of ETEC in adults in endemic areas and in traveller's diarrhoea. The antibiotic used depends upon susceptibility patterns in the particular geographical region (Hart *et al*., 2004).

Currently, the antibiotics of choice are currently fluoroquinolones or azithromycin, with an emerging role for rifaximin. Oral rifaximin, a semisynthetic rifamycin derivative is an effective and well-tolerated antibacterial for the management of adults with non-invasive traveller's diarrhoea. Rifaximin was significantly more effective and no less effective than ciprofloxacin in reducing the duration of diarrhoea. While rifaximin is effective in patients with *E. coli*-predominant traveller's diarrhoea, it appears ineffective in patients infected with inflammatory (Hart *et al*., 2004).

2.4 SALMONELLA

2.4.1 Background

Salmonella species are life-threatening bacteria that normally occur in developing and industrialized countries and are the leading cause of food-borne bacteria illnesses in humans (Sa nchez-Vargas *et al*.,2011). Infections caused by *Salmonella spp.* may lead to high morbidity rates in both industrial and developing countries as well as a high mortality rates in the poorest nations of the developing world (Sa nchez-Vargas *et al*.,2011).

Salmonella species are Gram-negative bacilli that are commonly associated with human and animal infections. *Salmonella* has been recognized as a causative agent

of intestinal diseases for many years and infections caused by this bacterium are difficult to treat because of its ability to tolerate environmental stress, ability to resist multiple drugs and adaptability as well as widespread distribution (Sa nchez-Vargas *et al*.,2011).

Salmonella comes second after *Campylobacter* as the most predominant bacterial cause of foodborne gastroenteritis worldwide (Mahmoud, 2012). *Salmonella* serotypes have the ability to grow in many different foods and the behaviour of *Salmonella* in foods is governed by different environmental and ecological factors (Mahmoud, 2012). *Salmonella* pathogens may be spread through faeces of wildlife and domestic animals, contaminated water and poor fertilization (as per usage of dungs as manure. (Sa nchez-Vargas *et al*.,2011).

2.4.2 Characteristics of *Salmonella*

According to Nchez *et al*. (2011), depending on the type of *Salmonella species* involved in the infection, the epidemiology varies amongst the species. *Salmonella typhimurium* and *Salmonella paratyphimurium* are the causative agents of enteric fever and lead to a severe disease that affects communities in undeserved nations. NTS (*Non-typhoid Salmonella*) infections are self-limiting and tend to affect communities worldwide (Sa nchez-Vargas *et al*.,2011).

2.4.3 Classification of *Salmonella*

The genus S*almonella* contains only two species namely *Salmonella enterica* and *Salmonella bongori.S. enterica* is further subdivided into six subspecies named as follows: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV)and *indica* (VI), while *S bongori* has no subspecies (CDC, 2006).

The genus *Salmonella* is composed of three species, *Salmonella enterica*, *Salmonella bongori* and *Salmonella subterranean* (Fookes *et al*., 2013). The species *Salmonella enterica* is then further classified into six subspecies which include; *Salmonella enterica subsp*. *Arizonae*; *Salmonella enterica subsp*. *Houtenae*; *Salmonella enterica subsp*. *Enterica*; *Salmonella enterica nsubsp*. *Houtenae*; *Salmonella enterica subsp*. *Indica* and *Salmonella enterica subsp* (Fookes *et al*., 2013).

Salmonella species belong to over 50 serogroups, and this is based on the O antigen; they have over 2500 serotypes each containing a unique combination of somatic O and flagellar H1 and H2 antigens that are pathogenic to both animals and humans

(Fookes *et al*., 2011). The predominant human *Salmonella* isolate is serotype *S. enterica subsp*. *enterica* is associated with a large percentage of enteric fever and gastroenteritis. *Serovars Typhi* and *Paratyphi* of the serotype are the causative agents of typhoid and paratyphoid fevers and this are generally found in the developing countries such as south America, Africa and parts of Asia (Fookes *et al*., 2013). Continuous genetic re-assortment in *Salmonella* has led to an increase in its virulence and its ability to resist multiple drugs and this is a significant public health concern (Fookes *et al*., 2013).

2.4.4 Pathogenesis of Salmonella infection

The pathogenesis of *Salmonella* in man and animals is affected by the degree of host adaptations that varies among different *Salmonella* serotypes (Langridge *et al*., 1998). Serotypes that are adapted to man include: *Salmonella* severe septic typhoid syndrome such as enteric fever in humans and are not pathogenic to animals (Fookes *et al*., 2011). The highly adapted serotypes to animal hosts include *Salmonella gallinarum* which commonly infects poultry and *Salmonella abortusovis* which infects sheep, these serotypes may only produce mild symptoms in humans.

The primary hosts of *Salmonella choleraesuis* is ~~a~~ swine however, they may also cause severe systemic illnesses in humans (Langridge *et al*., 1998). *Salmonella Dublin* also prefers cattle as their host and responsible for the systemic form of salmonellosis in humans. According to Neuert *et al* (2018), The studyreported that there are causative agents of gastrointenstinal infections namely: *Salmonella enteritidis* and *salmonella typhimurium*.They are also capable of causing the production of typhoid-like infections in mice and humans as well as asymptomatic intestinal colonization in chicken (Neuert *at al*., 2018).

The most remarkable characteristic in *Salmonella* pathogenesis is the invasion of non-phagocytic cells. *Salmonella* will penetrate into the intestinal epithelial cells by inducing their own uptake, in a complex and active process that morphologically resembles phagocytosis (Ohl *et al*., 2001). The virulence genes that are involved in invasion and required for intracellular survival are clustered in large chromosomal DNA regions designated *Salmonella* pathogenicity island (SPIs). SPI-1 and SPI-2 encode type III secretion systems, consisting of multi-protein complexes cell membranes, resulting in efficient translocation of bacterial effectors directly into the epithelial cell cytoplasm

(Ohl *et al*., 2001). The secreted effectors interact with eukaryotic proteins to activate signal transduction pathways and rearrange the actin cytoskeleton and lead to membrane ruffling and bacterial engulfment (Ohl *et al*., 2001).

2.4.5 Mode of transmission

Salmonella species are most commonly transmitted through ingestion of food that originates from an infected animal or contaminated by faeces of an infected animal or person (Humphrey *et al*., 2006). This includes egg products, meat, milk products, poultry and other food items that potentially use contaminated ingredients. The farm animals used to produce the mentioned ingredients may become infected by ~~aeting~~eating feed and (fertilizers do animals eat fertilizers??? recast) prepared from contaminated meat bones (Humphrey *et al*., 2006). The infection spread by bacterial multiplication during rearranging and slaughtering however it eventually leads to person-to-person fecal-oral transmission when a person comes into contact with contaminated faeces (Humphrey *et al*., 2006).

Humans are the only reservoirs of ~~s~~*Salmonella typhimurium* and ~~s~~*Salmonella paratyphimurium* and the route of transmission include ingestion of contaminated food and water with patients and carrier's faeces (Sánchez-Vargas et al., 2011). The mode of transmission of ~~s~~*Salmonellav paratyphimurium* is believed to be associated with consumption of street vendor's food (Sánchez-Vargas et al., 2011).

Non-Typhoidal-salmonella transmission to human occur by ingestion of animal products and non-animal products, they may occur through consumption of contaminated water or contact with animals. The rapid dissemination of pathogens to communities are caused by mass production and distribution of food products furthermore it is more difficult to control and prevent infections caused by *Non-Typhoidal-salmonella* organisms due to the antibiotic resistances (Humphrey *et al*., 2006.

Transmission may also occur if a person is in contact with pharmaceuticals of animal origin cite examples. The young children are at high risk of salmonellosis because of the hand- to- mouth and object-to-mouth object behaviour that is common among them. Sanchez-Vargas (2011) reported that transmission of *Salmonella* continues

especially in children worldwide and this happens regardless of significant advances in sanitation, providing of potable water and highly controlled food chain surveillance.

2.4.6 Diagnosis and cultivation of salmonella

Salmonella spp. may be isolated from bone marrow, blood, urine and stools. The main methods of diagnosing *Salmonella* include blood and stool cultures, serology as well as conventional microbiological identification (Sultana *et al*., 2016). Standard method of diagnosing enteric fever is the use of the bone marrow aspirate cultures because this type of culture has a high sensitivity (Sultana *et al*., 2016). Gilman *et al* (1975), stool and culture have low sensitivity due to the capability of the bacteria cell to shed and this requires multiple testing samples for *Salmonella* spp. to be evaluated. Urine culture also have low sensitivity and the positive culture in urine and stools may occur in chronic carriage and in an acute infection (Sánchez-Vargas et al., 2011).

Pulse-field gel electrophoresis (PFGE) and multiple loci sequencing typing (MLST) are the methods that are currently in use. PFGE has a reproducibility that is high between laboratories and is based on the separation of chromosomal DNA fragments after digesting by restriction enzymes (Farrari *et al*., 2017). MLST has an excellent data analytical capability as well as high discriminatory power and it is based on sequencing analyses of chromosomally located house-keeping genes (Farrari *et al*., 2017).

2.4.7 Prevention and control of *Salmonella* infection

Prevention of enteric infection include essential measures such as provision of safe food handling practices, sanitation measures and access to safe water as well as vaccination and public education. Since the primary goal of prevention is difficult to achieve in developing countries, chlorination is used in water that is suspected to be contaminated. Sa;nchez-Vargas *et al,* (2011) suggested that proper washing of hands after being in contact with animals and avoiding contact with animals known to be 90% colonized with *Salmonells spp.* is a measure used to limit NTS infections.

