Microbial analysis of spices and herbs obtained from market places around Gweru CBD

By

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A dissertation submitted in partial fulfillment of the requirements for the degree: Bachelor of Science Honors in Applied Biosciences and Biotechnology

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APPROVAL FORM

This is to certify that the dissertation titled “Microbial analysis of spices and herbs obtained from market places around Gweru CBD”, submitted in partial fulfillment of the requirements for Bachelor of Science Honors Degree in Applied Biosciences and Biotechnology at Midlands State University, is a record of the original research carried out by DIRK MANATSA HAMADZIRIPI R146288W under my supervision. No part of the dissertation has been submitted for any other degree or diploma.

The assistance received during the course of this research has been duly acknowledged. Therefore, I recommend that I will be accepted as fulfilling the dissertation requirements.

Name of supervisor……*Mr. G DOWO*

Signature……..

Chairperson ‘s signature……..

ABSTRACT

The importance of spices and herbs as dietary supplements and alternative medicines to Zimbabwean society has increased in recent times. The popularity of these substances has come largely as a result of the dire economic situation prevalent in Zimbabwe which has engendered the dramatic collapse of the country‘s healthcare system. In spite of the numerous therapeutic claims that have been associated with spices and herbs sold openly in the streets, they pose a genuine threat to the public if they become contaminated prior to their sale. Previous studies have shown that bacterial contamination is very common on substances vended openly in the streets. A study was carried out at Midlands State University to assess the safety of spices and herbs vended in the Gweru CBD area by analyzing their microbial quality. This study was done between June 2018 and August 2018. Samples of ginger, garlic, turmeric and basil were collected randomly from vendors operating outside OK supermarket, Pick ‘n pay supermarket, and TM. Enumeration, Isolation and identification of bacteria was carried out using inoculum prepared from the samples using standard microbiological methods. Three species of bacteria namely; *S. enterica*, *S. aureus*, and *E.coli* were isolated from the samples, and it was observed that 76.3% of all samples were contaminated. Bacterial counts were done and basil had the highest mean and range for the counts. *S. enterica* was the most prevalent bacteria isolated. Findings from this study show that spices and herbs sold openly in the Gweru CBD are unsafe for consumers, and more should be done by the vendors and public health officers to ensure that these substances are safe for consumption.

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DEDICATION

I dedicate this work to Tafadzwa F. Mtisi, a huge friend of mine with whom I started this journey in the life sciences.

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CHAPTER 1: INTRODUCTION

1.1 Background of the study

Spices are plant extracts (seed, fruit, bark, root), and herbs are the leaves, flowers, or stems of a plant. They have many age old applications including adding flavor to food, preserving meat and medicinal uses such as treating digestive disorders, arthritis, etc. While today herbs and spices are still used as natural remedies, studies by the US department of Agriculture (USDA) and Department of Health and Human services (HHS) have shown that culinary herbs and spices contain concentrations of antioxidants and phytonutrients (plant derived compounds important to human health) that provide long term health benefits far outweighing short term taste sensations (USDA and HHS, 2005). It is becoming clearer that spices and herbs should be a major constituent of human diet to promote healthy living, and dieticians around the world recommend their daily consumption (Tapsell *et al*.,2006).In many Asian countries, spices contribute to herbal medicines (HM) and they are an integral part of the health care delivery system on the same basis as orthodox medicine. In developing countries like Zimbabwe, spices and herbs are a big part of people‘s lives especially in the rural areas (which constitute 33% of the population) where there is limited access to modern medicines and other xenobiotic compounds that enhance our lives. As a result, the demand for spices and herbs is high, and food safety experts speculate that it is unlikely to decline any time soon (Mudadigwa, 2016).

1.2 Safety considerations of marketed products

The major problem that comes with a high demand of unregulated substances like spices and herbs is that they are supplied to the market poorly processed and usually contaminated.

Foodborne illness like salmonellosis can affect a population that is exposed to their contamination. Notwithstanding the fact that outbreaks of acute poisoning are frequent in Africa, individual countries have done little to implement surveillance systems for food borne diseases. Data concerning food borne diseases is scarce, but studies have shown that the most common causative agents in Zimbabwe are, *Salmonella, Shigella, Hepatitis, Brucella, Campylobacter, Staphylococcus, Escherichia coli,* and *rotavirus* (Gabida *et al*, 2012; MoHCC, 2014). In 2016, the Food and Drug Administration of the U.S introduced new preventive rules and controls in the food supply chain under the Food Safety Modernization Act (FSMA) in order to address persistent foodborne illnesses related to spices. Most developing countries do have regulatory bodies like the FDA, however they lack meaningful surveillance on the potential dangers that contaminated spices and herbs pose. City Councils in Zimbabwe have public health departments that are responsible for surveillance of food contaminants and upholding safety standards of substances sold on the streets. They concede that it is difficult to monitor and control hygienic standards of food substances sold by the vendors. As a result, Zimbabwe has not adequately adopted microbial standards and Good Manufacturing Practice (GMP) that guarantee safety and hygienic quality of non-sterile preparations like spices and herbs (Nashuuta, 2015; Kabak and Dobson, 2017).

1.3 Problem statement

The economic crisis that has plagued Zimbabwe for over a decade and a half is well documented (Mudadigwa, 2016). What is less understood is the effect of this political and economic turmoil on the health of the average Zimbabwean and the Government ‘s capacity to deliver an adequate healthcare system. Cash shortages and the liquidity crisis have resulted in shortages of drugs,

decline in sanitation, and inadequate healthcare delivery, among many other problems. High unemployment has pushed many to the CBDs to earn a living through vending foodstuffs, small gadgets, clothes etc. In recent times, spices and herbs have gained great popularity in Gweru as natural remedies to a wide variety of ailments. Spice and herb vendors have claimed that their products are not only healthy dietary supplements but can also cure hypertension, dysentery, arthritis, asthma, tuberculosis etc. Some even outrageously claim that their products can stop cancer development. As a result many people prefer these cheap alternatives to the pricy drugs and food supplements that are prescribed in the formal health sector. This, however, exposes the public to the risk of consuming unsafe substances that may be poorly processed prior to entering the market. Zimbabwe has historically suffered outbreaks of foodborne and waterborne diseases. Notwithstanding the Gweru typhoid outbreak of August 2018 which left nine dead, there have been many reported cases of foodborne illnesses, and the source of the illnesses is usually unestablished due to a lack of meaningful surveillance of the infections.

1.4 Justification

Reported cases of dysentery across Zimbabwe rose sharply from 42 626 in the year 2008 to 61 869 by end of 2013. It is likely that there were many unreported cases due to limited accessibility of health care facilities particularly in the rural areas. Diarrheal cases rose from 485 272 cases in 2008 to 817 787 in 2013 (MoHCC, 2014). The probable cause of one in every five case of dysentery and diarrhea is a food borne pathogen (CDC, 2011). The surge in reported cases of dysentery and diarrhea in recent times indicates the importance and need to increase hygienic quality measures in the food manufacturing industry (particularly unregulated substances). Currently, many substances enter the market without prior microbial quality control checks

leading to potential immediate and long term health hazards. Poorly processed and contaminated spices and herbs expose the clientele to a wide range of pathogenic agents like; *Salmonella* spp, *Escherichia coli* spp, *Shigella*, *Candida* and fungal secondary metabolites like aflatoxins, ochratoxins, and fumonisins (*Investigating outbreaks, 2012*). It is imperative to assess the microbial quality of spices and herbs due to their potential exposure to pathogenic agents. In order to protect the public from potential food borne diseases regular surveillance of contamination is required (*Eye Witness News*, 2017).

