An assessment on microbial quality of ready to eat cooked foods (Sadza, Rice and Relish), sold by vendors at Midlands State University (MSU) main campus bus terminus.

By

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ABSTRACT

Diarrheal diseases are the third leading cause of death in third world countries, resulting in 1.8 million deaths around the world per annum. A study was carried out to assess the microbial quality of ready to eat cooked foods sold at Midlands State University (MSU) bus terminus in March 2018. Type of samples which were microbially assessed were sadza, rice, chicken stew, beef stew, cabbage salads, pork chops and russian sausages. Sampling was done for three different days. A total of twenty one food samples were obtained from MSU bus terminus and transported to the MSU Biology Laboratory for all analyses. Total Bacterial Counts (TBC) and Total Coliform Counts (TCC) were done for each food sample in Plate Count Agar and Mackonkey Agar. Isolation and identification of specific groups of bacteria was carried out using inoculum prepared from the food samples. The mean TBC for sadza, rice, chicken stew, beef stew, cabbage salads, pork chops and russian sausages was 1.09×10^5 cfu/ml, 1.06×10^5 cfu/ml, 1.07×10^5 cfu/ml, 1.34×10^5 cfu/ml, 1.39×10^5 cfu/ml, 1.99×10^5 cfu/ml and 1.86×10^5 cfu/ml, respectively. The mean TCC of sadza, rice, chicken stew, beef stew, cabbage salads, pork chops and russian sausages was 1.26×10^5 cfu/ml, 1.09×10^5 cfu/ml, 1.18×10^5 cfu/ml, 1.85×10^5 cfu/ml, 1.52×10^5 cfu/ml, 2.05×10^5 cfu/ml and 1.98×10^5 cfu/ml, respectively. TBC counts for all food samples were below the recommended threshold of 1×10^6 cfu/ml and all TCC means for all the food samples were above the recommended threshold of 1×10^5 cfu/ml. This study showed that the type of food had no significant effect on the total bacterial count, ANOVA (P= 0.000). Cabbage salads, pork chops and Russian sausages had high TBC as compared to other samples. A total of three bacterial genera were isolated and identified and these were *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp. *Escherichia coli* was most prevalent (52.38 %) micro-organism in all the food samples. *Klebsiella* spp was isolated the least number of times with a prevalence of 38.1 %. *Staphylococcus aureus* had a prevalence of 42.85%. Identification of faecal coliforms in food which is consumed by people indicates poor quality of the food and a high degree of spoilage it has undergone. Isolation of *Staphylococcus aureus* showed poor personal hygiene of vendors and lack of knowledge on food safety. These bacterial isolates pose a great risk MSU students, staff members and the surrounding community, as they are constant customers of vendors at MSU bus terminus.

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DEDICATIONS

This dissertation is dedicated firstly to the Almighty, who has blessed me enough to do this degree without many challenges. I dedicate this body of work to my parents who always have faith in me, even when I doubt myself.

"If I have seen further than other men, it is because I stood on the shoulders of giants."

— Sir Isaac Newton

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CHAPTER 1: INTRODUCTION.

1.1 Background of the study

Across all continents, vendor food entrepreneurship is the most common source of income to a significant number of individuals, of all gender. Vendor foods are usually present at all times, mostly found at strategic areas where many people congest for a common cause (Muinde and Kuria, 2005). Most street vendors do not give precedence to customers, on the biological safety regulations implemented during food preparation and handling (Feglo, 2012).

It has been shown that Street foods have been implicated in outbreaks of foodborne illnesses all around the world (Dawson and Canet, 1991). In 1988, 14 deaths were reported in Malaysia because of foodborne diseases related to street foods and, in the same year 300 people became ill in Hong Kong after consumption of street vended foods. In 1987 in Singapore, an outbreak of cholera was also attributed to street foods (FAO, 1990). Poor knowledge on the effects improper food handling associated with certain foods, could explain the health and safety issues that street foods may pose (Rane, 2011).

Vendor food consumption is rapidly showing an increasing phenomenon in urbanized third world countries. This increase is due to high unemployment rates, low salaries and low work opportunities (Chauliac *et al.*, 1998). Customers usually opt to buy these types of foods because they are cheap, tasty, and easily available without spending most of time queuing. Individuals from low socioeconomic bracket and students at learning institutions are most popular consumers of vendor sold foods.

In the absence of running tap water for various activities, vendors may opt to re-use the water for cleaning utensils, hence bacterial contamination occurs. This has been reported in various continents like Asia, Africa and South America (Rane, 2011). Contamination in street food is of serious concern because it can cause food poisoning to human beings and death (Feglo, 2012).

Figure 1.0 A representative picture of sadza, beef stew, salads and vegetables sold by vendors on at MSU bus terminus

Cooked ready-to-eat street foods are usually sold near human public populated areas, for example, bus terminuses and near shopping centres. These places provide adequate conditions for emergence and are easily contaminated by biological pathogens (WHO, 2013). Most vendor populated areas do not have proper sanitation conditions like proper clean toilets and consistent clean water. The use of bush toilets also leads to easy transmission of disease at congested areas. At bus terminuses the presences of dust also triggers fast mobility of biological

pathogens from one individual to another, and transmission into food of *S. aureus*, *E. coli* and *Salmonella*. A study in India and Ghana on street vending showed that vendors do not follow food safety regulations and personal hygiene (WHO, 2010). Contaminated food and water is the most cause of illness and death in most of Africa's developing nations and the world (WHO, 2015).Antibiotic resistance of the food borne pathogens have increased due to overuse of β-lactam antimicrobial agents for treating infections (Tayebi *et al*., 2016). Some symptoms of consumption of contaminated food and water include nausea, abdominal pains, diarrhoea, headache and dehydration. Eating food that has been prepared at home also poses a large risk to customers as disease can be transported from home by street vendors.