Proper cooking of food that is suspected to be contaminated with *Salmonella spp.* as well as elimination of contaminated food products could also help in prevention of infections by the organism. Sa;nchez-Vargas *et al* (2011) reported that irradiation of food products may deduce the load of viable organisms . Restriction of indiscriminate use of antibiotic in livestock is another measure to prevent enhanced number of

antibiotic resistant NTS strain (Sa' nchez-Vargas *et al*., 2011). Treatment and prevention strategy of *Salmonella* are highlighted in **Table 2.1.**

Table 2.1. Treatment and prevention of salmonella infections (Sa'nchez-Vargas et al., 2011)

Measures	Enteric fever	NTS infections
Safe water, sanitation, education	Recommended	recommended
Measures during outbreaks	Search for cases, Eliminate contaminated food, Chlorinate suspected water and immunize people at risk	Eliminate food handling errors, Eliminate contaminated food
Antibiotic treatment	Recommended	Recommended for systemic disease
Treatment of carriers	Recommended	Not recommended
Salmonella vaccine	In endemic areas, during outbreaks for travellers	Not available

2.5 SHIGELLA

2.5.1 Background

Shigella spp. are short, Gram-negative rods, about 0.51–3 m in size. They are nonmotile, nonsporing, and noncapsulated. *Shigella species* with exceptions of *S. flexneri*, serotype 6, and some strains of other serotypes possess fimbriae (Krzywy, 1972).

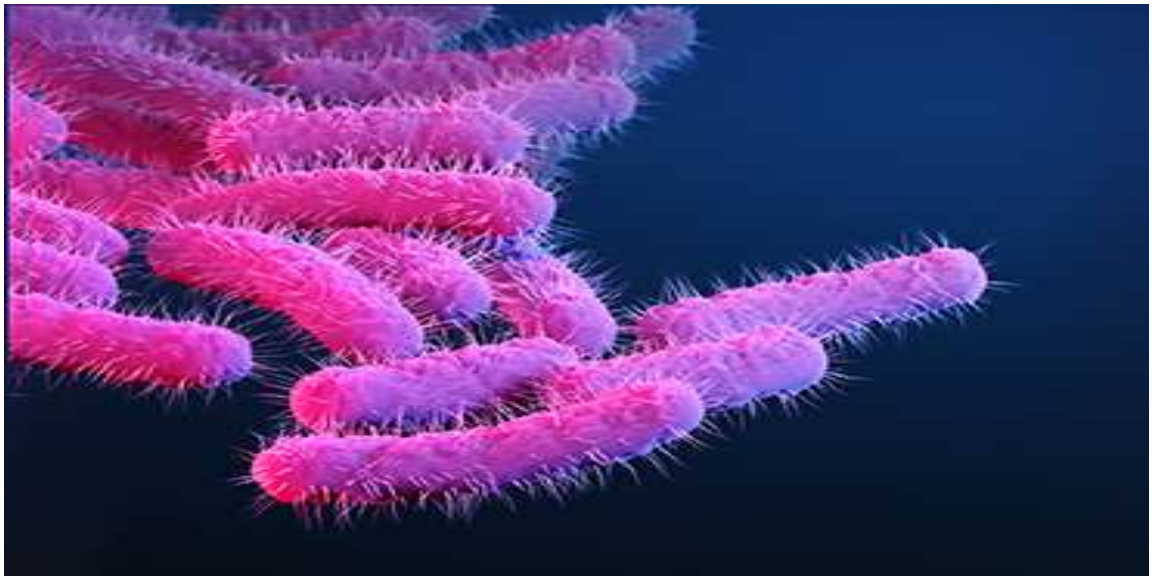

Figure 2.8. A picture showing the structure of *Shigella species* (*shigella* - Bing images).

Shigella spp. are aerobes and facultative anaerobes. They grow at a temperature range of 10–40°C with an optimum temperature of 37°C and pH 7.4. They grow on ordinary media, such as nutrient agar or Mueller–Hinton agar. *Shigella* colonies on nutrient agar, after overnight incubation, are small, circular, convex, smooth, and translucent. Occasionally on primary isolation and frequently in subcultures, a proportion of the colonies may be of the rough type. *Shigella spp.* on MacConkey agar produce nonlactose-fermenting pale, colorless colonies (Islam *et al*, .2011).

However, *S. sonnei* (which ferments lactose late) forms pale pink colonies on prolonged incubation. Deoxycholate citrate agar (DCA), xylose lysine deoxycholate (XLD) agar, *Salmonella–Shigella* (SS) agar, and Hektoen enteric (HE) agar are frequently used selective media for isolation of *Shigella species*. DCA is a useful selective medium for isolation of *Shigella* spp. from feces. On this medium, *Shigella spp.* produce small colonies, which on prolonged incubation produce lactose-fermenting pink colonies. XLD agar is another selective medium, which is less inhibitory to *S. dysenteriae* and *S. flexneri*. *Shigella spp.* forms red colonies on this medium. *Shigella spp.* on SS agar form colorless colonies. *Shigella spp.* on HE agar forms green colonies. Selenite F and Gram-negative (GN) broth are commonly used enrichment media. Enrichment of feces in GN broth for 4–6 hours followed by subculture on XLD or HE medium is useful for isolation of *Shigella* from clinical specimens (Islam *et al*, .2011).

2.5.2 Biochemical reactions

Shigella ferments mannitol, forming acid but no gas. Mannitol fermentation test is an important biochemical test, which is used to classify *shigellae* into mannitol-fermenting and -nonfermenting species. *S. flexneri*, *S. boydii*, and *S. sonnei* are mannitol-fermenting species, while *S. dysenteriae* is mannitol-nonfermenting species. However, exceptions are not that uncommon. *Shigella* also ferments glucose, producing acid but without gas. Newcastle and Manchester biotypes of *S. flexneri* type 6, and some strains of *S. boydii* types 13 and 14 are exceptions, which do not ferment glucose. They do not ferment lactose, sucrose, salicin, adonitol, or inositol. However, *S. sonnei* ferments lactose and sucrose late. They reduce nitrates to nitrites and do not form H_2S. They are MR positive, citrate ~~negative~~, and oxidase negative. They are catalase

positive with exception of *S. dysenteriae* type 1, which is catalase negative (Dodd *et al*, 1982).

2.5.3 Susceptibility to physical and chemical agents

Shigellae are killed at a temperature of 55°C in 1 hour or by 1% phenol in 30 minutes. In feces, they die within a few hours due to acidity produced by the growth of intestinal bacteria. They remain viable in moist environments for days but die rapidly on drying. *S. sonnei* is in general more resistant to unfavourable environmental conditions than the other *Shigella spp*. (Williams *et al*., 1964).

2.5.4 Virulence and pathogenesis

Virulence in *Shigella species* involves both chromosomal- and plasmid-coded genes, which express ~~for~~ many virulence factors. Endotoxins: The LPS moiety functions as an endotoxin and is an important component of the virulence of the bacteria. The endotoxin plays an important role in resistance of *Shigella* to nonspecific host defence encountered during tissue invasion (Burgos *et al*., 2012).

The toxin helps in invasion, multiplication, and resistance of *Shigella* to phagocytosis by tissue macrophages. The endotoxin increases the cytotoxic activity of Shiga toxin on human vascular endothelial cells. The endotoxin is expressed by chromosomal genes of the bacteria. Intestinal adherence factor: Intestinal adherence factor is a 97-kDa outer membrane protein encoded by each gene on chromosomes. This mediates colonization of *Shigella spp.* in infected human hosts and in animal models. Shiga toxin: Shiga toxin is an exotoxin produced by *S. dysenteriae.* It is a heat-labile protein and acts as enterotoxin and neurotoxin. Shiga toxin (*Stx*) is a group of cytotoxins that contain two majors immunologically non–cross-reactive groups called Stx1 and Stx2. Both *Stx1* and *Stx2* groups are encoded by a bacteriophage inserted into the chromosome of the bacteria (Burgos *et al*., 2012).

Shiga toxins have one A subunit and five B subunits: The main function of B subunit is to bind toxins to host cell glycolipid (Gb3) surface receptor, present on the brush border of epithelial cell of the intestines. It also mediates transfer of the A subunit into the cell. Subunit A is a 32-kDa polypeptide. It cleaves the 28S rRNA in the 60S ribosomal subunit, thereby preventing the binding of aminoacyl-transfer RNA and disrupting protein synthesis. The Shiga toxin shows three types of toxic activities:

Neurotoxic activity: This activity is demonstrable by paralysis and death of experimental animal following injection with the toxin (Burgos *et al*., 2012).

Although called neurotoxin, the primary site of its action is not the neural tissue but is the blood vessels, neurological manifestations being secondary. Enterotoxic activity: These toxins are enterotoxic for ligated rabbit intestinal segments with induction of fluid accumulation in ligated rabbit ileal loop. Two new *Shigella* enterotoxins, designated as S. ET-I and S. ET-2—the former confined to *S. flexneri* 2a and the latter more widespread— have been identified. Cytotoxic activity: This is demonstrated by cytotoxicity of toxin for vero, HeLa, and some selected endothelial cells, such as human renal vascular endothelial cells. This appears to be the same as vero toxin 1 (or Shiga-like toxin) produced by certain strains of *Escherichia coli* (VTEe). The primary manifestation of Shiga toxin is damage to the intestinal epithelium of the infected host, causing diarrhea and dysentery. However, in a small number of patients, Shiga toxin can mediate damage to the glomerular endothelial cells, resulting in hemolytic urinary syndrome (Burgos *et al*., 2012).