1.5 Objectives

1.5.1 Main Objective

- To investigate the presence of microbial pathogens on spices and herbs sold at market places around Gweru.

1.5.2 Specific objectives

- To isolate and identify specific bacterial species in the spices and herbs.
- To determine total bacterial count of the spices and herbs.
- To determine the prevalence of the foodborne pathogens on the spices and herbs.

CHAPTER 2: LITERATURE REVIEW

2.1 Significance of spices and herbs to society

The use of spices and herbs transcends cultural boundaries, and has been around since time immemorial. Such is the societal impact of these substances that the 15th century spice trade between Africa, Europe, and Asia represented the first major cross-cultural contact, and the birth of trade and global economics. Applications of spices and herbs included flavoring food, making perfume, embalming the dead, preserving meat and traditional medicines, the trade represented a highly lucrative part of early commerce (Sherman and Billing, 1999).

Today spices and herbs are still a vital aspect of our lives (though much less commercially lucrative) and their use is ubiquitous throughout the world. Though they retain their numerous age-old applications, advancements in technology and knowledge have served to further clarify their importance to human health and food science. While they are still used as natural remedies, studies have shown that they contain significant concentrations of antioxidants and phytonutrients (plant derived compounds important to human health) and may provide long term health benefits that outweigh short term taste sensations (Tapsell *et al*, 2006). It is becoming clearer that spices should be a major constituent of human diet in order to promote healthy living, and dieticians around the world encourage their daily consumption (Tapsell *et al*, 2006).

In Zimbabwe, many people resort to spices and herbal preparations as complementary medicine due to the unavailability and unaffordability of drugs in hospitals and pharmacies as official health facilities become a preserve of the wealthy (Mananavire, 2017). The government, through the Medicines Control Authority of Zimbabwe (MCAZ), intends to control commercialization of these complimentary medicines that have become increasingly popular. Their measures include the requirement of market authorization for products prior to distribution and sale in Zimbabwe. The government aims to ensure public safety, and their concerns are echoed by the Zimbabwe Traditional Healers' Association (ZINATHA) who bemoan the public sale of some traditional medicines and herbs in local markets (Mananavire, 2017; Chifera, 2015).

It is difficult to fully assess how much the vendors on the streets comply to requirements of MCAZ or public health standards set by city councils, and the public is potentially exposed to contaminated products. City Councils in Zimbabwe have public health departments that are responsible for surveillance of food contaminants and upholding safety standards of substances sold on the streets. They, along with regulatory bodies like the MCAZ, are mandated with ensuring the safety of spices and herbs sold openly on the streets. However, they concede that it is difficult to monitor and control hygienic standards of xenobiotics sold by the vendors. As a result, Zimbabwe has not adequately adopted microbial standards and Good Manufacturing Practice (GMP) that guarantees safety and hygienic quality of non-sterile preparations like spices and herbs (Nashuuta, 2015; Kabak and Dobson, 2015).

2.2 Implications on public health and safety

The popularity of spices and herbs as natural remedies and alternative medicines has always been very high in Africa. It is a big part of African culture and a source of identity for the indigenous people of the continent. However, its acceptance and integration into the orthodox national health service has not been without controversy due to the longstanding colonial legacy that most African countries experienced. During Zimbabwe ‘s 90 year colonial rule traditional medicines were widely shunned in the colonial system in preference of prescribed western medicines, despite the fact that most western medicines have active agents derived from plants. Since Zimbabwe ‘s independence in 1980, traditional medicines have achieved a level of acceptance and integration into the national health service (Waite 2000).

However, the economic decline of Zimbabwe resulted in the deterioration of the primary healthcare system. This coincided with a fall in demand for services, following the introduction of user fees and increasing costs of essential medicines (IRIN, 2014). As a result, many have turned to prescriptions made by street doctors for ailments like colds, flu, arthritis, and hypertension. Contamination of herbs and spices has been widely documented due to the perceived threat it poses. A study of the open markets of Ilala in Dar es Salaam has showed that the most likely sources of contamination are the use of untreated water and exposure to the environment including dust and particulate matter (Justin-Teemu *et al.*, 2009). In addition to this, previous studies have also shown that cultivation of spices and herbs in areas characterized by warm climate and high humidity provides conducive conditions for microorganisms to grow (Garbowska *et al.*, 2005).

2.3 Contamination by pathogenic microorganisms

Spices and herbs, like other xenobiotic products, can become contaminated during the manufacturing process (Sospedra *et al*., 2010). As stated earlier there are several sources of their contamination prior to entering the market, and the contaminants are usually bacteria, fungi and fungal metabolites (Sagoo *et al*., 2009). Of significance to this study are pathogenic microorganisms that are known to cause gastrointestinal illnesses.

2.4 *Salmonella* spp

Salmonella is a genus of rod-shaped, Gram-negative bacteria of the family Enterobacteriaceae. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori* , the first is divided into six subspecies: *S. e. enterica*, *S. e. salamae*, *S. e. arizonae*, *S. e. diarizonae*, *S. e. houtenae*, and *S. e. indica*. The taxonomic group contains more than 2 500 serovars (also called serotypes) defined on the basis of the somatic O (lipopolysaccharide) and flagellar H antigens. The *Salmonella* species are a significant group of intestinal pathogens that occur widely in the natural environment and in the global food chain, and they pose a significant challenge to food production (Boyle *et al*., 2007).

Table 2.1 Taxonomy of *Salmonella* spp (Boyle *et al*., 2007)

Species	Sub-species	Number of serovars
S.enterica	enterica	1443
	salamae	448
	arizonae	94
	diarizonae	323
	houtenae	70
	indica	20
S.bongori		20
Total		2449

Salmonella spp are facultatively anaerobic gram-negative rod-shaped bacteria belonging to the family *Enterobacteriaceae*. The biochemical identification of foodborne and clinical *Salmonella* isolates is generally coupled to serological confirmation, a complex and labor-intensive method involving the agglutination of bacterial surface antigens with *Salmonella*-specific antibodies (D'Aoust and Maurer 2007). *Salmonella enterica* serotypes are a major public health burden globally, accountable for over one billion human infections annually and being responsible for significant morbidity and mortality (Boyle et al., 2007).

2.4.2 Survival and growth characteristics of *Salmonella* spp

Table 2.2 Conditions affecting the growth of *Salmonella* spp (Boyle et al., 2007)

Conditions	Minimum	Optimum	Maximum
Temperature °c	5.2	35 – 43	46.2
pH	3.8	7 – 7.5	9.5
Water activity (a_w)	0.94	0.99	> 0.99

It is important to understand the survival and growth characteristics of *Salmonella* spp in order to appreciate the transmission and pathophysiology of salmonellosis.

2.4.3 Transmission of *Salmonella enterica*

In endemic areas, most infections are acquired via contaminated food or water – with water playing the significant role in endemic urban settings. Risk factors include drinking non-boiled water and eating food prepared outside the home. While direct contact with a typhoid fever patient is an infection risk factor, more than 80% of cases occur in individuals with no known contact (Boyle *et al*., 2007).

2.4.4 Pathology of Salmonellosis

Salmonella enterica serotype Typhi and serotypes Paratyphi A, B, or C respectively cause typhoid and paratyphoid fever. Both typhoid and paratyphoid fever are characterized by prolonged fever and sustained bacteremia (Boyle *et al*., 2007). Symptoms usually appear 12-36 hours after the ingestion of contaminated food. Symptoms include acute enterocolitis with sudden onset of headache, abdominal pain, diarrhoea, nausea, vomiting and fever. Dehydration

may occur particularly among vulnerable populations, e.g. infants, the immuno-compromised and the elderly. The condition usually lasts 2-5 days and is generally self limiting. Complications can lead to systemic infections and can result in various chronic conditions such as reactive arthritis. The infective dose can vary depending on the strain, the immuno-competence of the individual and the nature of the food. Data from outbreaks of foodborne diseases suggest that infections may be caused by the ingestion of as few as 10-45 cells (Ibarra and Steele-Mortimer, 2009).