The Food and Food Standards Act of Zimbabwe requires all meat companies, fisheries and abattoirs to bring all their meats to be inspected, before selling to consumers. Nhari, (2013) reported that, the total bacterial count in any kind of food for human consumption in Zimbabwe should be less than 1×10^6 cfu/g. Recently, Zimbabwe has been hit by many outbreaks of diseases like dysentery, typhoid and cholera. These disease are caused by poor sanitary conditions, food and water contamination (Rusare, 2015). An outbreak of cholera from the year 2008 to 2009 affected about 55 districts in Zimbabwe, infecting 99 704 people and killing 4 420 (NHS, 2009-2013).

1.2 Problem statement

The position of vendors at MSU terminus selling food is questionable. According to Mr Mhuka health director at MSU clinic, cases of diarrhoea are increasing, hence the need of an assessment of microbial quality of food. Sadza, rice, beef stew, chicken stew, salads, pork and sausages are the most popular type of food being sold by vendors at MSU bus terminus. However, at MSU vendors selling ready to eat cooked foods are ever increasing each semester.

Most of students at the campus prefer to go and buy food from these vendors because there are no long queues and there is variety of choice which is the opposite of the dining hall standards. On other hand the dining hall (DH) which is supposed to be the central point for students' meal is being neglected, leaving students opting for vendors' food. MSU bus terminus is a place where there is dust and it is an open space which is risky to buy food such as ready to eat cooked foods. The unhygienic nature of the bus terminuses where these ready to eat products are sold increases risk of food contamination as well as poisoning. The bulk sale of a single batch of these foods means they can be distributed to a large number of individuals very quickly, with the potential spread of contaminants easily.

1.3 Justification

The current economic climate in this country has led to people with less income being forced to do vending. This has led to popping up of unskilled enterprising personnels' selling the ready to eat cooked foods. These vendors usually prepare their food at home and when they are at the terminus point most of their food would be cold and this provides adequate breeding temperatures for bacteria. Most vendors do not subject their products to any quality body that would subject their products to any rigorous testing of any sort.

Ignorance and inadequate education leads to vendors selling their products in unsafe places, which compromises the health of the customers. Disregarding gender, not all of them are properly educated especially with regarding the aspects of food hygiene. There are no toilets and no tapped running water at MSU bus terminus. The washing of hands, utensils, and dishes is often done in open space. Another major concern is that the food is not adequately covered and protected from flies and dust. There is one litter bin at the MSU terminus hence people would litter everywhere. Studies on ready-to-eat cooked foods were carried out in most parts

of Zimbabwe but only a little or nothing, has been done on the microbial quality of ready to eat cooked foods sold by vendors at MSU bus terminus.

1.4 Objectives of study

1.4.1 Main objective:

- to assess the microbial quality of ready to eat cooked foods being sold by vendors at MSU main campus bus terminus.

1.4.2 Specific objectives:

- to isolate and identify the bacteria present in ready to eat cooked foods sold by vendors at MSU main campus bus terminus,
- to enumerate the bacteria present in the food sold by vendors at MSU main campus bus terminus,
- to determine the prevalence of pathogenic bacteria in the food sold by vendors at MSU main campus bus terminus, and
- to determine the antibiotic susceptibility pattern of the isolated microorganisms.

CHAPTER 2: LITERATURE REVIEW.

2.1 Food safety knowledge

It is estimated that 48 million cases of foodborne diseases occur each year in the United States of America (USA) alone, resulting in 128,000 hospitalizations and 3,000 deaths (CDC, 2013). In Europe a total of 5,262 foodborne disease outbreaks were reported in 2011, causing 43,473 human cases, 4,695 hospitalizations and 25 deaths (EFSA and CDC, 2012). Unfortunately, in most of the developing countries, data regarding foodborne illnesses remain scarce (WHO, 2013). Diarrheal diseases are the third leading cause of death in third world countries resulting in 1.8 million deaths around the world in 2005 alone (WHO, 2013). In general most of the cases result from the consumption of contaminated food and water. All over the world, public health agencies are concerned with food safety assurance due to globalization of food markets, and increasing numbers of meals saved outside the home by vendors. Sales of minimally processed ready-to-eat cooked foods have increased rapidly in the last decade because of a busy life style, convenience and freshness (Campbell, 2011).

The preparation of minimally processed ready-to-eat cooked foods typically involves peeling, cutting, slicing or shredding (Bore *et al.*, 2007). These steps increase the possible contamination of these products by foodborne pathogens if done by unskilled personnel. The majority of vendors do not know the basic rules of food hygiene (Sockett, 1995). It has been shown that 691 food poisoning cases and 49 deaths from 1983 to 1992 in Shandong (China) were caused by street foods (Lianghui *et al.,* 1993). It has been observed that the same knife without being cleaned was used to cut raw meat and poultry as well as cutting vegetables for making salad (Mensah *et al,* 2002). In recent years, there has was a steady increase in the production and consumption of processed meat products worldwide because of their high nutritive value and convenience (Bore *et al.,* 2007). Processed food products may at times

constitute a public health hazard due to the possible presence of foodborne pathogenic bacteria which cause illness, intoxication and sometimes outbreak of deadly *listeriosis* as recently observed in South Africa (WHO, 2018).

Staphylococcus aureus, E. coli, Klebsiella spp, *Listeria* and *Salmonella* are important causes of food intoxication throughout the world (Gran *et al.*, 2003). These bacterial species can contaminate several foods, including minimally processed ready-to-eat vegetables, salads and processed meat products. *Escherichia coli*, as enteric pathogens, is becoming increasingly important from the view point of public health, particularly psychrotrophic strains. *E. coli O157:H7* can grow on unprocessed vegetables, salads and processed meat products at 4–12 ^{o}C causing haemorrhagic colitis (FAO, 1998). Routine isolation, identification and enumeration of foodborne pathogens are usually carried out by conventional methods based on the use of selective media, following identification by biochemical tests, respectively.