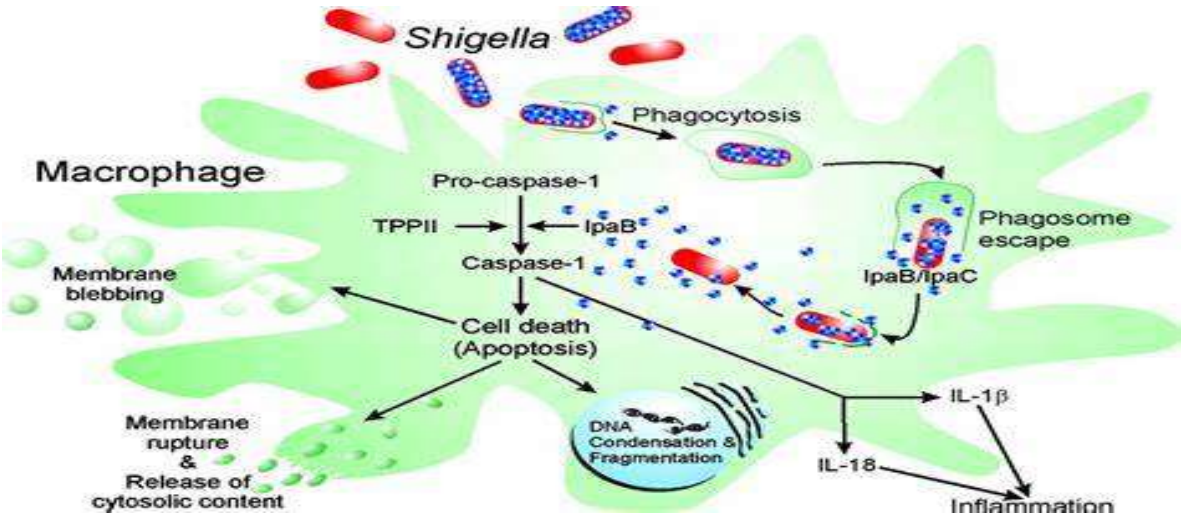

Figure 2.9. A picture showing the general pathogenesis of *Shigella species* (pathogenesis of *shigella* - Bing images).

2.5.5 Epidemiology

Shigellosis occurs worldwide. Estimated 150 million cases occur annually worldwide. The incidence of shigellosis in developing countries is nearly 20 times more than in developed countries. It is estimated that 30% of these infections are caused by *S.*

dysenteriae. S. flexneri is the most common cause of shigellosis in developing countries. *S. sonnei* is the most common cause in the industrial world. Shigellosis is worldwide in distribution but shows a lot of variations epidemiologically between the nature and extent of the infection in the industrially advanced and in the developing and poor countries (Kotloff et al., 1999).

Endemic shigellosis is found in all age groups and is caused by all species in developing countries where environmental sanitation is poor. In India, *S. flexneri* is the major species and constitutes 50–85% of all *Shigella* isolates followed by *S. dysenteriae* (8–25%), *S. sonnei* (2–24%), and *S. boydii* (0–8%). *Shigella species* are strict human pathogens. They are found in the large intestine of infected human hosts. They are not found in any other animal hosts. Reservoir, source, and transmission of infection Infected patients or, less often, carriers are reservoirs of infections for shigellosis. Chronic carriage is rare, because the bacilli are not excreted in feces within a few weeks, except in some malnourished children or in patients with AIDS (Kotloff et al., 1999).

Shigellosis is transmitted by: Fecal–oral route by hand-to-mouth infection through contaminated fingers. Because as few as 10–200 bacilli can cause disease, shigellosis spreads rapidly in areas where sanitary standards and the level of personal hygiene are low. Contaminated food and water: Food and water contaminated with human feces containing *Shigella spp*. is the main source of infection. Fomites such as door handles, water taps, lavatory seats. Flies, which may transmit the infection as mechanical vectors. Sexually among young male homosexuals due to oro-anal contact. Shigellosis is primarily a disease of children (Kotloff et al., 1999).

Nearly, 70% of all infections occur in children younger than 15 years. Patients at highest risk for disease are malnourished children, young children in daycare centers, nurseries, and custodial institutions, siblings, and parents of these children. *Shigella* infection in malnourished children often causes a vicious cycle of further impaired nutrition, recurrent infection, and further growth retardation. Endemic disease in adults is common in household contacts of infected children and in male homosexuals (Kotloff et al., 1999).

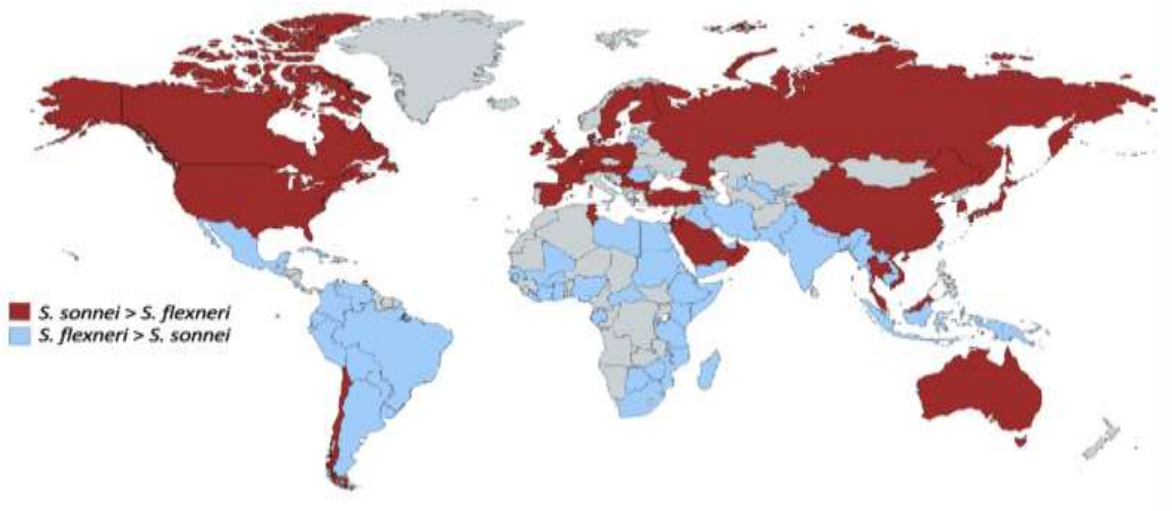

Figure 2.10. A map showing the cases of *S. sonnei* versus Cases of *Shigella flexneri.* Worldwide (Epidemiology of Shigella sonnei. (A) Cases of *S*. sonnei versus *Shigella*... | Download Scientific Diagram (researchgate.net))

2.5.6 Laboratory Diagnosis

Specimens, stool is the specimen of choice. Diagnosis of shigellosis is made by isolating *Shigella spp.* from feces. Fresh feces are inoculated without delay or transported in a suitable medium, such as Sachs' buffered glycerol saline, pH 7.0–7.4. Also, rectal swabs may be taken from the site of ulcer by sigmoidoscopy. However, rectal swabs that do not contain copious volume of stool or mucus are not satisfactory.

Routine microscopy of stool may reveal clumps of polymorphonuclear leukocytes. Fecal blood or leukocytes are detectable in the stool in approximately 70% of cases of shigellosis. A sample for stool is obtained in all suspected cases of shigellosis for culture. Usually, more than one stool or rectal swab is collected and inoculated immediately on at least two different culture media, such as MacConkey, XLD, DCA, or eosin-methylene blue agars.

For enrichment, one tube each of selenite F and GN broth are inoculated and incubated at 37°C for 12–18 hours before subculture onto selective media. After overnight incubation, *Shigella* produces pale nonlactose fermenting colonies on MacConkey and DCA media and red colonies on XLD medium and colorless colonies on SS agar. Identification of bacteria:- Pale non–lactose-fermenting colonies on MacConkey agar are identified by carrying out motility test, biochemical tests, and

slide agglutination test with specific *Shigella antisera* (polyvalent and monovalent sera). Serological tests are not useful in the diagnosis of *shigellosis* (Hunt, 1989).

2.5.7 Treatment

Uncomplicated *shigellosis* is a self-limited condition and patients usually recover spontaneously in a few days. Hence, no antibiotics are recommended for these cases. The dehydration observed in acute cases, particularly in infants and young children, needs adequate replacement of fluids and electrolytes by oral fluid and salts. Antibiotic treatment for *Shigella* infection is recommended (i) for severe or toxic cases and (ii) for the very young, debilitated and the aged individuals. Antibiotic treatment is recommended to decrease the duration of illness, person-to-person spread, and cases in household contacts. In developing countries, treatment in malnourished children is likely to reduce the risk of worsening malnutrition morbidity following shigellosis. Prophylactic antibiotics are not recommended for contacts (Ciccarelli *et al*., 2013).

Trimethoprim–sulfamethoxazole, ampicillin, tetracycline, and the quinolones, such as nalidixic acid and ciprofloxacin are frequently used antibiotics. Trimethoprim–sulfamethoxazole is very effective for shigellosis. The antibiotics act against *Shigella* by producing a sequential blockade in folic acid synthesis. This is the drug of choice when antibiotics susceptibility of the bacteria is not known. However, ampicillin is still the drug of choice if *Shigella* isolate is susceptible to this drug. Antibiotics-resistant *Shigella*: Multiple-antibiotics-resistant plasmids are widely prevalent in *shigella* and were first documented in Japan during early 1950s. Most of these strains were resistant to streptomycin, chloramphenicol, and sulfonamides. Indiscriminate use of the antibiotics has worsened the problem. Hence, it is essential to treat the cases of shigellosis with the results of in vitro antibiotic susceptibility testing of *Shigella* (Ciccarelli *et al*., 2013).