2.4.5 Virulence factors of *Salmonella enterica*

Salmonella enterica is found within a variety of phagocytotic and non-phagocytotic cells in vivo. Intracellular survival and replication are important virulence determinants of the bacteria. Following colonization of the human gut the bacteria enter enterocytes, M cells, and dendritic cells in the intestinal epithelium (Salcedo *et al*., 2001). Internalization into a wide variety of hosts is vital to the success of *Salmonella enterica* as a pathogen, it achieves this through at least two distinct processes including utilization of a type III secretion system (T3SS) called T3SS1-mediated invasion, and a T3SS independent invasion (McGhie *et al*.,2009). T3SS1-mediated invasion involves a secretion of a set of proteins (SipA, SipC, SopB/SigD, SopD, SopE2 and SptP) that induce a rearrangement of the actin cytoskeleton causing massive localized membrane ruffles and internalization of the bacteria (McGhie *et al.,* 2009). The T3SS independent invasion involves utilisation of fimbriae and/or non-fimbrial adhesins bound to the surface of *Salmonella enterica* spp that mediate attachment to the host. Within the host the bacteria makes use of virulence determinants that enable intracellular survival, and most of these factors are encoded from genes found on the Salmonella Pathogenicity Islands (SPI) on the chromosome. These factors enable nutrient acquisition, ion acquisition, and avoidance of the

host's antibacterial mechanisms within the cell. They also confer protection of the bacteria from extracellular reactive oxygen species (Muller *et al*., 2009; Ibarra and Steele-Mortimer, 2009).

2.5 *Staphylococcus* spp

Bacteria in the genus *Staphylococcus* are pathogens of man and other mammals, they are facultatively anaerobic, gram positive cocci that are 1 µm in diameter. They were traditionally classified into two distinct groups based on their ability to clot blood plasma (the coagulase reaction) namely; coagulase-negative staphylococci and coagulase-positive staphylococci (Forster, 1996). This classification has since proven to be artificial and misleading leading to classification based on DNA-ribosomal RNA (rRNA) hybridization and comparative oligonucleotide analysis of 16s rRNA. Under this method at least 40 species of *Staphylococcus* exist in clusters of 11 (Takahashi *et al*., 1999; Forster, 1996).

Table 2.3 Taxonomy of *Staphylococcus* spp (Taylor and Unakal, 2017)

Cluster	Species
S. aureus group	*S. argenteus, S. aureus, S. schweitzeri, S. simiae*
S. auricularis group	*S. auricularis*
S. carnosus group	*S. carnosus, S. condimenti, S. massiliensis, S. piscifermentans, S. simulans*
S. epidermidis group	*S. capitis, S. caprae, S. epidermidis, S. saccharolyticus*
S. haemolyticus group	*S. devriesei, S. haemolyticus, S. hominis*
S. hyicus-intermedius group	*S. agnetis, S. chromogenes, S. cornubiensis, S. felis, S. delphini, S. hyicus, S. intermedius, S. lutrae, S. microti, S. muscae, S. pseudintermedius, S. rostri, S. schleiferi*
S. lugdunensis group	*S. lugdunensis*
S. saprophyticus group	*S. arlettae, S. cohnii, S. equorum, S. gallinarum, S. kloosii, S. leei, S. nepalensis, S. saprophyticus, S. succinus, S. xylosus*
S. sciuri group	*S. fleurettii, S. lentus, S. sciuri, S. stepanovicii, S. vitulinus*
S. simulans group	*S. simulans*
S. warneri group	*S. pasteuri, S. warneri*

Of the 40 species, 16 are known to colonize humans, and can cause a variety of diseases through penetration or toxin production. Staphylococcal toxins are a common cause of food poisoning. Of the species described *S. aureus* and *S. epidermidis* are common commensals and possess the greatest pathogenic potential. *Staphylococcus aureus* infections cause a variety of human infections including bacteremia, infective endocarditis, skin and soft tissue infections (e.g impetigo, and folliculitis), gastroenteritis etc (Taylor and Unakal, 2017).

2.5.2 Survival and growth characteristics of *S. aureus*

Staphylococcus aureus can grow in a rather wide range of temperatures, 7 ºC to 48.5 ºC with an optimum of 30 ºC to 37 ºC. They also thrive in pH ranging between 4.2 to 9.3 with an optimum of 7 to 7.5, and sodium chloride concentrations of up to 15% NaCl. These characteristics enable the pathogen to survive in different food stuffs and promote their occurrence in foodstuffs that require manipulation during processing including foodstuffs like cheeses (Le Loir *et al,* 2003).

2.5.3 Transmission and epidemiology of *S. aureus*

Staphylococcus aureus is a pathogen of humans and other mammals, it can be transmitted from person to person via direct contact or by formites. The bacteria can live on the skin which is one of its primary mode of transmission. Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions. *Staphylococcus aureus* does not compete well with indigenous microbiota in raw foods, as a result contamination is mainly associated with improper handling of cooked or processed foods, followed by storage under conditions which allow growth of *S. aureus* and production of the enterotoxins. However, *S. aureus* is also present

in domestic animals including dairy cattle, sheep and goats, particularly if affected by subclinical mastitis, and are likely contaminants of milk (Stewart, 2005).

2.5.4 *Staphylococcus aureus* and gastroenteritis

Some strains of *S.aureus* are capable of producing staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisoning. *Staphylococcus aureus* can express two different types of toxin with superantigen activity, enterotoxins, of which there are six serotypes (A, B, C, D, E and G) and toxic shock syndrome toxin (TSST-1). Enterotoxins cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning. When expressed systemically, enterotoxins can cause toxic shock syndrome (TSS) (Stewart, 2005).

Several staphylococcal species other than *S.aureus* reportedly produce staphylococcal enterotoxins. For instance, among the coagulase negative species; *S.cohnii, S.haemolyticus, S. epidermis and S.xylosus* have been isolated from ewe‘s milk and found to produce one or several staphylococcal enterotoxins. *Staphylococcus intermedius* is the only non-*S. aureus* species that has been clearly involved in staphylococcal food poisoning. Staphylococcal enterotoxins are highly stable, resist most proteolytic enzymes (such as pepsin and trypsin), and thus keep their activity in the digestive tract after ingestion. Genes encoding staphylococcal enterotoxins have variable genetic supports most of which are mobile genetic elements. The main regulatory system controlling the gene expression of virulence factors in *S. aureus* is the accesory gene regulator (*Agr)* which works in combination with the staphylococcal accesory regulator (*Sar).* The *arg* system is one of the most important and well characterised operons in *S.aureus* biology (Stewart, 2005).

2.6 Pathogenic *Escherichia coli*

E. coli is a Gram-negative, oxidase-negative, rod-shaped bacterium from the family Enterobacteriaceae. It is able to grow both aerobically and anaerobically, preferably at 37°C, and can either be non-motile or motile, with peritrichous flagella. *Escherichia coli* is readily isolated from fecal samples by plating on selective media. The change in pH due to lactose fermentation can be used to differentiate between lactose-fermenting and non-lactose-fermenting strains, as lactose-positive E. coli colonies will appear red or pink on media such as MacConkey agar (Croxen *et al*., 2013).