This traditional method is cumbersome and time consuming as compared to the recent one, polymerase chain reaction (PCR). Furthermore, the former method can frequently lead to ambiguous results in some tests (Tayebi *et al.*, 2016). Fast and sensitive methods for identification of foodborne pathogens are very crucial for microbiological safety throughout the food production chain. In the last 10 years, a considerable number of detection methods using molecular tools have been proposed and set in place (Tayebi *et al.*, 2016). The polymerase chain reaction (PCR) method based on 16Sr RNA gene for the detection and identification of pathogenic bacteria in food presents sensitive and fast method (WHO, 2014). Some of the signs which are detectable of food spoilage may include a change in colour, a change in texture, an unpleasant odour, and undesirable taste (Mensah *et al*., 2002). On food contamination, in Zimbabwe, the total bacterial count in all foods for human consumption should not exceed 1 x 10^6 cfu/g (Nhari, 2013). In Gauteng, South Africa a survey was

conducted among street food vendors and most of them maintained a high standard of hygiene during food preparation and serving (Nyenje *et al.,* 2012). It has been observed in Ghana that a large proportion of street food are contaminated with unacceptable levels of bacteria (Mensah *et al.,* 2002).

Studies by FAO (1995) in Sudan and Democratic Republic of Congo (DRC) showed poor hygienic knowledge and practices in food handling in the assessment of microbial contamination of food sold by vendors. This work was conducted to study the level of food safety knowledge, practices in food handling and assessment of microbial contamination of food sold by vendors. Also a study has been carried out on street food in Ghana that most of the foods are not effectively protected from flies and dust (Chukuezi, 2010). In Bangladesh, street foods are mostly prepared and processed manually and sold to the public at various lorry and bus terminals, by the roadside or by itinerant vendors (Heymann *et al.*, 2006)

Spoilage is the process in which food deteriorates to the point in which it is not fit for consumption by humans or its quality of edibility becomes reduced (Nyenje *et al.,* 2012). Food safety was one of World health Organisation's (WHO) 13 strategic main aim for 2008 to 2013. The importance of food safety is that, it involves taking actions aimed at ensuring that all food is safe for consumption. When bacteria breaks down the food, acids and other waste products are created in the process (Burt *et al.,* 2003). The bacteria itself may or may not be harmful, the waste products may be unpleasant to taste or may even be harmful to one's health. The International Standardization Organizations protocol 22 000 (ISO 22000) is the international standard, which deals with protocols of food safety handling standards and management (Schelin *et al.,* 2011; CFS and EHD, 2014).

Not all bacteria are harmful to humans and some are quite beneficial, such as those found in yoghurt and cheese production. Bacteria can be found everywhere and are impossible to see with the naked eye. However, food processors and handlers, are supposed to control the spread of harmful bacteria by maintaining food safety and practising extreme hygiene especially vendors (Kumar *et al.,* 2014). Bacteria need a constant source of food for their survival, especially products with more protein (Heymann *et al*., 2006). This type of products needs to be monitored constantly as recently seen in the deadly outbreak of *Listeria* in South Africa on the processed meats (WHO, 2018).

2.2.1 Rice spoilage

Rice serves as the staple food of over half the world's population currently. It is the predominant dietary energy source in approximately 17 countries in Asia and the Pacific, nine countries in North and South America and eight countries in Africa (Jahn *et al.*, 2004). Rice is known to provide 20% of the world's dietary energy supply, while wheat supplies 19% and maize (corn) 5% (Jahn *et al.*, 2004). When cooked, unenriched, white, long-grained rice is composed of 68% water, 28% carbohydrates, 3% protein, and negligible amounts of fat. In a 100 gram serving, it provides 130 calories and contains no micronutrients in significant amounts, with all less than 10% of the Daily Value (DV) (Jahn *et al.*, 2004).

Cooked rice can contain *Bacillus cereus* spores, which produce an emetic toxin when left at temperature of 4–60 °C (39–140 °F). When storing cooked rice for use the next day, rapid cooling is advised to reduce the risk of emetic toxin production. One of the enterotoxins produced by *Bacillus cereus* is heat resistant, reheating contaminated rice kills the bacteria, but does not destroy the toxin already present (Jahn *et al.*, 2004). However, this may be the challenge which faces mobile street vendors of this type of food, as they usually cook their food at home then bring it to sell at market places (FAO, 2005).

2.2.2 Sadza spoilage

Sadza is made with finely ground dry maize that is mealie meal. This maize meal is referred to as hupfu in Shona or impuphu in Ndebele. Despite the fact that maize is actually an imported food crop to Zimbabwe in 1890, it has become the chief source of carbohydrate and the most popular meal for indigenous people (Douglas, 2009). Locals either purchase the mealie meal in retail outlets or produce it in a grinding mill from their own maize. Zimbabweans prefer white maize meal. The microflora of cereals and cereal products including maize meal varies, and it includes moulds, yeasts, lactic acid bacteria, rope forming bacteria (*Bacillus* spp.), bacterial pathogens, coliforms, and *Enterococci*. Coliforms and enterococci also occur as indicators of unsanitary handling and processing conditions and possible faecal contamination (Gadaga *et al.*, 1999).

2.3 Foodborne Bacterial pathogens associated with vendor prepared foods

2.3.1 *Salmonella* species

Salmonella spp genus, is rod shaped , bacillus and gram-negative bacteria which is of the family *Enterobacteriaceae*, with a peritrichous flagella that is 0.5 to 0.7 μm wide and 1.0 to 3.0 μm long. *Salmonella* common species are *Salmonella enterica* and *Salmonella bongori* (Heymann *et al.*, 2006). Those species are usually found in the gut of warm blooded animals, while *S. bongori* is restricted to cold blooded animals, particularly reptiles found in environments polluted with human or animal excreta (Janda and Abbott, 2006). *Salmonella* spp are facultative anaerobes, intracellular pathogens and can grow in the temperature range of 7 to 48^{o}C and pH range of 3.8 to 9.5.