2.5.8 Prevention and Control

In developed countries, person-to-person transmission is the most common source of infection. Water contaminated with human excreta is the most common source of infection. Therefore, control consists essentially in improving personal and environmental sanitation. Antibiotics are not used in prophylaxis, and they are not effective (Ahmed *et al*., 1993).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

Thohoyandou is a town located within Vhembe District in the province of Limpopo South Africa. It is a commercial, legislative and administrative centre for the surrounding villages in the District municipality. Vhembe District municipality was selected because no similar study has been conducted to assess the microbial quality and safety of street vended ready-to-eat meat. It is also a home to the University of Venda which is in close proximity (about 2km) to a shopping complex with established retailers. Thohoyandou is known as the capital of Venda homeland. People from different villages in Thulamela municipality use this area for shopping. Street vended foods are sold as alternative for lunch at an affordable price.

Figure 3.1. The map of Vhembe District municipality (https://www.google.com/maps/@-22.932497,28.2262351,8z)

3.2 SAMPLE COLLECTION AND PREPARATION

3.2.1 Sample collection

A total of 168 samples of street vended meats consisting of chicken (n=84) and beef (n=84), were collected from the local street vendors around Thohoyandou area in the Vhembe district. Samples were selected using simple random sampling method. Purchased meat samples were transferred from vendor packaging into sterile lunch boxes at the point of purchase. The packed samples were placed in a cooler box and immediately transported to the Microbiology laboratory, University of Venda for further analysis. Microbiological analysis of all samples was carried out within 4h of purchase as recommended by (Gomes *et al*., 2003).

3.2.2 Preparation of samples according to ISO 6887/2/2003

Ten grams (10g) of chicken and beef samples were transferred into separate zip lock bags containing 90 ml of peptone buffered water (Oxoid) to provide a dilution of 10^{-1}. The content was then macerated to aid the recovery of microorganism. The macerated solution was allowed to stand for 5 minutes at room temperature and then serial dilutions (10^{-4}) were prepared using buffered peptone water.

3.3 ISOLATION AND IDENTIFICATION OF *STAPHYLOCOCCUS SPP*, *E. COLI*, *SALMONELLA* AND *SHIGELLA*

Selective media were used for the cultivation of specific bacteria.

3.3.1 Staphylococcus spp.

A spreading stick was used to culture 0.5ml from both (10^{-1} to 10^{-4} dilutions) Mannitol salt agar (MSA) (Oxoid Ltd) agar for *Staphylococcus spp.* To ensure that uniform distributions of microbes were present, the tubes were shaken before plating. The plates were then labelled as per dilution and incubated at 37^{o}C for 24 hours. The presumptive colonies of *Staphylococcus spp*. appeared as yellow colonies on MSA agar. All presumptive colonies were then counted and expressed as colony forming units per ml of the sample.

3.3.2 E. coli spp.

A spreading stick was used to culture 0.5ml from both (10^{-1} to 10^{-4} dilutions) on Eosin Methylene Blue (EMB) agar (Davies Diagnostics Pty Limited) for *E.coli*. To ensure that uniform distributions of microbes were present, the tubes were shaken before plating. The plates were then labelled as per dilution and incubated at 37^{o}C for 24 hours. The presumptive colonies of *E. coli* appear as metallic green sheen on EMB. All presumptive colonies were then counted and expressed as colony forming units per ml of the sample.

3.3.3 Salmonella spp.

3.3.3.1 Primary and secondary enrichment

Primary and secondary enrichment of *Salmonella* was done according to a method described by Andrews et al. (1995). Briefly, zip-lock bags (after maceration) were placed into beakers and incubated at 37^{o}C for 24 hours. Secondary enrichment was then achieved by inoculation of 10 µL from the primary enriched solution into ten millilitres (10ml) of RVS (Rappaport-Vassiliadis Soya Peptone) broth (Oxoid) and incubated at 42 ^{o}C for 48 hours.

3.3.3.2 Culturing of *Salmonella*

The samples were then plated on XLD and MacConkey agar (Davis Diagnostics Pty Limited) and incubated at 37 ^{o}C for 24 hours. The presumptive colonies of *Salmonella* should appear as colorless. All presumptive colonies were then counted and expressed as colony forming units per ml of the samples.

3.3.4 Shigella

A spreading stick was used to culture 0.5ml from both (10^{-1} to 10^{-4} dilutions) on MacConkey agar (Davies Diagnostics Pty Limited) for *Shigella*. To ensure that uniform distributions of microbes were present, the tubes were shaken before plating. The plates were then labelled as per dilution and incubated at 37^{o}C for 24 hours. The presumptive colonies of *Shigella* appear as colorless on MacConkey. All presumptive colonies were then counted and expressed as colony forming units per ml of the sample.

3.4 IDENTIFICATION

After sub-culturing, the isolates were identified and confirmed using Gram staining method and biochemical testing such as Catalase test, VITEK 2, and API 20E.

3.4.1 Gram staining method

Gram staining was done according to the protocol depicted by Beveridge (2001) to categorize isolates into Gram positive or Gram negative found on their cell wall composition. To prepare a heat fixed smear, a drop of water was placed on a slide and mixed with an isolated colony from Nutrient agar plate using a sterile inoculation loop. The emulsion-containing slide was passed through the flame of a Bunsen burner three times. A drop of Crystal violet was added to the heat fixed smear and allowed to stand for 1 minute followed by rinsing with tap water. A drop of Gram's iodine was then be added and allowed to stand for 1 minute followed by rinsing with tap water.

Decolourization was done using few drops of 95% Ethyl alcohol and allowed to stand for 5-10 seconds before rinsing with tap water. A drop of Sefranin were then added and the slides were allowed to stand for 45 seconds before rinsing with tap water. The slides were gently dried with paper towel. The slides were then viewed under a light microscope. Gram negative bacteria stained red or pink and Gram-positive bacteria stained blue or purple. The shape of the bacteria was also observed under the light microscope.

3.5 BIOCHEMICAL TEST

3.5.1 Catalase test

The test was carried out by flooding an agar culture with several drops of 3% hydrogen peroxide as described by Global Khan, 2003. Catalase mediates the breaking down of hydrogen peroxide into oxygen and water. Inoculum of bacterial isolates in a small amount was mixed into hydrogen peroxide solution (3%) and observed for the quick elaboration of oxygen bubbles. Lacking catalase is evident by a lack of weak bubble production. Catalase positive bacteria have the capacity to breathe utilizing oxygen as a terminal electron acceptor. Catalase negative bacteria can be anaerobes that exclusively ferment and do not breathe using oxygen as a terminal electron acceptor. Briefly, an enzyme produced by microorganism living in oxygenated environments to neutralize toxic forms of oxygen metabolites is referred to as Catalase.

3.5.2 Kligler Iron Agar Test

Kligler Iron Agar test was performed according to the protocol described by Khalil. (2017), to differentiate members of Enterobacteriaceae by their capacity to ferment dextrose, lactose and to produce hydrogen sulfide and gas.

3.5.3 Biochemical test API 20E

All the presumptive colonies of *E.coli* were subjected to API 20E biochemical test and this was performed as explained according to the API 20E manual pamphlet (protocol) of the Identification System of *Enterobacteriaceae* and other non-fastidious Gram-negative rods.

The biochemical test API 20E was used to confirm the presumptive colonies of *E. coli.* Pure cultures were obtained by sub-culturing the presumptive colonies onto Nutrient agar then the plates were incubated at 37^{o}C for 24 hours. A single colony was emulsified in 5ml of distilled water and turbidity was compared with the 0.5M McFarland concentration. The solution was then poured into API strips as explained in the API 20E manual. For the CIT, VP and GEL tests, both the tube and cupule were filled with inoculum. For the other tests, only the tube was filled. An anaerobic environment was then created by overlaying (ADH, LDC, ODC, URE and H2S tests) with paraffin oil. The biochemical test was then done after adding a drop of API20E reagents (TDA in the TDA test, VP 1 and VP 2 in the VP test and James solution in the IND test) as explained according to the API20E manual. The results were recorded based on the colour changes as it is described by the manufacturer (Swiss based company **Lonza** Ltd).

3.5.4 VITEK assessment of Staphylococcus spp.

An automated bacterial identification system (VITEK 2; Biomerieux) was used at the Water and Health Research Unit (University of Johannesburg) for the detection of *Staphylococcus spp*. It is an advanced colorimetric system that uses ID cards. The cards were inoculated with standardized bacterial suspension from pure culture, incubated in the VITEK 2 and read with internal optics.

3.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING OF STAPHYLOCOCCUS SPP, E. COLI, SALMONELLA AND SHIGELLA

Antimicrobial susceptibility testing was done according to a Kirby Bauer Disc Diffusion method described by (Bauer et al.,1966).

Mueller-Hinton agar (MHA) (CSD) was poured 4mm in a petri-dish (plate). Several colonies of similar appearances were emulsified in 5ml of distilled water. The turbidity of the subculture was then matched against the turbidity standard (McFarland) with concentration of 0.5M. A sterile dry cotton swab was then used to apply the inoculum evenly across the Mueller Hinton plates. The plates were allowed to dry for a few minutes with the petri-dish lid in place. A sterile forcep was then used to place the antimicrobial discs into the inoculated plates. After the discs were applied onto the plates for 30 minutes, the plates were then incubated aerobically at 35-37°C fro 24 hours.

The presumptive colonies of *E.coli* and *Staphylococcus spp*. isolates were tested for susceptibilityto 9 antimicrobial agents, namely: kanamycin (K 30 μg), chloramphenicol (C 30 μg), amoxicillin (AMC 30 μg). Streptomycin (S 10 μg), tetracycline (TE 30 μg), penicillin (PG 10 μg) and ampicillin (AMP 10 μg).