Enteric *E. coli* are both natural flora of humans and important pathogens causing significant morbidity and mortality worldwide. Many pathogenic strains cause a wide spectrum of diseases, ranging from self-limiting to life threatening intestinal and extra-intestinal illnesses. Enteric *E. coli* infections are traditionally divided into 6 pathotypes based on their pathogenicity profiles (virulence factors, clinical disease and phylogenetic profile): Enteropathogenic *E. coli* (EPEC), Enterohamerrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC) and Diffusely Adherent *E. coli* (DAEC). Two further pathotypes have recently emerged; Adherent Invasive *E. coli* (AIEC) which has been implicated with Crohn disease but does not cause diarrhogenic infection, and the Shiga Toxin (Stx) producing Enteroaggregative *E. coli* (STEAEC) responsible for the 2011 Germany *E. coli* outbreak. As a population, *E. coli* strains can be assigned phylogenetically to 5 main groups namely; A, B1, B2, D, and E. Commensal isolates are mostly grouped in phylogroup A (Kaper *et al*., 2004; Croxen *et al*., 2013).

Table 2.5 Overview of enteric *E. coli* pathotypes (Croxen *et al.*, 2013)

Pathotype	adhesin	toxin	T3SS	SPATE	disease
ETEC	Colonization factors (CF) Porcine A/E associated adhesin (Paa)	Heat-labile enterotoxin (LT) Heat-stable enterotoxin (ST), Cytolysin A	-	ETEC autotransporter A (EatA)	Acute watery diarrhea (< 5yo) Travelers' diarrhea
EAEC	Aggregative adherence fimbriae (AAF) (I, II, III, Hda) Toxigenic invasion loci A (Tia)	EAEC heat-stable enterotoxin 1 (EAST1) Shigella enterotoxin (ShET)1 Hemolysin E	+/-		Travelers' diarrhea Infant diarrhea
DAEC	afimbrial (Afa), or fimbrial (Dr) adhesins	-	-	Sat	Acute diarrhea (< 5yo)
EPEC	Intimin Bundle forming pili (BFP)	-	LEE encode-d	EspC	Infant diarrhea
EIEC	-	ShET1/2	pINV encoded	Shigella extracellular protein (Sep)A SigA	Shigellosis
EHEC	Intimin Paa Toxin B (ToxB), E. coli factor for adherence (Efa)-1, LPF STEC autoagglutinating adhesin (Saa), E.coli Ig protein (EibG), Outer membrane protein A (OmpA) Iha	Stx	LEE encoded	EspP	Food poisoning

2.6.2 Survival and growth characteristics of pathogenic *E. coli*

The extent to which *E. coli* (particularly the pathogenic strains) can survive in open environments and which factors affect this survival rate are crucial issues from a fundamental perspective. However little is known about their lifestyle outside a living host, instead much focus has been directed to how they survive in the host ‘s alimentary tract. All pathogenic strains of *E. coli* contain genomic regions (islands) loaded with a suite of virulence genes that encode important factors for adherence/colonization, invasion, secretion of toxic compounds and transport functions, as well as siderophore production (Touchon *et al.*, 2009). *Escherichia coli* that enter the human/animal stomach with ingested food are confronted with severe stress due to the often low pH in the stomach, but they are able to survive the gastric stresses. Recent studies have shown that pathogenic *E. coli* are able to survive pH 2.5 in the stomach due to secretion of the ‘shiga toxin’. This toxin is expressed by phage infected bacteria. Optimum growth of E. coli occurs at 37 °C, but some laboratory strains can multiply at temperatures up to 49 °C. *E. coli* is a facultative anaerobe, meaning that it uses oxygen when it is present and available. Notwithstanding their capabilities to tolerate extreme conditions in the digestive tract, some *E. coli* strains produce filamentous structures that extend from the cell surface and help cells to attach to surfaces (e.g plant surfaces). Owing to this ability, *E. coli* coming from external sources like soil, manure, irrigation water or contaminated seeds may colonize (and thus find a refuge niche) plants such as radish and lettuce (Van Elsas *et al*., 2011).

2.6.3 Virulence factors in pathogenic *E. coli*

Virulence associated properties of *E. coli* enable the bacteria to harmfully parasitize the host. Adherence factors enable the bacteria to directly interact with the epithelial cells, and this is mediated by surface structures or molecules , like the fimbrial adhesins, the afimbrial adhesins, and/or outer membrane proteins. The exotoxins (aptly named although not all are actually excreted into the extracellular environment) are proteins that can target either the cell skeleton (type 3-secreted effectors), or the cell metabolism (intracellular AB toxins with enzymatic activity; oligopeptide toxins activating a metabolic cascade after fixation on the cell cytoplasmic membrane), or the cell cytoplasmic membrane (enzymatic and pore-forming cytolysins). Besides the STa and STb oligopeptide enterotoxins, porcine and human ETEC strains can also produce an A_1B_1 heat-labile enterotoxin or LT. The heat-labile enterotoxins are produced by the type 2 secretion system. The action of heat-labile enterotoxins results in the opening of the cystic fibrosis transmembrane conductance regulator (CTFR) with increased secretion of chloride and carbonate ions, and of water and the inhibition of sodium absorption, leading to watery cholera-like diarrhea (Kaper *et al.*, 2004).

2.6.4 Epidermiology and pathogenesis of *E. coli*

Human infections occur through consumption of contaminated food products (undercooked meat, or contaminated fresh produce such as salad leaves), drinking water contaminated with animal or human waste, or through direct person-to-person spread from poor hygiene. In the developing world ETEC, EPEC and EAEC appear to be major causes of infantile diarrhea with potentially fatal consequences when untreated, while in the developed world these infections are

mild and self-limiting. EHEC and more recently EAEC and STEAEC are the main E. coli pathotypes associated with food poisoning outbreaks in the developed world (Clements *et al*., 2012). ETEC is reportedly the most commonly isolated bacterial enteropathogen in children under 5 years of age in developing countries, representing approximately 20% of cases, equivalent to several hundred million cases of diarrhea and tens of thousands of deaths each year (Allocati *et al*., 2013). ETEC is the most common cause of traveler's diarrhea and is known to cause disease in animals. EAEC is the second most common cause of travelers' diarrhea after ETEC and its prevalence in endemic and epidemic disease is becoming well recognized. It is responsible for persistent diarrhea in children in developing countries. Three pathotypes of E. coli (EHEC, EPEC and EIEC) employ a type 3 secretion system to translocate bacterial proteins, known as effectors, directly into the eukaryotic host cell in order to subvert host cell processes. As indicated by table 2.5 there are numerous virulence strategies, including secretion of enterotoxins, and watery diarrhea is a common clinical manifestation among the pathotypes (Allocati *et al*., 2013).

2.7.1 Other notable pathogens of clinical significance : *Shigella*

Shigella is a genus of Gram-negative, facultative aerobic, nonspore-forming, nonmotile, rod-shaped bacteria genetically closely related to E. coli. *Shigella* species, members of the family Enterobacteriaceae, are responsible for causing acute gastroenteritis which is one of the most common causes of morbidity and mortality in children in developing countries. Shigellosis (disease caused by shigella) is ubiquitous in impoverished populations of Asian and African countries and antibiotic-resistant strains of different *Shigella* species and serotypes have emerged all over the world. It comprises four species, S. *dysenteriae, S. flexneri, S. sonnei,* and *S. boydii,*

which are further classified into serotypes based on biochemical differences and variations in their O-antigen (Marteyn *et al.,* 2012). The primary mode of transmission is by faeco-oral route and as low as 10-100 bacteria can cause infection - Such a low infective dose enables *Shigella* to cause large outbreaks. The high incidence of *Shigella* in the developing world is generally attributed to lack of clean water, poor hygiene, malnutrition and close personal contact. Shigellosis is associated with a few medical complications and diagnosis is made by culture isolation of *Shigella* from faeces or rectal swabs (Marteyn *et al*., 2012).