Salmonella enterica is further divided into six subspecies that include over 2,500 serotypes. The serogroups are grouped into typhoidal form species *S. typhi* and *S. paratyphi* and non

typhoidal species *Salmonella pnteritidis* and *Salmonella typhimurium* as the most common isolates. *S. typhi* and *Salmonella paratyphi* have no reservoirs other than humans only and can cause disease even with very low inoculum. *S. typhi* usually causes a febrile illness called typhoid fever (Campos, 2013 and Chandler, 2011). After passing through the intestinal cell lining, *S. typhi* is engulfed by macrophages, where it is able to replicate, and get transported to the liver, spleen and bone marrow (Shanson, 1999). After about 5 to 21 days, patients experience a fever with headache, malaise, mayalgias, and salmon pink rash on the abdomen.

Severe manifestations such as sepsis and intestinal bleeding may also develop. *S. enteritidis* colonizes the gastrointestinal tract of virtually all animals (Hamilton *et al.,* 2006). Infection in humans usually occurs when contaminated foods are improperly stored, thus allowing bacteria to proliferate. The infectious dose of *S. enteritidis*, however, is lower in certain high risk populations such as the elderly, immunosuppressed and HIV- infected people. *S enteritidis* infection is characterized by fever, nausea, vomiting, bloody or non-bloody diarrhoea and abdominal cramps (Shanson, 1999).

Symptoms of *Salmonella* spp food poisoning include diarrhoea, fever, and abdominal cramps. They develop 12 to 72 hours after infection, and the illness usually lasts four to seven days. Children younger than five years, the elderly, pregnant women and people with weakened immune systems are at a higher risk (Musah and Akande, 2003; Clark, 2015). A smaller number of people who are infected with salmonellosis develop Reiter's syndrome, a disease that can last for months or years and can lead to chronic arthritis (CDC, 2015). *Salmonella* food poisoning has been known to infect, on average, 20 000 people in the United States of America and causing 400 deaths annually (Centres for Disease Control and Prevention Report, 2011).

2.3.2 *Klebsiella* species

Klebsiella spp is a member of the family *Enterobacteriaceae*. *Klebsiellae* spp are non-motile, rod shaped, gram negative bacteria with a prominent polysaccharide capsule (Brisse *et al.,* 2006). The capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defence mechanisms. The *Klebsiella* genus typically express two types of antigens on their cell surface. The first is a lipopolysaccharide (O antigen), the other is a capsular polysaccharide (K antigen). Both of these antigens contribute to pathogenicity (Brisse *et al.,* 2006 and Knirel *et al.*, 2002).

The structural variability of these antigens forms the basis for classification into various serotypes. The virulence of all serotypes appears to be similar. Three species in the genus *Klebsiella* are associated with illness in humans: *Klebsiella pneumoniae, Klebsiella oxytoca,* and *Klebsiella granulomatis* (CDC, 2015). *K. pneumoniae* was also recognised as an important food borne pathogen in fresh produce (Brisse *et al.,* 2006). High contamination rate of salad vegetables with food pathogens, it is essential to control the hygienic level associated with these products to reduce or minimize risk of food borne disease. Salad vegetables can also be contaminated with *Klebsiella* (Brisse *et al.*, 2006).

2.3.3 *Staphylococcus aureus*

Staphylococcus aureus can cause food poisoning when a food handler contaminates food and then the food is improperly stored for later consumption. *Staphylococcus* food poisoning also leads to gastro-intestinal illnesses. *S. aureus* is a gram positive *cocci* occurring in irregular clumps that are 0.7- 0.9 μm in diameter (Kumar *et al.*, 2014). Its optimum temperature of growth is 37^{o}C, though it grows at a temperature range of 5,5^{o}C to 48^{o}C. *S. aureus* is free living and found on the human body and in the mammalian respiratory tract. *S. aureus* infections may cause disease which include skin lesions, pimples, and skin infections such as furunculosis, toxic shock syndrome and food poisoning (Schelin *et al*., 2011; Lucas and Ferraz, 2006).

A total number of 293 cases of food poisoning caused by *S. aureus* was reported in Europe, in the year of 2009 (EFSA and ECDC, 2015). *S. aureus* can survive in salty environments. When multiplying in food, it realises protein enterotoxins which cause food poisoning when ingested. *Staphylococcus* food poisoning can occur on consumption of only 20-100 mg of enterotoxins in 30 minutes. The symptoms include diarrhoea, profuse vomiting, nausea and abdominal pains. Staphylococcal enterotoxins are heat resistant and are not destroyed during cooking (Todar, 2014).

2.3.4 Escherichia Coli

E. coli is a gram negative bacillus and a facultative anaerobe which colonizes the gastrointestinal tract of warm blooded animals and is one of most important bacteria medically. Some strains of *E. coli* are capable of causing disease under certain conditions when the immune system is compromised or disease may result from environmental exposure (Rane, 2011; Ayulo *et al.*, 1994). *E. coli* may also give rise to infections in wounds, the urinary tract, biliary tract, and abdominal cavity. *E. coli* may cause septicaemia, neonatal meningitis, infantile gastroenteritis and haemorrhagic diarrhoea (Ayulo *et al.,* 1994).

Other known *E. coli* infections may include renal failure, pancreatitis, and diabetes mellitus. Some neurological symptoms such as drowsiness, seizure and coma may also occur (CDC, 2015). Infections with this type of bacteria pose a serious threat to public health with outbreaks arising from food and water that has been contaminated with human and animal faeces or sewage. Transmission can also be through unhygienic practices such as not washing hands and lack of personal hygiene (Musah and Akande, 2003).

There are more than 700 serotypes of *E. coli* that have been identified and these are distinguished by their "O" somatic and "H" flagellar antigens both of which are found on the cell surface of the bacteria (Todar, 2014; Hamilton *et al.*, 2006). More than 50 serogroups of *E. coli* have been isolated, however, serotype O157:H7 is the most dangerous strain as it has been associated with a particularly severe form of diarrhoea (Foodsafety.gov, 2015; Gillespie and Hawkey, 2006). This bacterium produces Shiga toxin which blocks protein synthesis in cells especially the endothelial cell of blood vessels (Foodsafety.gov, 2015). *E. coli* O157:H7 responsible for most of the *E. coli* associated gastroenteritis in the United States. EHEC disease is most common in summer, and can be attributed to the consumption of under cooked meat products, water, unpasteurized milk or fruit juice (Shanson, 1999). The bacteria constantly evolve and mutate attaining new virulent features hence treating infections of *E. coli* O157: H7 becomes complex (Todar, 2014).