Table 3.1. Antimicrobial susceptibility testing table of the antimicrobial agent and the different potencies that are used for the treatment of infections caused by *Enterobacteriaceae spp*. as well as the zones of inhibition in milimetres (mm).

Antimicrobial agent	Disc code	Potency	Resistance (in mm)	Intermediate (in mm)	Susceptible (in mm)
Amoxicillin	AMC	30 μg	≤ 13	14-17	≥ 15
Ampicillin	AMP	10 μg	≤13-	14-16	≥17
chloramphenicol	C	30 μg	≤12	13-17	≥18
kanamycin	K	30 μg	≤ 13	14-17	≥18
Streptomycin	S	10 μg	≤ 11	12-14	≥15
Tetracycline	TE	30 μg	≤11	12-14	≥15
Neomycin	N	30 μg	≤12	12-14	≥17

Adapted in part from CLSI document M100 523 (M02 A11).” Disc supplemental tables.” Performance standards for antimicrobial susceptibility testing.

Table 3.2. A table of antibiotics with concentration used to determine the antibiotic susceptibility profile of *Staphylococcus spp*. using Kirby Bauer Disc Diffusion method following the guideline of the NCLSI (2013).

Antimicrobial agent	Disc Code	Antibiotic Class	potency	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
Cefoxitin	FOX	Cephalosporins	30 µg	≤21	-	≥22
Tetracycline	TE	Tetracycline	30 µg	≤14	15-18	≥19
Trimethoprim-sulfamethoxazole	TS	Sulfonamides	25 µg	≤10	11-15	≥16
Vancomycin	VA	Glycopeptide	30 µg	≤14	15-16	≥17
Ampicillin	AMP	B-lactams	10 µg	≤19	-	≥20
Oxacillin	OX		1 µg	≤10	11-12	≥13
Chloramphenicol	C	Amphenicols	30 µg	≤12	13-17	≥18

*Adapted in part from CLSI document M100 523 (M02 A11). "Disc supplemental tables." Performance standards for antimicrobial susceptibility testing.

The antibiotic susceptibility of *E. coli* and *Staphylococcus spp*. was then achieved by measuring the zones of inhibition and this was based on the zone diameters formed against each individual antibiotic agent. The results were then interpreted according to the guidelines (represented in Table 3.2) of the National Committee for Clinical Laboratory Standards for anti-microbial susceptibility testing (Rubin, 2013).

3.7 MOLECULAR CHARACTERISATION USING MULTIPLEX PCR

Multiplex PCR was done as described by Omar *et al*. (2014) to determine different strains and pathotypes of *E. coli*, using different primers. To do multiplex PCR, DNA extraction was done using Qiagen Kit according to the protocol described in the manufacturer's instructions.

3.7.1 DNA extraction

DNA extraction was done as described by Omar *et al* (2014). The enriched single colony in 2ml Eppendorf was centrifuged for 2 min at 1300 rpm that was done to pellet the cells of bacteria from which the DNA was extracted, the supernatant was discarded. The amount of 700 µL of the lysis buffer was added to the Eppendorf tube which have pellet then mixtures was incubated at 70^{o}C for ten minutes. The amount of 250 µL of 100 % ethanol was added to the lysis buffer to enhance the binding of

DNA to the celite after that incubation was done for 10 minutes at 56ºC. The amount of 50 µL of celite was then added to the mixture then put in the vortex and incubated for 10 minutes at room temperature. The celite that bounded the DNA was washed twice with 400 µL of the wash buffer. The 70% ethanol was added two times following wash buffer and spin columns were then dried by centrifugation at 13000 rpm for 2 minutes. The DNA was then eluted by DNA was eluted with 50 µL Qiagen elution buffer and transferred in clean Eppendorf tubes. The DNA was finally stored at the temperature of 20ºC.

3.7.2 Gene amplification by Multiplex PCR

Multiplex polymerase reactions were performed in a Biorad MycyclerTM Thermal cycler in a volume of 20 µL. The multiplex PCR kit Qiagen® were used for the provision of the multiplex PCR protocol which were used. The reactions consist of 1X Qiagen® PCR mix which comprise of HotstartTaq® DNA polymerase, m-PCR buffer, and dNTP mix; 10 x primer mix, 4 µL of sample DNA and 4 µL of grade water. The subjection of the reactions to an initial step were done at 95ºC for 15 minutes, followed by 35 cycles which consist of denaturing steps for 45 seconds, annealing step at 57ºC for 45 seconds, the extension step was done at 68 ºC for 2 minutes and final elongation was done at 72ºC for 10 minutes.

Table 3.3. Primers sequences used in m-PCR reactions

Pathogen	Primer	Sequence (5'-3')	Size (bp)	Conce.(µm)	Reference
E. coli	*mdh* (F) *mdh*	*GGT ATG GAT GCT TCC GAC CT*	304	0.1	Tarr *et al.* (2002)
	(R)	*GGC AGA ATG GTA ACA CCA GAG T*			
EIEC	*ial* (F)	*CTG AAC GGC GAT TAC GCG AA*	630	0.2	Lopez-Sasucedo *et al* . (2003)
	ial (R)	*CCA GAC GAT ACG ATC CAG*			
EHEC/Atypical EPEC	*eae*A (F)	*CTG AAC GGC GAT TAC GCG AA*	917	0.3	Aranda et al(2004)

	*eae*A (R)	*CCA GAC GAT ACG ATC CAG*			
Typical EPEC	*bfp*A (F)	*AAT GGT GCT TGC GCT TGC TGC*	410	0.3	Aranda *et al*. (2004)
	bfpM (R)	*TAT TAA CAC CGT AGC CTT TCG CTG AAG TAC CT*			
EAEC	*eagg* (F)	*AGA CTC TGG CGA AAG ACT GTA TC*	194	0.2	Pass *et al*. (2000)
	eagg (R)	*ATG GCT GTC TGT AAT AGA TGA GAA C*			
EHEC	*stx*1 (F)	*ACA CTG GAT GAT CTC AGT GG*	614	0.5	Moses *et al*. (2006)
	*stx*1 (R)	*CTG AAT CCC CCT CCA TTA TG*	779	0.3	Moses *et al*. (2006)
	*stx*2 (F)	*CCA TGA CAA CGG ACA GCA GTT*			
	*stx*2 (R)	*CCT GTC AAC TGA GCA CTT TG*			
ETEC	*Lt-1* (F)	*GGC GAC AGA TTA TAC CGT GC*	360	0.1	Pass *et al*. (2000)
	Lt-1 (R)	*CGG TCT CTA TAT TCC CTG TT*	160	0.5	Pass et al (2000)
	St-a(F)	*TTT CCC CTC TTT TAG TCA GTC AAC TG*			
	St-a (R)	*GGC AGG ATT ACA ACA AAG TTC ACA*			
E. coli toxin	*ast*A (F)	*GCC ATC AAC ACA GTA TAT CC*	106	0.3	Kimata *et al.* (2005)

	*ast*A (R)	*GAG TGA CGG CTT TGT AGT CC*			
External control	*gapdh* (F)	*GAG TCA ACG GAT TTG GTC GT*	238	0.3	Mbene *et al*. (2009)
	gapdh (R)	*TTG ATT TTG GAG GGA TCT GC*			

3.7.3 Gel electrophoresis

DNA was analysed on a horizontal agarose slab gel of 2.5 % with ethidium bromide 0.5mℓ in TAE buffer. Electrophoresis was performed for 2 hours in the electric field strength of 80 V then DNA visualization was done under UV light. The relative sizes of the DNA fragments were estimated by comparing their electrophoretic mobility with that of the standards DNA ladder with the samples on each gel. (Omar *et al*., 2010).

3.7.4 Statistical and Data Analysis

Excel was used for the statistical analysis of results. The data was then interpreted in the form of graphs and tables.

CHAPTER 4

RESULTS AND DISCUSSION

Meat is prone to contamination at various stages from primary production to when it is ready for consumption. Its composition is ideal for the growth of pathogenic bacteria (Sofos, 2014). One of the major causes of food-borne illnesses is contaminated meat (Akbar *et al*., 2011). The quality and safety of meat is a major concern in the meat industry, with foodborne pathogens as the utmost concern (Abuelnaga *et al*., 2021).

4.1 BACTERIAL LOAD COUNTS

Determination of meat quality parameters has always been very essential throughout all processes of the food industry because consumers are always demanding superior quality of meat and meat products (Elmmasry *et al*., 2012). Meat quality is driven by the need to supply the consumer with a consistently high-quality product at an affordable price. High quality is a key factor for the modern meat industry because a high-quality product is the basis for success in today's highly competitive market.

Samples were cultured to determine the total bacterial count. The total bacterial count ranged from $1.4x10^6$ to $9.2\ x10^7$ with a mean $8.7x10^7$ in fried chicken, followed by cooked beef with the mean of $1.1x10^7$ cfu/ml, respectively. Presumptive colonies were counted and colony forming unit was calculated. The results are shown below in **Table 4.1.**

Table 4.1. Bacteriological loud and mean counts of bacterial isolated from beef and chicken (CFU/ml).