2.7.2 Hepatitis A virus

Hepatitis A virus (HAV) is a positive-stranded RNA virus of the Herpavirus genus of Picornaviridae that is 27 nm across, non-enveloped, and icosahedral. It causes a viral liver disease, it is transmitted through ingestion of contaminated food and water or through direct contact with an infectious person. Hepatitis A is highly distributed to Africa, South America, and Asia, the risk of infection is associated with a lack of safe water, and poor sanitation and hygiene (such as dirty hands). Diagnosis is made by the serological detection of Hepatitis A virus specific immunoglobulin antibodies in the blood (Franco *et al*., 2012).

2.7.3 Norovirus

Norovirus is a very contagious virus that causes vomiting and diarrhea, it sometimes referred to as the winter vomiting bug. It is transmitted from person to person and indirectly via contaminated food and water. Studies have shown that very few virus particles can cause infection, and the virus can be aerosolized from vomit or diarrhea. The virus is diagnosed by polymerase chain reaction (PCR), and tests such as ELISA although less specific and sensitive.

The virus replicates in the small intestines and symptoms of infection are watery diarrhea, forceful vomiting, nausea and abdominal cramps (Robilotti *et al*., 2015).

CHAPTER 3 : MATERIALS AND METHODS

3.1 Study site

The study was carried out at Midlands state university‘s applied biosciences laboratories, situated in Gweru in the midlands province of Zimbabwe. Midlands State University is an institute of higher learning, its biosciences laboratories are located at the main campus in the Senga area.

3.2 Study design

The study was done in the months of July and August 2018, it was anonymous and non-invasive. Spices from different vendors in the Gweru CBD area were considered for this study. A total number of 40 samples (*viz* 12 turmeric, 9 garlic, 9 ginger, and 10 basil) were randomly selected from the vendors, each sample contained in a 10 gram plastic sachet.

Fig 3.1 Vendors operating outside a supermarket in Gweru CBD

3.3 Sample collection

Samples were given unique identifiers upon collection. Samples from the turmeric class were given identifiers that started with the letter T, and numbers that represented the order with which they were collected. Based on this criteria the 12 samples were assigned identifiers that began from T1 up to T12. The same criteria was used to issue identifiers to the basil group, and samples from this group were labelled B1 up to B10. Ginger samples were labelled G1 to G9, and Garlic samples were labelled GC1 to GC9. The samples were collected randomly five different vendors all operating in and around the Gweru CBD.

3.4 Laboratory analyses

3.4.1 Primary sample analysis

Upon entering the laboratory the samples were inoculated onto the surface of MacConkey agar and Mannitol salt agar. Two loopfuls of sample were extracted from each 10 gram sachet, and immediately streaked onto MacConkey agar and Mannitol salt agar respectively. All inoculated samples were incubated at 37 ºC for 24 hours, and the work was done aseptically inside biological safety cabinets. The laboratory samples were assigned revised identifiers due to the fact that two loopfuls were extracted from each sample sachet for inoculation onto the surfaces of MacConkey agar and Mannitol salt agar respectively.

3.4.2 Laboratory sample identifiers

Twelve turmeric sub-samples to be inoculated in MacConkey agar were assigned the labels TA1 to TA12, another 12 to be inoculated in Mannitol salt agar were assigned labels TB1 to TB12. Nine ginger sub-samples to be inoculated in MacConkey agar were labelled GA1 to GA9, while the other 9 to be inoculated into Mannitol salt agar were labelled GB1 to GB9. Ten basil sub-samples were labelled BA1 to BA10 prior to inoculation onto the surface of MacConkey agar, and another 10 sub-samples labelled BB1 to BB10 for inoculation onto the surface of Mannitol salt agar. 9 garlic sub-samples were labelled GCA1 to GCA9 for inoculation onto MacConkey agar, and another 9 sub-samples were labelled GCB1 to GCB9 prior to treatment in Mannitol salt agar.

3.4.3 Enrichment and preparation of serial dilutions

A loopful (about 1ml) from each sub-sample was inoculated in 9 ml buffered peptone water and incubated at 37 ºC for 18 hours for enrichment. After enrichment, 1 ml of sample enriched

nutrient broth was pipetted into 9 ml of sterile peptone water. This represented a dilution factor of 10^1. A final dilution factor of 10^3 was achieved by pipetting 1 ml from each dilution into 9 ml sterile peptone water blanks (i.e twofold serial dilutions).

3.5 Total bacterial counts

After the serial dilutions 1 ml from each 10ml dilution was pipetted onto a corresponding plate count agar dish and incubated at 35 ºC for 25 hours.

3.6 Bacterial enumeration

After the incubation the petri dishes were examined for bacterial counts. Plates with colonies ranging between 30 and 300 were considered, and the number of colonies counted was represented as colony forming units per milliliter (cfu/ml).

3.7 Isolation of presumptive pathogenic bacteria

All annotated samples were inoculated in appropriate culture media for selective growth of *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus.*

3.7.1 Primary identification of *Salmonella enterica* and *Escherichia coli*

Samples taken from turmeric, ginger, garlic, and basil were inoculated on MacConkey agar for selective growth of *Salmonella enterica* and *Escherichia coli.* Following inoculation the inoculum was streaked with a sterile wire loop from the point of inoculation to other quadrants on the petri dish. These steps were performed in a laminar flow hood under aseptic conditions. Samples cultured on MacConkey agar were then incubated at 37 ºC for 24 hours, along with two petri dishes serving as negative controls. Following incubation the cultures were observed for the growth of lactose fermenting coliforms (*E. coli* colonies) and non lactose fermenting coliforms (*salmonella* spp).

3.7.2 Secondary screening of *Salmonella enterica* : citrate test

Simmons citrate agar was prepared according to specifications by the manufacturer, and poured into a test tube to cool and solidify. The medium was inoculated lightly on the slant by transferring a colony with the aid of a sterile inoculating loop. A negative control was set up in a test tube containing citrate agar without inoculation. The test tubes were observed after an incubation period of 24 hours for blue coloration which denotes alkalinisation.

3.7.3 Lysine decarboxylase test

Lysine decarboxylase broth was prepared according to the instructions by the manufacturer, and poured into a sterile test tube. A presumptive *Salmonella enterica* colony was transferred to the

broth with the aid of a sterile inoculating loop and the test tube was sealed with a new cap. The culture was incubated at 37 ºc for 24 hours before the first observations were made. Observations were then made after another 24 hours to make it a total of 48 hours incubation period. Aseptic conditions were maintained throughout, the medium was observed for a yellow coloration at 24 hours and a purple coloration at 48 hours.

3.7.4 Indole test

Tryptophan broth was prepared in accordance to the instructions provided by the manufacturer. It was poured into glass bottle and inoculated with a presumptive *Salmonella enterica* colony. The colony was transferred from the agar plate with aid of a sterile inoculating loop that was exposed to the flame of a Bunsen burner. A negative control was set up in another glass bottle and the tryptophan broth was not inoculated. These procedures were undertaken in a laminar flow cabinet and aseptic conditions were maintained. After an incubation period of 24 hours 0.5 ml of Kovac‘s reagent was added to the broth culture of the test organism and of the control. The broths were observed for a pink colour change which denotes the presence of the compound indole and a positive test.

3.7.5 Secondary screening of *Escherichia coli*

Presumptive colonies of *E. coli* were screened for the ability to produce the compound indole and to utilize sodium citrate in the same ways described in sections 3.7.4 and 3.7.2 respectively. Aseptic conditions were maintained throughout. A further test was performed to confirm the presumptive organism‘s ability to hydrolyze urea (i.e urease test).