2.4 Economic implications of food related diseases

The vendor sold foods play an important socio-economic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income groups. They are appreciated for their unique flavours and convenience. Street foods also assure food security for low income urban population and livelihood for a significant proportion of the population in many Africa's developing countries **(**Pehrsson *et al.*, 2000**)**. These food items are usually sold by vendors and hawkers in the streets. While street vended foods are appreciated for their unique flavours as well as their convenience, they are also important in contributing to the nutritional status of the population (Ferron and Morgan, 2000).

In contrast to these potential benefits, it is also recognized and assumed that street food vendors are often poor, uneducated, and lack knowledge in safe food handling, environment, sanitation and hygiene, mode of food display, food service and hand washing, sources of raw materials,

and use of potable water (European food safety.org, 2012). Consequently, street foods are perceived to be a major public health risk. Foodborne illnesses of microbial origin are a major health problem associated with street foods. In addition, resistance of foodborne microorganisms has made the food safety situation more vulnerable in public health **(**FAO, 2012).

The World Health Organization (WHO) in 2006 launched a scheme termed, "Estimating the global burden of food borne diseases." This was done to provide policy makers in different countries worldwide with data to set appropriate priorities for food safety (WHO, 2015). The FAO Corporate Document of 2005 has put a report that food borne diseases such as Cholera, acute *aflotoxicosis, Salmonellosis, Listeriosis*, and many others claim on average 700 000 lives in Africa annually. Low educational qualifications, socio-economic status, lack of knowledge of safe food handling, vendor's mobility, diversity, temporary nature also contribute to public health risk (WHO, 1996).

2.4.1 Food Legislation and Regulatory Aspects

Food legislation and regulatory control of street food vending varies from country to country. The food control and regulatory system in Zimbabwe is beset by many challenges. The system is fragmented and consists of many entities in the Ministry of Health, the Ministry of Agriculture and in local authorities. In chapter 15:04 of food and food standards act, there are no clear mechanisms to coordinate the activities of these different entities. In practice, they act independently except in times of a national food safety challenge (https://www.researchgate.net/publication/262806750_Food_control_in_Zimbabwe_A_situational_analysis). This, therefore, makes it difficult to ensure food safety throughout the food chain. Lack of the requisite resources is a major contributory factor to weaknesses in the food control system.

The proposed Food Control Bill 2011, has provisions for a coordinated approach to food safety which would strengthen and improve food regulation in Zimbabwe. It includes proposals to bring together all inspection and analytical services under the supervision of one organisation. However, the priority given to enhancing the overall supply of food and the lack of resources to develop a modern food control system is resulting to continuing delays in implementing an enhanced food control system for food safety and quality in Zimbabwe. (https://www.researchgate.net/publication/262806750_Food_control_in_Zimbabwe_A_situational_analysis).

A recent review of the situation in Asia found great diversity among the legal instruments developed to control the street food trade. Some countries had no specific legislation or control systems at all (Lloyd, 2011). In those countries where street food activities were regulated by law, the regulations or by-laws affecting the street food trade were part of a larger body of legislation dealing with food, health, or environmental sanitation. Licensing or registration systems, inspection systems, and codes of practice are other forms of regulation that are in effect in some countries. A number of pieces of legislation relating to the preparation and sale of safe street foods have been established also, by some governments. The Bangladesh Pure Food Ordinance 1959 (2005) has several sections dealing with the safety of street food, adulteration of food, prohibition of calcium carbide, formalin, and insecticide, selling unwholesome food, uncovered foods, and unhygienic premises and violations of the health code (Lloyd, 2011).

In contrast, key constraints to the effective management of street foods, are the lack of awareness of personal hygiene and safe food among street food vendors and consumers. Also insufficient awareness among the consumers about the Consumer Rights Act lack of clarity in existing legislation and standards on street food, no specific regulatory body for licensing, provision of ID card, no medical fitness, and no dress codes concerning street food vendors,. Other constrains include no demarcation of specific areas by local municipalities for street food vendors, insufficient number of sanitary inspectors, the inability of sanitary inspectors to take penal actions against street food vendors and absence of appropriate training and supervision of street food vendors (Pehrsson *et al.,* 2000**)** .

CHAPTER 3: MATERIALS AND METHODS.

3.1 Study Site

This study was carried out at Midlands State University (MSU), bus terminus. It is located in Gweru Zimbabwe, 19 45'S latitude and 29 84' longitude. The area falls under natural region three of Zimbabwe. The site is located 10km southeast of Gweru CBD, at an altitude of 1428 meters above sea level. The average mean temperature is 20-28 ºC. All the experiments were done at Midlands State University main campus biology laboratories.