Samples	Minimum	Maximum	Mean	Standard
Fried chicken N=42	$2.2x10^6$	$1.8x10^7$	$8.7x10^7$	10^2 cfu/g
Cooked chicken n= 42	$1.4x10^6$	$9.2x10^7$	$1.1x10^7$	10^2 cfu/g
Cooked beef N=42	$6.0x10^6$	$1.8x10^7$	$1.1x10^7$	10^2 cfu/g
Fried beef n=42	$2.0x10^6$	$1.8x10^7$	$1.1x10^7$	10^2 cfu/g

In this study, the total bacteria count (TBC) ranged from 1.4×10^6 to $9.2\text{x}10^7$ CFU/ml, with the mean of $8.7\text{x}10^7$ and the highest count was observed in fried chicken. This was higher compared to the microbial standards of total bacterial count of ready to eat meat which is 10^2 cfu/g. Levels exceeding 2 Log_{10} CFU/g are outside of microbial limits, which may indicate poor hygiene practice, poor food handling and likelihood of the presence of pathogens (Shingenin, 2019). The total bacterial count of this study was higher compared to the TBC reported in Ethiopia which was $3.98\text{x}10^3$ CFU/g (Zerabruk *et al*., 2019). The results of the current study were also higher compared to studies conducted in Lagos, Nigeria by Abera *et al.* (2016 and in Tirumala, India by Madueke *et al*. (2014), which showed lower counts of $4.09\text{x}10^4$ cfu/g and $5.81\text{x}10^5$ cfu/g, respectively. Another study carried out in Zaria, Nigeria, reported a bacterial count of street food samples of about 4.7 log CFU/g (Masupye *et al*., 2000).

Total bacterial counts in the meat samples analysed in the present study were found to be very high and agreed with similar studies that had been carried out elsewhere. For instance, a study carried out in the Dominican Republic reported bacterial counts of between 5 and 9 log cfu/g in street-vended fried, chicken, beef and meat stews (Kubheka *et al*., 2001). In Pakistan, total bacterial counts as low as 8 log CFU/g, reported on cooked ground meat sold on the streets (Masupye *et al*., 2000). In Nigeria, APCs and coliform counts of between 7 and 9 log CFU/g and between 2 and 3 log cfu/g, respectively, were reported in meat products sold on the streets (Manguiat *et al*., 2013). Similar study done by Igene, (1983), reported $4.78\text{x}10^5$ cfu/g of fried chicken meat which is high and is in agreement with the present study. The occurrence of organisms in ready-to-eat meat (Fried chicken) shows an inacceptable state of poor hygiene practices employed in the processing and sales of these food products. In the open market, the products are left exposed without any form of packaging or covering as often as the customers' demand and attracts sporadic visits by flies (Varela *et al*., 2011). This is in line with the results of Hussin *et al*. (2018), who detected that street vendors in Haiti were not covering their foods hence exposing them to dust containing microbes which contaminated the foods. Another reason of this findings could be the fact that, the vending sites lacked available hand washing and sanitary facilities as the vending sites were along the roads. High counts may indicate the presence of other pathogenic microbes due to improper handling of food, as these microorganisms are indicators in the evaluation of food safety.

4.2 PREVALENCE OF E*SCHERICHIA COLI* AND *STAPHYLOCOCCUS SPP*, *SALMONELLA* AND *SHIGELLA* IN EXAMINED STREET VENDED MEATS

A total of 168 different street vended samples were collected and analysed. The results indicated that 29/168 (17%) were positive for *E. coli* and 32/168 (19%) were positive for *Staphylococcus spp*, while *Salmonella* and *Shigella* were not detected in the meat samples. The prevalence of *E. coli* and *Staphylococcus spp*. were high in fried and in cooked chicken and very low in fried beef. The results are presented in **Table 4.2.**

Table 4.2. Prevalence of different pathogenic bacteria found on beef and chicken (n=168 samples).

Samples Type	No of (+) *E. coli* (%)	No of (+) *Staphylococcus aureus* (%)	No of (+) Salmonella (%)	No of (+) Shigella (%)
Cooked beef N=42	4(9.5%)	5(11.9%)	Not detected	Not detected
Fried beef N=42	3(7.1%)	8(19%)	Not detected	Not detected
Cooked chicken N=42	8(19%)	11(26.2%)	Not detected	Not detected
Fried chicken N=42	14(33.3%)	8(19%)	Not detected	Not detected
Total=168	29(17.3%)	32(19%)	Not detected	Not detected

Prevalence of Staphylococcus spp.

It was found that 32 (19%) samples were positive for *Staphylococcus spp*. with highest percentage found in cooked chicken meat. This prevalence rate was lower compared to the study conducted by Incili *et al*. (2022) from Shaanxi province in China. The results showed the prevalence of 25% in cooked meat (chicken and beef). Another study done by Ayenew (2012), in Ethiopia showed the prevalence of 51.8%, which is higher compared to the current study. However, the prevalence of *Staphylococcus spp*. in our study was comparable to the findings reported by Kerekes, (2015), which showed 19% of Staphylococcus spp. found in cooked chicken meat while Azanaw et al. (2021)in another study reported 17.9% out of 120 samples. Our findings were in contrasts with the findings of the study conducted in Thailand by Huang *et al*. (2013), which showed higher isolation rate of 39.1% of *Staphylococcus spp*. out of 144 samples.

Detection of Staphylococcus spp.in cooked chicken in this study might be associated with improper personal hygiene and contaminated hands of vendors as this bacterium is usually related to human skin and clothing (Birgen *et al*., 2020). The reason of this high rate of *Staphylococcus spp*. could be the fact that, *Staphylococcus spp*. are common environmental bacteria and could thus have been introduced into the food after cooking through cross-contamination (for instance, from utensils used by the vendors to serve food since vendors commonly stored their serving utensils on the tables (Birgen *et al*., 2020).

The reason of these findings could be the fact that, vendors served chicken products while simultaneously managing money. Another study in Kenya reported that street vendors handled both money and food with their bare hands (Birgen *et al*., 2020). Hands are potential vehicles for transmitting pathogenic microbes. It is recommended that food handlers should not handle food with bare hands and handle money simultaneously to avoid incidences of cross contamination that can be health risk (Munoz *et al*., 2012). To prevent instances of cross contamination that pose a health risk, it is advised that food workers avoid handling both food and money with bare hands.

Staphylococcus spp can be isolated from humans (respiratory passage, skins and superficial wounds) and also in the environment (Von, 1997). previous sentence the inherent danger of *Stapylococcus spp*. with or without their metabolic products in various foods and without further heat treatment incurs possible outbreaks of serious food borne illness (Eromo *et al*., 2016).

4.3 IDENTIFICATION OF STAPHYLOCOCCUS AUREUS USING VITEK 2 SYSTEM

38 presumptive *staphylococcus spp* isolates were further subjected to VITEK 2 system for the identification of *Staphylococcus species*. Out of 38 isolates tested, 15 isolates were found to be *staphylococcus* and the remaining failed to grow and were not recovered. *Staphylococcus xylosus* were found to be the most prevalent species with 13.2%, followed by *staphylococcus saprophyticus* with 10.5%. The results are presented in the table below.

Table 4.3. Confirmatory results from VITEK (n=38)

Staphylococcus spp	**No**	**Percentage (%)**

1. *S cohnii spp urealyticus*	2	5.3
2. S *haemolyticus*	2	5.3
3. *S saprophyticus*	4	10.5
4. *S xylosus*	5	13.2
5. *S epidermidis*	1	2.6
6. *S hominis spp hominis*	1	2.6

Similar study done by Tshipamba *et al*. (2018), showed the isolation of Staphylococcus spp. such as *S. aureus*, *S. Vitulinus* and *S. equorum*, which were isolated from chicken meat. The findings of this present study indicated that *Staphylococcus* was higher than the results observed in a study done by Osakue *et al*., (2016), which showed 2% of *Staphylococcus xylosus*. *S. xylosus* is a source of proteolytic enzymes frequently used in food handling. *S xylosus* is identified as a cause of human infection (Giordano *et al*. 2016). Acute endocarditis, acute pyelonephritis, urinary tract infection, secondary root canal infection, corneal external infections, otogenic brain abscess, orthopedic implant infections, erosive esophagitis, and filarial lymphedema superinfection are among the few conditions currently reported in the literature that indicate the pathogenetic role of S. xylosus (Giordano *et al*. 2016).

Staphylococcus aureus was not detected in our study, and this was in contrast with studies conducted by Sina *et al*. (2011) and Tshipamba *et al*. (2018) which reported higher prevalence of *S. aureus* in street vended ready to eat meat of 18% and 52%, respectively. The presence of *Staphylococcus aureus* in meat have been found to be the main cause of the foodborne illnesses in humans (Tessew *et al*., 2010).

Prevalence of E. coli

The prevalence of *E. coli* in this study was found to be 17.3 % with the highest percentage obtained in fried chicken meat samples. This clearly indicates that, the street-vended ready to-eat meats in Thohoyandou were contaminated with *E. coli*.

The prevalence of E. coli in this research may be attributed to inadequate heat processing, post-processing contamination, feces contamination, and inadequate food

handlers' hygienic practices (Bereda et al., 2016). The lack of knowledge based on the significance of disinfecting and sanitizing continuously also plays a significant role in the higher levels of contamination (Bereda et al., 2016).

These bacteria could cause health issues to the customers if they are present in food. A study done by Gallo *et al*. (2020), showed that the incidences of *E .coli* were 46.7%, 40% and 33.3% of the examined ready to eat meat in hawawshi, Kofta and shawarma samples, respectively, which supported the results of the present study. The higher incidence of *E. coli* had been reported in other studies, Eromo *et al*., (2016) in Ethiopia reported that 42% out of 96 samples they examined were positive for *E.coli*. Another study conducted in Mexico by Diaz-Lopez *et al.* (2011), in which *E. coli* was detected in 5 of 43 fried chicken samples (11.6%) from street vendors was lower compared to the findings of this study. Another study conducted in Taiwan by Le et al. (2015) showed that *E. coli* was detected in 10(72%) of the cooked chicken samples, this result was higher compared to the current study. This variation is mostly brought by the ingredients used during cooking (such as special spices), and by using the same utensils without in-between cleaning which may results in cross-contamination between different raw materials and products as prepared.