3.7.6 Urease test

Urea broth was prepared and poured into two glass tubes. The first tube was inoculated with presumptive colonies of *E. coli* on the slanting surface of the medium with the aid of a sterile inoculating loop inside a laminar flow cabinet. The second tube was not inoculated with the test organism. The slants were incubated at 36 ºC for 24 hours, after the incubation period they were observed for a colour change from yellow to magenta.

3.7.7 Primary screening of *Staphylococcus aureus*

Samples taken from turmeric, ginger, garlic, and basil were inoculated on Mannitol salt agar for the selective culture of *Staphylococcus aureus.* The culture medium was prepared according to the manufacturer‘s specifications, and the sample inoculum was pipetted from the enrichment broth onto the medium. After inoculation the inoculum was streaked to form three quadrants in the petri with the aid of a sterile inoculating loop. A negative control was established in a petri dish that contained culture without inoculum. The cultures were incubated at 37 ºc for 24 hours. Following incubation the cultures were observed for the growth of yellow colonies (i.e Mannitol fermenting coliforms).

3.7.8 Secondary screening of *Staphylococcus aureus*

Presumptive colonies of *Staphylococcus aureus* were screened for the ability to produce indole, utilize sodium citrate and hydrolyse urea in the ways described in sections 3.7.4, 3.7.2, and 3.7.6 respectively. The results of the tests were noted and interpreted.

3.8 Data analysis

Data from the total bacterial counts, prevalence of the four groups of pathogens, and percentages of contamination were presented on tables, charts, and graphs. Bacterial counts across the four sample groups conformed to normality (Shapiro-wilk) and therefore were analyzed using One Way Analysis of Variance (One-Way ANOVA) SPSS package v 20.

3.9 Microbiological specifications for ready to eat food on the market

Table 3 Hygiene guidelines for ready to eat food

Indicator organism	Cfu/ml		
	Satisfactory	Borderline	unsatisfactory
enterobacteriaceae	$< 10^2$	10^2 - $\leq 10^4$	$> 10^4$
Escherichia coli	<20	20 - $<10^2$	$> 10^2$

Source : Food safety guideline according to the Centre for food safety in Hong Kong.

CHAPTER 4 : RESULTS

4.1 Primary and secondary screening of pathogenic bacteria

Confirmatory biochemical tests were done to establish the identity of each pathogenic organism in accordance with Bergey‘s manual of systematic bacteriology. Prior to the biochemical tests, presumptive organisms were primarily identified on the basis of colony morphology in the culture media.

4.2 Identification of *Salmonella enterica*

On MacConkey agar presumptive *Salmonella enterica* colonies were observed as whitish, lactose negative colonies. The presumptive colonies were inoculated on citrate agar slant to observe alkalinisation, and a positive result was obtained from the citrate test. After the citrate test, presumptive *Salmonella enterica* colonies were further subjected to Lysine decarboxylase and Indole tests, positive and negative results were obtained respectively. The results are summarized in Table 4.1 below. A total of 29 isolates were suspected to be *Salmonella enterica* and turmeric had 10 isolates, ginger had 6 isolates, garlic had 6 isolates, and basil had 7 isolates.

Table 4.1 Identification of *Salmonella enterica*

MAC	**Citrate**	**Lysine decarboxylase**	**Indole**	**Suspected bacteria**
+	+	+	_	*Salmonella enterica*

Key: MacConkey agar (MAC) Positive (+) Negative (-)

4.3 Identification of *Staphylococcus aureus*

On the surface of Mannitol salt agar presumptive *Staphylococcus aureus* colonies were observed as yellow, and milky in growth zones. This indicated a positive test on Mannitol salt agar. The presumptive colonies were then subjected to citrate test, blue coloration (denoting alkalinisation) was observed showing a positive test. The presumptive *Staphylococcus aureus* colonies were further subjected to urease and Indole tests. In the urease test a colour change from yellow to magenta was observed signifying a positive test. In the Indole test a pink colour change was not observed showing the absence of the compound Indole and consequently showing a negative test. The results are summarized below in Table 4.2

Table 4.2 Identification of *Staphylococcus aureus*

MSA	Citrate	Urease	Indole	Suspected bacteria
+	+	+	-	*Staphylococcus aureus*

Key : Mannitol salt agar (MSA) Positive (+) Negative (-)

A total of 25 isolates were suspected to be *Staphylococcus aureus,* 10 were isolated from turmeric samples, 3 from ginger samples, 5 from garlic samples, and 7 from basil samples.

4.4 Identification of *Escherichia coli*

Primary screening of *Escherichia coli* was done on MacConkey agar. Presumptive *Escherichia coli* colonies were observed as red, lactose positive colonies. The presumptive colonies were further subjected to the following biochemical screens for confirmation; citrate test, urease test, and indole test. In the citrate test no colour change was observed, the result could not be

differentiated from the control, hence a negative test result was obtained. In the urease test a colour change was also not observed and a negative test result was obtained. The presumptive colonies were able to produce Indole in the Indole test meaning that a positive test was obtained. *Escherichia coli* was the least prevalent organism isolated and only 7 isolates were obtained across all four sample types. The results are summarized in table 4.3 below

Table 4.3 Identification of *Escherichia coli*

MAC (colony appearance)	**Citrate**	**Urease**	**Indole**	**Suspected bacteria**
red, cloudy	_	_	+	*Escherichia coli*

Key : Positive (+) Negative (-)

4.5 Total bacterial counts

The total bacterial counts for turmeric ranged from 3.5 x 10^4 cfu/ml to 1.75 x 10^5 cfu/ml. The total bacterial counts for basil ranged from 6.9 x 10^4 cfu/ml to 2.5 x 10^5 cfu/ml. The total bacterial counts for ginger ranged from 4.1 x 10^4 cfu/ml to 1.17 x 10^5 cfu/ml. Total bacterial counts for garlic ranged from 5.4 x 10^4 cfu/ml to 9.6 x 10^4 cfu/ml. The mean counts for each class of sample were as follows; turmeric had 9.4 x 10^4 cfu/ml, ginger had 7.3 x 10^4 cfu/ml, garlic had 7.5 x 10^4, and basil 1.53 x 10^5 (Fig 4.1 below).