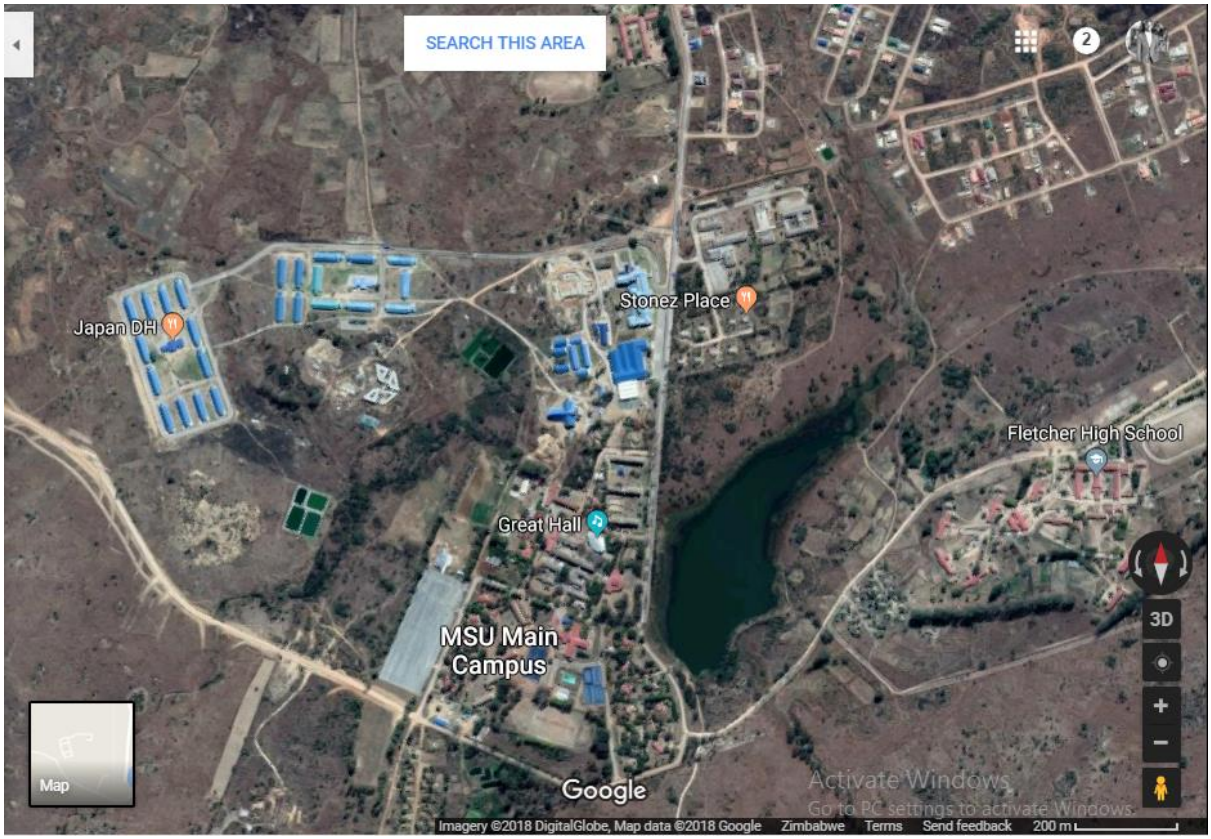

Figure 3.0 Satellite map image of MSU Main Campus, (Accessed 22/03/2018).

3.1.2 Sampling

Three vendors who sell ready-to-eat food were randomly selected and from these, seven food samples were purchased which are sadza, rice, chicken stew, beef stew, cabbage salads, russian sausages and pork chops. Sampling was done three times. Once the samples were purchased they were promptly put in a sterile box to prevent contamination of these samples, hence safer transportation. Samples were given identification codes for convenience, for example A1 represented the first sample of plain sadza, A2 for the second sample of plain sadza, A3 for third sample of sadza, B1 for first sample of plain rice, B2 for the second sample of plain rice, B3 third sample of plain rice and so on up to G3 for all samples.

3.2 Laboratory Analyses

3.2.1 Inoculum preparations

At the biology laboratory, 10g of each sample was measured using an analytical balance. These were placed each into conical flask containing 30ml sterile ringers' solution. The conical flasks were labelled with sample name and number. The samples were then placed on the orbital shacking incubator to homogenise the solution for 20 minutes at 25ºC.

3.2.2 Total Bacterial Counts

3.2.2.1 Serial dilutions

Four test tubes were filled with 9ml ringers' solution each. A measurement of 1ml was collected using a micropipette for each sample per serial dilution. It was put into the first test tube. The solution in the test tube was mixed to make concentration of $10^{-1.}$ A volume of 1ml was drawn from test tube of 10^{-1} concentration into the next test tube. A volume of 1ml of the 10^{-1} dilution was transferred into the 10^{-2} test tube using a micropipette and mixed. A volume of 1ml of the 10^{-2} dilution was then transferred into the 10^{-3} bottle, 1 ml of 10^{-3} dilution was then transferred into the 10^{-4} bottle and then 1ml was drawn from 10^{-4} and discarded. The serial dilutions were done for each of the samples, respectively.

3.2.2.3 Inoculation

For each sample, four sterile petri dishes were labelled according to dilution from 10^{-1} up to 10^{-4}, respectively. One more plate was labelled as the negative control. A volume of 1ml of each dilution was inoculated into the relevant petri dish using a micropipette. A volume 1ml of sterile ringers' solution was then pipetted into the negative control plate. Plate Count Agar (PCA) was poured into each petri dish and was mixed with the each sample dilution by doing several slow rotations. The PCA was left to set for 30 minutes at room temperature then incubated at 30^{o}C for 72 hours.

3.2.2.4 Enumeration

After 72 hours, petri dishes were removed from the incubator for enumeration. Samples having thirty to approximately three hundred colonies were selected and counted from the plate count agar plates only. A black marker pen was used to help in identification of the bacteria already counted. The results were recorded, tabulated and calculations of colony forming units per millilitre (cfu/ml) were done as follows;

Cfu/ml = number of colonies X reciprocal of volume X reciprocal of the dilution factor

3.2.3 Total coliform counts

3.2.3.1 Serial dilutions

A total number of four test tubes were filled with 9ml ringers' solution each. A measurement of 1ml of each respective inoculum was drawn from a conical flask and put into the first test tube. The solution in the test tube were mixed and concentration becomes $10^{-1.}$ A volume of 1ml is drawn from test tube of 10^{-1} concentration into the next test tube. A volume of 1ml of the 10^{-1} dilution was transferred into the 10^{-2} test tube using a micropipette and mixed. A volume of 1ml of the 10^{-2} dilution was then transferred into the 10^{-3} tube, 1ml of 10^{-3} dilution was then transferred into the 10^{-4} tube and then 1ml was discarded. The serial dilutions were done for each of the samples, respectively.