4.4 ANTIBIOTIC SUSCEPTIBILITY TEST

Antimicrobial susceptibility of *E. coli* and *Staphylococcus spp*. was determined, and the findings were interpreted in accordance with the National Committee for Clinical Laboratory Standards' recommendations for anti-microbial susceptibility testing (CLSI, 2013). An antibiotic susceptibility test for the isolates was performed using the Kirby-Bauer disk diffusion method. The diameter of inhibition zones around antibiotic disks was measured using a 300 mm ruler and expressed as Susceptible (S), Intermediate (I) or Resistance (R) according to the criteria recommended by the clinical and Laboratory Standards institute (Bemis, 2009). The antimicrobial agents were selected based on the availability and frequency of prescription for the treatment of bacterial infections in south Africa (Morgan *et al*.,2011). *Staphylococcus spp*. were highly resistant to Ampicillin (AMP) and h also highly susceptible to Neomycin(N). The results are presented in **Table 4.3 and Table 4.4.**

Table 4.4. Antimicrobial susceptibility profile of *Staphylococcus spp*. to different microbial agents.

Antibiotics	Disc content (µg)	Resistance (R)	Intermediate (I)	Susceptible (S)
Ampicillin (AMP) N=42	10(µg)	≤ 28 28(100%)	0	≥ 29 0
Chloramphenicol(C) N=42	30(µg)	≤ 12 13(46%)	_ 0	≥ 18 15(54%)
Tetracycline(T) N=42	30(µg)	≤11 15(54%)	12-14 1(4%)	≥15 12(42%)
Gentamycin (GM) N=42	10(µg)	≤12 9(32%)	13-14 3(11%)	≥15 16(57%)
Neomycin(N) N=42	10(µg)	≤12 0	13-17 0	≥17 9(100%)

Results expressed as number and percentage of *E. coli* isolates **(S)** susceptible, **(I)** intermediate/moderately susceptible and **(R)** resistant, respectively for each antimicrobial agent.

The results of this study showed that *Staphylococcus spp*. were highly susceptible to neomycin and highly resistant to ampicillin. A study done by Eromo *et al*. (2016), showed that *Staphylococcus spp*. were susceptible to ciprofloxacin, gentamycin, naldixic acid, norfloxacillin, Neomycin and trimethoprim sulphamethoxazole. This means that these antimicrobials are still drug of choice for the management of food borne illnesses in that locality. On the other hand, *Staphylococcus spp*. showed resistance to ampicillin, cloxacillin, ceftriaxone and other tested antimicrobial drugs which would make the treatment of *Staphylococcus spp*. infections difficult, which agrees with the present study. The presence of resistant *Staphylococcus spp*. strains in food is a big health risk (Attien et al., 2013).

The ability of Staphylococcus spp. to create an exopolysaccharide barrier and their location within micro abscesses, restricts the effect of medications, which is well known for developing resistance to antimicrobials. (Ortega *et al*., 2010). The resistance of *Staphylococcus spp*. to Ampicillin obtained from this study is supported by a study done in Morocco by Bernado *et al*. (2005), which showed that 42.86% of the isolated *S. aureus* were resistant to ampicillin. *Staphylococcus spp*. pose a danger to human health on a global scale, primarily due to their ability to spread zoonotic infections and broad-spectrum antibiotic resistance (Sugar *et al*., 2019).

The susceptibility of *Staphylococcus spp* to neomycin obtained from the current study is also supported by the results of a study done by Penna *et al*. (2010), which showed that all isolated strains of *Staphylococcus aureus* were sensitive to neomycin. The results of this study showed that *Staphylococcus spp* were sensitive to chloramphenicol by 54%. This is contrary to study by Ed-Dra *et al*. (2018) which showed that 38.1% of *Staphylococcus spp* isolates were resistance to penicillin, while 19.05% were resistant to chloramphenicol and this is lower compared to the current study.

The antibiotic susceptibility profile of *E. coli* isolates showed higher resistance to ampicillin (AMP), tetracyclin (T) and penicillin (PG) and higher susceptibility to neomycin (N), The results are shown below in Table 4.5.

Table 4.5. Antimicrobial susceptibility profile of *E. coli* to different microbial agents.

Antibiotics	**Disc content (µg)**	**Resistance (R)**	**Intermediate (I)**	**Susceptible (S)**
Ampicillin (AMP) N=42	10(µg)	≤ 14 42(100%)	0	≥14 0
Chloramphenicol(C) N=42	30(µg)	≤12 38(88%)	13-16 5(12%)	≥18 0
Tetracycline(T) N=42	30(µg)	≤ 11 42(100%)	12-14 0	≥ 15 0
Gentamycin (GM) N=42	10(µg)	≤12 32(74%)	13-17 9(20%)	≥15 3(6%)
Penicillin (PG) N=42	10(µg)	≤ 28 42(100%)	- 0	≥ 29 0
Neomycin(N) N=42	30(µg)	≤12 0	13-17 0	≥17 42(100%)

Results expressed as number and percentage of *E. coli* isolates **(S)** susceptible, **(I)** intermediate/moderately susceptible and **(R)** resistant, respectively for each antimicrobial agent.

The antibiotic susceptibility profile for *E. coli* isolates showed that 100 % of the tested *E. coli* isolates were resistant ampicillin (AMP), tetracyclin (P), penicillin (PG) and 100% were susceptible to neomycin (N). The results of antibiotic susceptibility profile

of E. coli obtained in this study are in agreement with study done by Adzitey *et al*. (2020) in Ghana on antimicrobial resistance of *Escherichia coli* Isolated from various meat types. The study revealed that 71.67% of *E. coli* isolates were resistant to ampicillin, penicillin and tetracycline and other antibiotics (Adzitey *et al*., 2020). Moreover, Ayamah *et al*. (2021), reported that, *E. coli* were 100% resistant to ampicillin, tetracycline, gentamicin, cefuroxime, ceftriaxone, and cefotaxime, which agrees with the present study. The results of the present study are in line with those of Ahmadi *et al*. (2015), who found that *E. coli* isolates from meat and meat products were resistant to ampicillin, tetracycline, and other antibiotics.

Ayamah *et al*. (2021) also reported a higher percentage of isolates resistant to ampicillin. Similar findings reported by Somda et al. (2018) described E. coli isolates from grilled, flamed, and fumed chicken that were resistant to ampicillin (42.86%), tetracycline (64.3%), ceftriaxone (7.14%), cefotaxime, and other antibiotics. The current findings also agree with work carried out by Apun *et al*. (2008), who reported *E. coli* isolates from Malaysian broiler chickens resistant to ampicillin, tetracycline, and gentamicin ranging from 11 to 95%. Wayne. (2006) reported 43% of *E. coli* isolates resistant to ampicillin and other antibiotics that support the current results, and this partly agrees with the current findings.

Despite samples coming from various locations near Thohoyandou, *E. coli* and *Staphylococcus spp*. showed a comparable pattern of antibiotic resistance. This is in agreement with the study done by Achi *et al*. (2007) who found that *E. coli* isolates from various vendors in food samples shared comparable patterns of antibiotic resistance. These antibiotic-resistant bacterial isolates from the Thohoyandou samples suggest that people who purchase meat from street vendors may experience severe health issues. Umoh *et al*. (1990) claim that the overuse and abuse of antibiotics in the environment is what has led to the current degree of antimicrobial resistance.

Moreover, the practice of giving antibiotics to domestic livestock in order to cure and avoid illnesses as well as to promote growth may cause bacteria resistant to antibiotics (Caly *et al*., 2015). Foodborne bacteria have become increasingly resistant to antibiotics in recent years. Allison *et al*. (1995) hypothesized that the rise in cases could be ascribed to the selection pressure brought on by the use of antibiotics in food-

producing animals and the unrestricted use of antibiotics by people in developing nations. According to 000000000000000), the widespread use of these antibiotics in veterinary medicine is anticipated to lead to an increase in antibiotic resistance.

In addition, neomycin obtained from this study agrees with the results obtained in a study done by Bag *et al.* (2021) in Bangladesh. Bag *et al*. (2021) also reported similar finding in antimicrobial resistance of *Escherichia coli* isolated from milk, beef and chicken meat, and revealed that all *E. coli* isolates tested were sensitive to neomycin which is the same results obtained from the current study. Neomycin has activity against most Gram-negative aerobes and inhibits protein synthesis via binding to the 30S ribosomal unit (Dowling *et al*., 2017). The antibiotic susceptibility profile of *E. coli* against penicillin and other antibiotics, support the results obtained from this study which was 100% resistance, this finding is in agreement with the results reported by Kazemnia *et al.* (2014) in Iran, which showed that 100% of *E. coli* isolates were resistant to penicillin.

4.5. MOLECULAR CHARACTERIZATION OF E*. COLI*

Thirty *E. coli* isolates were further characterized using multi-plex PCR of which 27/30 (90%) of the isolates were successfully amplified and identified whereas 3 isolates were not amplified probably they do not harbour the genes profiled. The virulence genes ranged from 13,33% to 86,67% with *asta*, *stx1* and *eae* being the most prevalent. The pathotypes that were detected in this study were EPEC, EHEC, ETEC, EAEC, and EIEC was the least detected pathotype. The results are shown in Tables 4.6, 4.7 and 4.8.

Table 4.6. Amplification of *E. coli* genes by multiplex PCR assay.