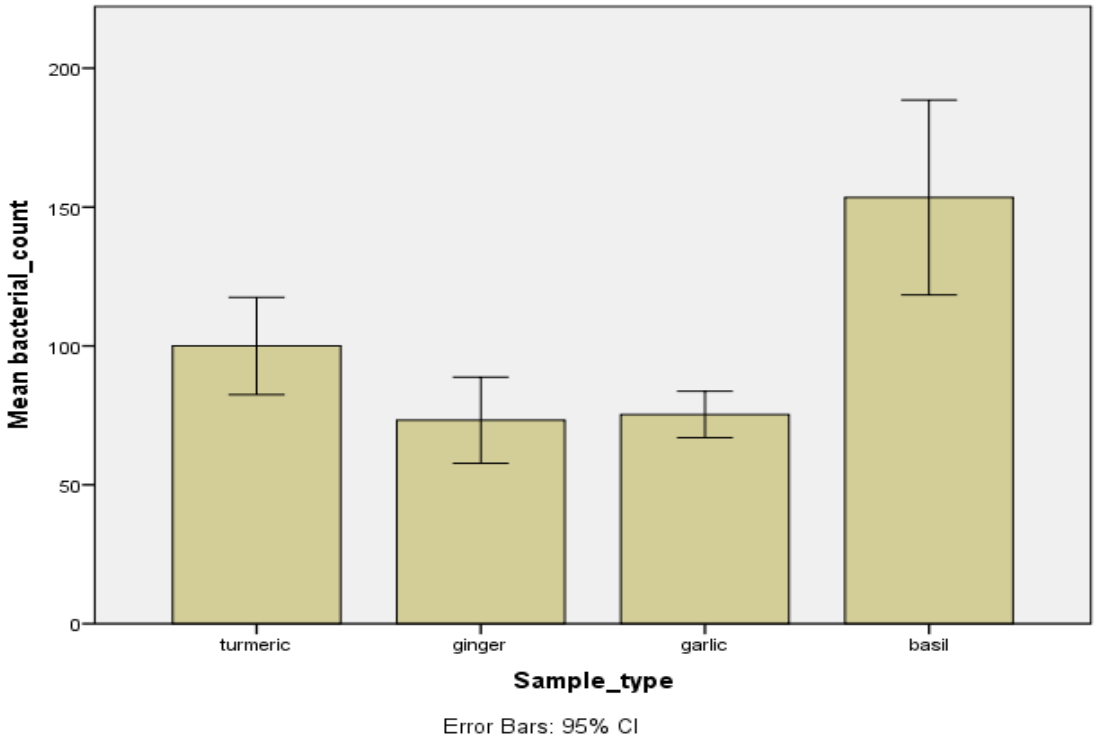

Fig 4.1 Mean total bacteria count (cfu/ml)

4.6 Prevalence of specific bacteria across different sample classes

Three genera of bacteria were isolated from the four sample types and these were suspected to be *Salmonella enterica, Staphylococcus aureus,* and *Escherichia coli.* Primary identification and recovery was done by analyzing the bacterial colonies on selective media. Secondary confirmatory screening was done for each presumptive organism as described in section 3.7. From the results prevalence of specific bacterial species across all the sample types was determined. *Salmonella enterica* was established to be the most prevalent organism amongst the contaminated samples contributing to 47.5 % of the contamination. *Staphylococcus aureus* was the second most prevalent organism among the contaminated samples contributing 40.9 % of the contamination. *Escherichia coli* was the least common organism contributing 11.5 % of all

contaminated samples. Below is a graphical presentation of the overall prevalence of the pathogenic organisms in the samples.

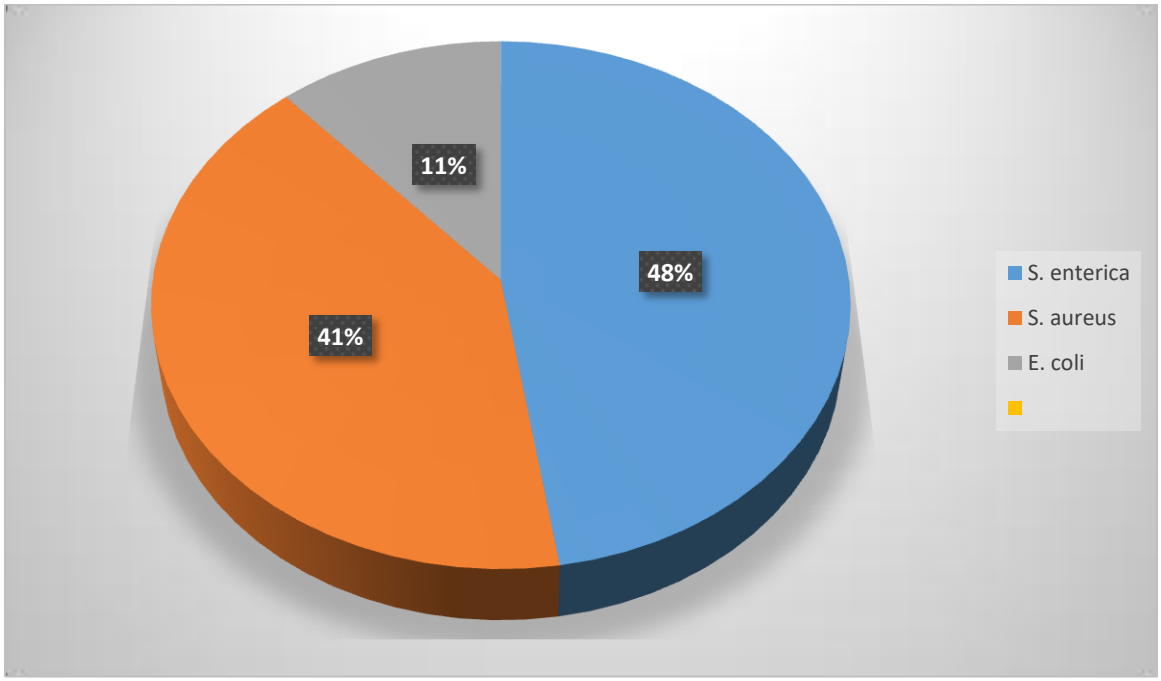

Figure 4.2 showing overall prevalence of the three pathogenic organisms across all the samples

76.25 % of the samples were contaminated and there was significant difference observed between total bacterial count and sample type (ANOVA p = 0.00).

CHAPTER 5 : DISCUSSION

5.1 Total bacterial counts

The bacterial load of the sampled spices and herbs represents the measurable amount of bacteria for each sample, and provides insight on the levels of contamination. The results of this study show that 76.25% (61 out of 80) of all samples were contaminated, and among the contaminated samples *S. enterica* was the most prevalent (Figure 4.2). The Food Safety standards of Hong Kong set the maximum limit for total aerobic mesophilic bacteria in ready to eat substances (Table 3, section 3.9) at $>10^4$. The borderline range is between 10^2 -$\leq 10^4$, and the acceptable range is $<10^2$. As per these specifications, 52.5% of the contaminated samples were within the borderline range while 47.5% were beyond the $>10^4$ limit which renders them unacceptable. All the samples that yielded *E. coli* had counts greater than 10^2, and were thereby unacceptably contaminated. *Escherichia coli* should never be found in food substances and it is an indicator of fecal contamination. The other enterobacteriaceae species are used more generally as indicators of hygienic quality rather than of fecal contamination and therefore say more about general microbiological quality than possible health risks posed by the product (Adams & Moss, 1995).

It was observed that basil had the greatest mean and range for bacterial counts, followed by turmeric, garlic and ginger that were some way off. The connection between basil in particular and the high bacterial load could be attributed to different factors (particularly contamination during processing). However, significant incidents of food poisoning have been linked with basil in the past. In 2007, basil exported from Israel was established to have been the cause of 32 cases of salmonellosis in England and Wales according to the UK government (Smith, 2008). It

is possible that the various phytochemical constituents of the basil plant are not as hostile to the bacteria as are other samples. It is unsurprising that *S. enterica* was the most prevalent organism isolated across all four sample types because of the typhoid outbreak that hit Gweru during the month of August in 2018. Typhoid fever is an acute illness associated with fever caused by the *S. enterica* serotype Typhi bacteria. The prevalence of this pathogenic organism in all the spices and herbs suggests that most food processing environments were contaminated with *S. enterica*. According to Boyle et al (2007) contaminated water plays a significant role in the transmission of *S. enterica* in urban settings. Water plays an important role during processing of these spices and herbs, and it is unlikely that the vendors use gloves or other specialized equipment when they pack the products into sachets. Gloves are expensive and unpopular for such operations and as such the vendors just wash their hands and get on proceed to pack the substances. However, as of August 8, 2018 the Ministry of health and child welfare declared Gweru water unsafe for consumption, overruling all previous assertions in the process (Mrewa, 2018). In essence, unsafe water was used during the processing of these products. Considering the food safety guidelines on total bacteria counts from Hong Kong ‘s food safety centre the spices and herbs were grossly contaminated.