3.2.3.2 Inoculation

For each sample, a sterile petri dish containing Mackonkey agar was labelled with the sample code and tittle. A volume of 100µl of each dilution was inoculated into the respective petri dish using a micropipette, using the spread plate method. A plate was added and labelled as negative. However, 100µl of sterile ringers' solution was then pipetted into the negative control plate. After inoculation, all the petri dishes were placed in the incubator at 37^{O}C for 24 hrs.

3.2.3.3 Enumeration

After 24hrs plates were removed from the incubator and cross examined. For each sample with approximately thirty to three hundred colonies were selected and counted. A black marker pen was used to indicate those counted. The results were recorded, calculated and tabulated of colony forming units per millilitre (cfu/ml).The equation is as follows;

Cfu/ml = number of colonies X reciprocal of volume X reciprocal of the dilution factor

3.2.4 Microbial Isolation and Identification

3.2.4.1 Isolation of *Staphylococcus aureus*

A volume of 100µl of the inoculum for each of the samples was measured using a micropipette and inoculated onto labelled petri dishes of Blood agar using streak plating method. Plates were incubated for 24 hours in a 37°C incubator.

3.2.4.2 Gram staining

Blood agar plates with growth were selected and different colonies in each plate were selected for gram staining. Microscope glass slides were labelled with respective sample codes in preparation for gram staining. Using a dropper, ringers' solution was put onto each slide and using a flamed inoculating loop, colonies of interest were picked to make a suspension on the glass slides. Heat fixation was done by passing the slide through a flame. They were then flooded with Crystal Violet dye for 120 seconds and washed with distilled water, then flooded with Iodine Solution for sixty seconds, then washed with distilled water. Acetone was then

added onto each slide to decolourize the bacteria with thin cell walls for 10 seconds then washed off with distilled water. The slides were then flooded with a counter stain that was safranin for 120 seconds then washed with distilled water and placed in the drying oven at 27^{o}C. A drop of immersion oil was added onto each slide and observed under high power lens (X 100) of a compound light microscope. All observed results were recorded and tabulated.

3.2.4.3 Sub culturing

Blood agar plates with mixed growth were sub cultured. This was done by selecting single colonies of interest using a sterilized inoculating loop and inoculating on labelled petri dishes with fresh Blood agar plates using the streak plate method. The plates were incubated at 37^{o}C for 24hours.

3.2.4.4 Catalase test

Glass slides were labelled with respective sample code and colony type. Sterile ringer's solution was placed onto the glass slides using dropper. Gram positive colonies were picked from Mannitol Salt Agar using a sterilised inoculating loop and suspended in the ringers' solution on the glass slides. A drop of 3 % H_2O_2 (Hydrogen peroxide) was added onto each slide using a dropper and slides were observed for effervescence. No bacteria was added to the negative control.

3.2.4.5 Coagulase

Glass slides were labelled with respective sample codes. Sterile ringers' solution was placed onto the glass slides using a sterilised inoculating loop then Gram positive colonies were picked from Blood agar and suspended in the saline. A drop of blood plasma was put on each slide and mixed with the bacterial suspension. The slides were gently rocked and observed for agglutination. A negative control was also included in which no bacteria was added.

3.2.4.6 Mannitol Salt Test

Gram positive colonies were picked from sub-cultured blood agar plates and inoculated using a flamed sterile loop onto the labelled petri dishes of mannitol salt agar. The petri dishes were incubated for 24 hours in an incubator set to 37 ^{o}C. The negative control, no bacteria was added. After incubation, the plates were examined for any yellow colouration on the inoculated areas.

3.2.5 Isolation of *Salmonella*

A volume of 100µl of the inoculum of each sample was measured using a micropipette and inoculated onto labelled petri dishes of lysine iron agar. It was cultured using the streak plating method and were incubated for 48 hours in a 37°C incubator. One more plate was labelled as the negative control. A volume of 100µl of sterile ringers' solution was then pipetted into the negative control plate.

3.2.5.1 Oxidase Test

An oxidase test strip was inoculated with isolated bacterial colony from lysine iron agar using a sterilised inoculation loop. The test part of the strip was viewed for any colour change.

3.2.6 Isolation of Coliforms

Using a micropipette, 100µl of the samples inoculum were drawn and inoculated onto labelled petri dishes of MacConkey agar, using a sterile inoculating loop. The streak plate method was used. The plates were incubated for 24 hours at 37^{o}C in an incubator.

3.2.6.1 Identification of coliforms through Biochemical tests

3.2.6.1.1 Indole Test

Colonies which were gram negative and pink coloured were picked from the Mackonkey agar plates using a sterilised inoculating loop and a suspension was made in the peptone water. These were then incubated at 37^{0}C for 24 hours and three drops of Kovac's reagent were added into each test tube. Samples were observed for the presence of a reddish ring or a brown ring and checked against the negative control.

3.2.6.1.2 Citrate test

Gram negative lactose fermenting pink colonies were picked from MacConkey agar using a sterilized inoculating loop. These were inoculated onto Simmon's Citrate agar .The media was put in the incubator at 37^{o}C for 24 hours, labelled with the respective sample name and colony type. Samples were observed for a blue colour change and checked against the negative controls.

3.2.7 Antibiotic sensitivity testing

The identified bacteria were picked and inoculated into labelled 15ml vile containing ringer's solution. The vile was labelled with the name of the isolated bacterial genera. Mixture, for each sample was then spread onto petri dishes of Mueller Hinton agar using a sterile swab. The antibiotic discs were put onto the plates to determine their antibiotic susceptibility. The petri dish were sectioned and labelled by a marker pen each section with the name of the antibiotic disk. The antibiotics used were penicillin, ampicillin, ciprofloxacin and amikacin. The plates were then put into an incubator set to 37^{o}C for 24 hours. The results were then recorded and tabulated.

3.2.8 Data Analyses

All data on Total Bacterial Counts, Total Coliform Counts and antibiotic sensitivity were presented in tables and bar graphs. The Total Bacterial Counts and Total Coliform Counts were analysed using One Way Analysis of Variances (One-way ANOVA), SPSS Package version 21.

CHAPTER 4: RESULTS.