				EHEC								
Sample				EPEC				EIEC	ETEC		EAEC	**Pathotype**
	Sample Name	*Asta*	*mdh*	*bfp*	*Eae*	*Stx1*	*Stx2*	*ial*	*Lt*	*St*	*Eagg*	
1	CB1	1	1		1		1					EPEC, EHEC
2	CC2		0				1		1		0	EHEC, ETEC
3	CC4	0	0	0	0	0	0	0	0	0		No id
4	CC6		1		1							EPEC
5	CC11		1		1							EPEC
6	CC12		1									ECOM
7	CC13	0	0	0	0	0	0	0	0	0	0	Not id
8	FB1		1		1	1	1		1	1	1	EPEC, EHEC, ETEC, EAEC
9	FB2		1	1						1	1	EPEC, ETEC, EAEC
10	FB3		1				1			1	1	EHEC, ETEC, EAEC
11	FB4		1		1		1			1	1	EPEC, EHEC, ETEC, EIEC
12	FB5		1	1		1	1			1	1	EPEC, EHEC, ETEC
13	FB6	1	1				1					EHEC, EAEC
14	FB14	1	1		1		1	1	1			EPEC, EHEC, EIEC, ETEC
15												
16	FC1	1	1	1	1		1					EPEC, EHEC, EAEC
17	FC3	1	1				1	1	1			EHEC, EIEC, ETEC, EAEC
18	FC4	1	1	1		1	1		1			EPEC, EHEC, ETEC, EAEC
19	FC5	1	1									EAEC
20	FC6	1	1									ECOM
21	FC7	1	1		1			1	1			EPEC, EIEC, ETEC
22	FC8		1			1	1	1			1	EHEC, EIEC, EAEC
23	FC9		1		1		1		1			EPEC, EHEC, ETEC
24	FC10		1		1	1						EPEC, EHEC
25	FC12		1									ECOM

26	FC13		1			1						EHEC
27	FC14		1	1	1	1						EPEC, EHEC
28	FC15		1	1	1						1	EPEC, EAEC
29	FC16		1	1	1							EPEC
30	FC17		1		1							EPEC

CEC *-Commensal E. coli ;* **A-EPEC**-*Atypical enteropathogenic E. coli (only eae) ;* **T-EPEC**-*Typical enteropathogenic E. coli (bfp and eae) ;* **EHEC** – *Enterohaemorrhagic E. Coli ;* **EAEC**-*Enteroaggregative E. Coli ;* **ETEC**-*Enterotoxigenic E. Coli ;* **EIEC**-*Enteroinvasice E. coli and/or Shigella*, E.COM

(1) = Positive results, (0) = No Identity

Table 4.7. Prevalence of virulence genes of *E. coli (*n=30)

Virulence genes	Number of virulence genes	Percentage (%)
Asta	9	30
Mdh	26	86.67
Bfp	7	23.33
Eae	14	46.67
Stx1	7	23.33
Stx2	13	43.33
Ial	4	13.33
It	7	23.33
St	5	16.67
Eagg	7	23.33

Table 4.8. Prevalence of pathotypes

Pathotypes	No of (+) strains N= 30(%)
EPEC, EHEC	2(7%)
EHEC, ETEC	1(3%)
EPEC	2(7%)
ECOM	2(7%)
EPEC, EHEC, ETEC, EAEC	2(7%)
EPEC, ETEC, EAEC	2(7%)
EHEC, ETEC, EAEC	1(3%)
EPEC, EHEC, ETEC, EIEC	2(7%)
EPEC, EHEC, ETEC	2(7%)
EHEC, EAEC	1(3%)
EHEC, EIEC, ETEC, EAEC	1(3%)
EAEC	1(3%)
EPEC, EIEC, ETEC	1(3%)
EHEC, EIEC, EAEC	1(3%)
EHEC	1(3%)

In the current study the most prevalent virulent genes were *Eae* (46.67%), followed by *stx2* (43.33%) and *Asta* (30%). Comparing to other studies, the results are high

compared to the study done by Martinez *et al*. (2018), which showed *Eae* (19.2%), *Stx2* (17.7%) and *Asta* (13.5%). Another study done by Modgil *et al*. (2020), showed the rate of *Eae* (14%%), *Stx2* (6.8%) and *Asta* (4,9%), theseis results are lower compared to the present study. The *eae* gene encodes for intimin that facilitates intimate attachment of *E. coli* O157:H7 to the host cell by producing attaching and effacing intestinal lesions. The *astA* gene was first identified as a structural gene that encodes a distinct low-molecular weight putative enterotoxin (Yatsunyanagi *et al*., 2003). Soto *et al*. (2009) reported that the *astA* gene is a structural gene that encodes an enteroaggregative heat stable toxin 1 (EAST-1). This toxin is thought to play a role in toxicity as it has been associated with the pathogenicity of EAEC and it has been detected also in EPEC, atypical EPEC, ETEC and EIEC strains (Yatsuyanagi *et al*., 2003). According to a study reported by Omar *et al*. (2015), in 1996 gastrointestinal illness outbreak was caused by *E. coli* 0166:H15 possessing an *astA* gene. The *stx2* gene is usually more prevalent in *E. coli* O157:H7 (Ateba, 2011; Johnsen *et al*., 2001; Villani *et al*., 2005). However, Previous reports indicated that the *stx2* gene was more strongly associated with disease in humans (Ateba *et al*., 2011).

The most identified pathotypes in this study were coinfections, wherein EHEC and EPEC dominated. The prevalence of EHEC (3%) and EPEC (7%) in the current study is lower compared to the study reported by Martinez et al. (2018) in Japan where the results of EHEC showed (16.7%) and EPEC showed (8.33%). Similar studies done in South Korea by Hoang et al (2015) showed (17%) of EHEC and (5%) of EPEC, another study done by Ateba *et al*. (2015) in South Africa showed the results of (23.7%) of EHEC and (7%) of EPEC, respectively.

The higher rate of pathotypes was also observed in a study done by Slinger *et al.* (2017), which reported EPEC (18%) and EHEC (9%). Another study done by Martinez *et al* .(2018), showed 31(19%) of EHEC and 19% of EPEC.

The existence of EHEC and EPEC pathotypes raises serious concerns because they have been linked to human infections and illnesses. The eneterohaemorrhagic *E. coli* (EHEC) and *enteropathogenic E. coli* (EPEC) are strains that are considered a high health risk because they can cause diarrhoea and serious conditions such as haemolytic-uraemic syndrome (HUS) and in some cases, even death (Moynihan, 2011). Estrada *et al.* (2010) detected STEC as the most prevalent pathotype. The

variation is mostly brought by hygienic conditions and selling undercooked meat products. It is very important to observe control measures to reduce contamination. Additionally, employing such precautions when cooking beef may lower the risk of human food-borne infections (Asime *et al*., 2020).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The main objective of this study was to determine the microbial quality and safety of street vended ready-to-eat meat sold around Thohoyandou area. This was achieved by resolving secondary objectives.

The study first objective was to isolate and determine the prevalence of selected pathogenic bacteria (*E. coli*, *Staphylococcus aureus, Shigella,* and *Salmonella spp*) from meat using standard culture methods. Out of 168 samples tested, 32 (19%) were found to be positive for *Staphylococcus spp* with highest percentage found in cooked chicken meat. The most prevalent *Staphylococcus* species identified in this study were *S. xylosus* (13.2%) and *S. saprophyticus (10.5%).* The prevalence of *E. coli* was found to be 19.3 % in which highest percentage was found in fried chicken.

The second objective was to determine the antimicrobial susceptibility profiles of *E. coli*, *Staphylococcus aureus, Shigella* and *Salmonella spp* using Kirby-Bauer disk diffusion method. The antibiotic susceptibility profile of *E. coli* isolated showed that 100% were Resistant to ampicillin (AMP), tetracyclin (T) and penicillin (PG) and 100% were susceptible to neomycin (N). *Staphylococcus spp* isolates showed that 100% were resistance to ampicillin (AMP) and 100% were also susceptible to neomycin(N).

The third objective was to determine the pathotypes of the detected enteric bacteria isolated from meat using Multiplex PCR. All the positive *E. coli* isolates (30) were further characterized using multi-plex PCR and 27/30(90%) of the isolates were identified whereas 3 isolates were not amplified due to none possession of the targeted genes. The virulence genes ranged from 13,33% to 86,67% with asta, stx1, and eae being the most prevalent. The pathotypes that were detected in this study were EPEC, EHEC, ETEC, EAEC, and EIEC and majority of the isolates were positive for mixed pathotypes (co-infection).

In conclusion, the microbial quality and safety of street vended meat is inadequate and therefore not acceptable for consumption. Street vended meat contained various pathogenic bacteria that may trigger dangerous health issues. The detection of these organisms in all beef and chicken meats investigated serves as a warning danger of

foodborne diseases that could be associated with poor personal hygiene, and poor food preparation.

LIMITATIONS

The limitation of the study was that due to financial constraints, some antibiotics were not equally distributed among the different isolates and few isolates were identified and characterised using Multiplex PCR or Vitek 2 system.

RECOMMENDATIONS

The study recommends that:

- Further studies need to be conducted to fully identify Staphylococcus species and characterise pathogenic *E. coli* isolated from street vended ready to eat meat,
- To control pathogenic bacteria, good manufacturing practices for the processing and handling of food should be applied.

Good hygiene plays a huge role in elimination of pathogens that are capable of prevailing in street vended meat. Therefore, education for vendors on food safety and hygienic practices is essential to reduce contamination rate. In addition, regular inspection on food vending practices and safety of street foods is required to improve the health standards of consumers. The use of various antibiotics on animals should be investigated as it is the source of antibiotic resistance development and therefore antibiotic profiling of pathogenic bacteria is recommended.

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