5.2 Potential sources of contamination

In addition to unsafe water, there are other potential sources of contamination of the spices and herbs before they enter the market. At harvest, the products can become contaminated with pathogenic microorganisms through several mechanisms. They can become contaminated during picking, or through contaminated tools like scissors, knives and chippers. The manure used as fertilizer can also be a source of contamination. A number of studies have demonstrated the long

term survival of E. coli 0157.H7 and salmonella in manure (Okafor et al., 2003). Pre-harvest contamination of produce can also take place through migratory or feral animals. Outbreak investigations have revealed direct link between produce and animal reservoirs. Several recent produce-associated outbreaks have followed wildlife intrusion into growing fields or fecal contamination from nearby animal production facilities that likely led to produce contamination (Okafor et al., 2003). Distribution, marketing and storage also play important roles in microbial contamination of spices and herbs. Contamination can occur via storage in the market in contaminated bins and other containers, and possible contact with decaying products. Contamination also occurs when the products are not stored at the right temperature, thus promoting the temperature danger zone (i.e. the temperature in which bacteria can be the most widely spread).

5.3 Isolation of the pathogenic microorganisms

Isolation of the pathogenic organisms was primarily based on observation of different colony morphologies in MacConkey agar and Mannitol salt agar. Biochemical confirmation of the presumptive organisms was performed in accordance to Bergey‘s manual of systematic bacteriology. However, laboratory limitations prevented further classification of pathogens like *S. enterica* and *S. aureus* down to serotypes and species level respectfully. Molecular techniques like real time pcr are ideal for species level classification and determination of bacterial load.

Previous studies on the microbiological safety of spices and herbs have shown that foodborne pathogens are a common occurrence when the products are sold in the streets. A study of herbal preparations marketed openly in south eastern Nigeria revealed very high degrees of

contamination. *S. aureus, S. enterica*, and *E. coli* were among a host of indicator organisms (Esimone et al., 2001). This implies that government regulations on the safety standards of substances sold on the streets are either serially breached, or inadequately enforced. The government of Zimbabwe has attempted to rectify this by chasing vendors away from the streets. However, due to deep economic problems plaguing the country this solution has resulted in violent confrontations between the police and the vendors and it does not appear to be the feasible long term solution (Chadenga, 2018). As a result, the problems associated with foodborne illnesses may persist. In Gweru vendors have been successfully removed from the streets in the CBD area but the crux of the matter remains unsolved.

5.4 Conclusion and recommendations

The reliable safe supply of spices and herbs that are free from harmful contaminants is important for the people‘s general health and daily life, economic development and social stability and the government ‘s image. It is recommended that the government (through the city councils and the Medicines Control Authority of Zimbabwe) ensure the safety of spices and herbs that enter the markets through vendors. The government should also embark in education and awareness campaigns about the dangers posed by selling substances that are unsafe for human consumption. This would encourage the vendors to engage in Good Manufacturing Practice and thereby play their role in keeping the consumers safe. The government should engage the private sector and establish vending markets on the periphery of CBD areas without compromising the vendors themselves or their businesses. This would also help in regulating the microbiological safety of vended xenobiotics by increasing their surveillance.

The study revealed the presence of three pathogenic organisms namely *S. enterica, S. aureus,* and *E. coli*, however it is possible that a wider range of organisms would have been isolated (including fungi and their mycotoxins). The laboratory facilities were the limiting factor to this possibility. As stated in previous sections molecular techniques would have enabled greater profiling of the pathogenic organisms. This would even make it possible to do parsimony studies and establish relatedness between the *S. enterica* isolated in the samples and the *S. enterica* that caused the Gweru typhoid outbreak of 2018. From the results, it is clear that the spices and herbs sold on the markets are contaminated and unsafe for human consumption.

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APPENDICES

APPENDIX 1 TOTAL BACTERIAL COUNTS FOR TURMERIC, GINGER, GARLIC AND BASIL

Bacterial counts for Turmeric samples

Sample	Bacteria	Bacterial count (cfu/ml)	Sample	Bacteria	Bacterial count (cfu/ml)
TA1	*S. enterica*	4.7×10^4	TB1	*S. aureus*	3.5×10^4
TA2	*S. enterica*	5.8×10^4	TB2	*S. aureus*	5.1×10^4
TA3	*S. enterica*	1×10^5	TB3	*S. aureus*	1.55×10^5
.1TA4	*S. enterica*	1.28×10^5	TB4	*S. aureus*	9.9×10^4
TA5	*S. enterica*	9.3×10^4	TB5	*S. aureus*	7.5×10^4
TA7	*S. enterica*	6.4×10^4	TB6	*S. aureus*	8.2×10^4
TA8	*S. enterica*	1.75×10^5	TB8	*S. aureus*	1.06×10^5
TA9	*S. enterica*	1.5×10^5	TB9	*S. aureus*	1.24×10^5
TA10	*S. enterica*	1.41×10^5	TB11	*S. aureus*	7.9×10^4
TA11	*E. coli*	1.13×10^5	TB12	*S. aureus*	9.1×10^4
TA12	*S. enterica*	1.34×10^5			

Bacterial counts for Ginger samples

Sample	Bacteria	Bacterial count (cfu/ml)	Sample	Bacteria	Bacterial count (cfu/ml)
GA1	*S. enterica*	6.8×10^4	GB1	*S. aureus*	7×10^4
GA2	*S. enterica*	1.09×10^5	GB2	*S. aureus*	1.17×10^5
GA3	*S. enterica*	8.1×10^4	GB3	*S. aureus*	4.4×10^4
GA4	*S. enterica*	8×10^4			
GA5	*E. coli*	6.6×10^4			
GA6	*E. coli*	4.1×10^4			
GA7	*S. enterica*	9.5×10^4			
GA8	*S. enterica*	5.9×10^4			
GA9	*E. coli*	4.9×10^4			

Bacterial counts for Garlic samples

Sample	Bacteria	Bacterial count(cfu/ml)	Sample	Bacteria	Bacterial count(cfu/ml)
GCA1	*S. enterica*	5.7×10^4	GCB3	*S. aureus*	9×10^4
GCA2	*S. enterica*	6.2×10^4	GCB4	*S. aureus*	9.4×10^4
GCA3	*S. enterica*	6.3×10^4	GCB5	*S. aureus*	7.6×10^4
GCA4	*S. enterica*	8.7×10^4	GCB7	*S. aureus*	7.9×10^4
GCA5	*S. enterica*	6.1×10^4	GCB9	*S. aureus*	5.9×10^4
GCA6	*E. coli*	9.6×10^4			
GCA8	*S. enterica*	7.5×10^4			
GCA9	*E. coli*	8×10^4			

Bacterial counts for Basil samples

Sample	Bacteria	Bacterial count(cfu/ml)	Sample	Bacteria	Bacterial count(cfu/ml)
BA1	*S. enterica*	7.4×10^4	BB1	*S. aureus*	2.43×10^5
BA2	*S. enterica*	1.08×10^5	BB3	*S. aureus*	1.43×10^5
BA3	*S. enterica*	1.04×10^5	BB4	*S. aureus*	2×10^5
BA4	*S. enterica*	1.73×10^5	BB5	*S. aureus*	2.38×10^5
BA6	*S. enterica*	1.28×10^5	BB7	*S. aureus*	1.65×10^5
BA7	*S. enterica*	6.9×10^4	BB9	*S. aureus*	2.5×10^5
BA8	*E. coli*	1.06×10^5	BB10	*S. aureus*	2.14×10^5
BA9	*S. enterica*	8.7×10^4			

KEY TA (Turmeric in MacConkey) TB (Turmeric in Mannitol salt agar) GA (Ginger in MacConkey) GB (Ginger in Mannitol SA) GCA (Garlic in MacConkey) GCB (Garlic in Mannitol SA) BA (Basil in MacConkey) BB (Basil in Mannitol SA)