4.1 Bacterial isolation

A total of three bacterial species were isolated and identified from all seven food samples. The identified species include *Staphylococcus aureus*, *Escherichia Coli* and *Klebsiella* spp (Table 4.0). No *Salmonella* species was isolated. *Staphylococcus aureus* isolates were identified as gram positive *cocci* bacteria, as catalase positive, coagulase positive, MSA positive. *E.coli* colonies were identified as gram negative rods, indole positive, citrate negative and were observed as pink colonies on MacConkey Agar. *Klebsiella* spp were identified as gram negative rods, indole negative, Citrate positive and were observed as pink colonies on MacConkey Agar.

Table 4.0 Summary of isolated bacteria from food samples.

Sample type	Isolated bacteria
Sadza	*Escherichia coli, Staphylococcus aureus*
Rice	*Staphylococcus aureus, Klebsiella* spp
Beef stew	*Escherichia coli, Staphylococcus aureus, Klebsiella* spp
Chicken stew	*Escherichia coli*
Pork chops	*Escherichia coli, Staphylococcus aureus, Klebsiella* spp
Russian sausages	*Escherichia coli, Staphylococcus aureus, Klebsiella* spp
Cabbage salad	*Escherichia coli, Staphylococcus aureus, Klebsiella* spp

4.1.2 Isolation of *salmonella* spp

Salmonella spp colonies were not isolated on the lysine iron agar. Thus a negative result for *Salmonella* spp.

4.1.3 Isolation and identification of *Staphylococcus aureus*

Out of 21 food samples, a total of nine food samples were infected with of *Staphylococcus aureus*. *S.aureus* isolates produced effervescence, when hydrogen peroxide (H_2O_2) was added on the glass slide during catalase test. It grew positive to salt mannitol test by fermenting MSA from phenol-red to yellow. The isolates were able to coagulate blood plasma, a positive coagulase test (Table 4.1).

Table 4.1 Identification of *Staphylococcus aureus.*

Blood agar (BA)	Catalase	MSA	Coagulase	Suspected Bacteria
Small, white, β-haemolytic	+	+	+	*Staphylococcus aureus*

Key: Blood Agar (BA), Positive (+), Negative (-)

4.1.4 Isolation of coliforms

Out of 21 food samples, a total of eleven food samples were infected with *E. coli* (Table 4.3). The *E. coli* isolates tested positive for indole test and negative for citrate test. A total of eight food samples were infected with *Klebsiella* spp. *Klebsiella* spp isolates tested negative on indole tests and positive citrate test (Table 4.1).

Table 4.2 Identification of Isolated Coliforms

MAC	Gram status	Indole	Citrate	Suspected Bacteria
Pink and bulgy	Negative rods	+	_	*Escherichia coli*
Pink and mucoid	Negative rods	_	+	*Klebsiella spp*

Key: MacConkey Agar (MAC), Positive (+), Negative (-)

Table 4.3 Prevalence of isolated microorganisms in different food samples

Sample Type	Bacteria Specie	Contaminated samples.	Prevalence %
Sadza	*Staphylococcus Aureus*	1/3	33.3
	Klebsiella Spp	0/3	0
	Escherichia Coli	1/3	*33.3*
Rice	*Staphylococcus Aureus*	2/3	*66.6*
	Escherichia Coli	0/3	*0*
	Klebsiella Spp	1/3	*33.6*
Chicken Stew	*Klebsiella Spp*	0/3	*0*
	Escherichia Coli	2/3	*66.6*
	Staphylococcus Aureus	0/3	*0*
Beef Stew	*Staphylococcus Aureus*	1/3	*33.3*
	Escherichia Coli	1/3	*33.3*
	Klebsiella Spp	1/3	*33.3*
Russian Sausages	*Klebsiella Spp*	2/3	*66.6*
	Escherichia Coli	2/3	*66.6*
	Staphylococcus Aureus	1/3	*33.3*
Pork Chops	*Staphylococcus Aureus*	3/3	*100*
	Escherichia Coli	3/3	*100*
	Klebsiella Spp	2/3	*66.6.3*
Cabbage Salads	*Klebsiella Spp*	2/3	*66.6*
	Escherichia Coli	2/3	*66.6*
	Staphylococcus Aureus	1/3	*33.3*

4.1.6 Prevalence of bacterial isolates in overall food samples

Escherichia coli was most prevalent in all the food samples with a prevalence of 52.38 %. *Klebsiella* spp was isolated the least number of times of all the three isolated bacterial species with 38.1 %. *Staphylococcus aureus* was the second prevalent isolate with 42.85 %. (Table 4.3)

Table 4. 4 Prevalence of isolated species for all food samples

Organism	Isolates	Prevalence (%)
Escherichia coli	11/21	52.38
Klebsiella spp	8/21	38.1
Staphylococcus aureus	9/21	42.85

4.2 Bacterial Enumeration

4.2.1 Total bacterial count and total coliform count

The mean total bacterial counts (TBC) for sadza was 1.09 x 10^5 cfu/ml and the mean total coliform counts (TCC) was 1.26 x 10^5 cfu/ml. The mean total bacterial counts for rice samples, was 1.06 x 10^5 cfu/ml and the mean total coliform counts was 1.09 x 10^5 cfu/ml. Mean total bacterial count for beef stew samples was 1.34 x 10^5 cfu/ml and mean total coliform count was 1.85 x 10^5 cfu/ml . Mean total bacterial counts for chicken stew was 1.07 x 10^5 cfu/ml and total coliform counts mean was 1.18 x 10^5 cfu/ml. The mean bacterial counts for cabbage salads was 1.39 x 10^5 cfu/ml, total coliforms counts was 1.52 x 10^5 cfu/ml. On pork chops samples the mean total bacterial counts was 1.99 x 10^5 cfu/ml and total coliform counts mean was 2.05 x 10^5 cfu/ml. The mean total TBC of russian sausages was 1.86 x 10^5 cfu/ml and also mean TCC was 1.98 x 10^5 cfu/ml.

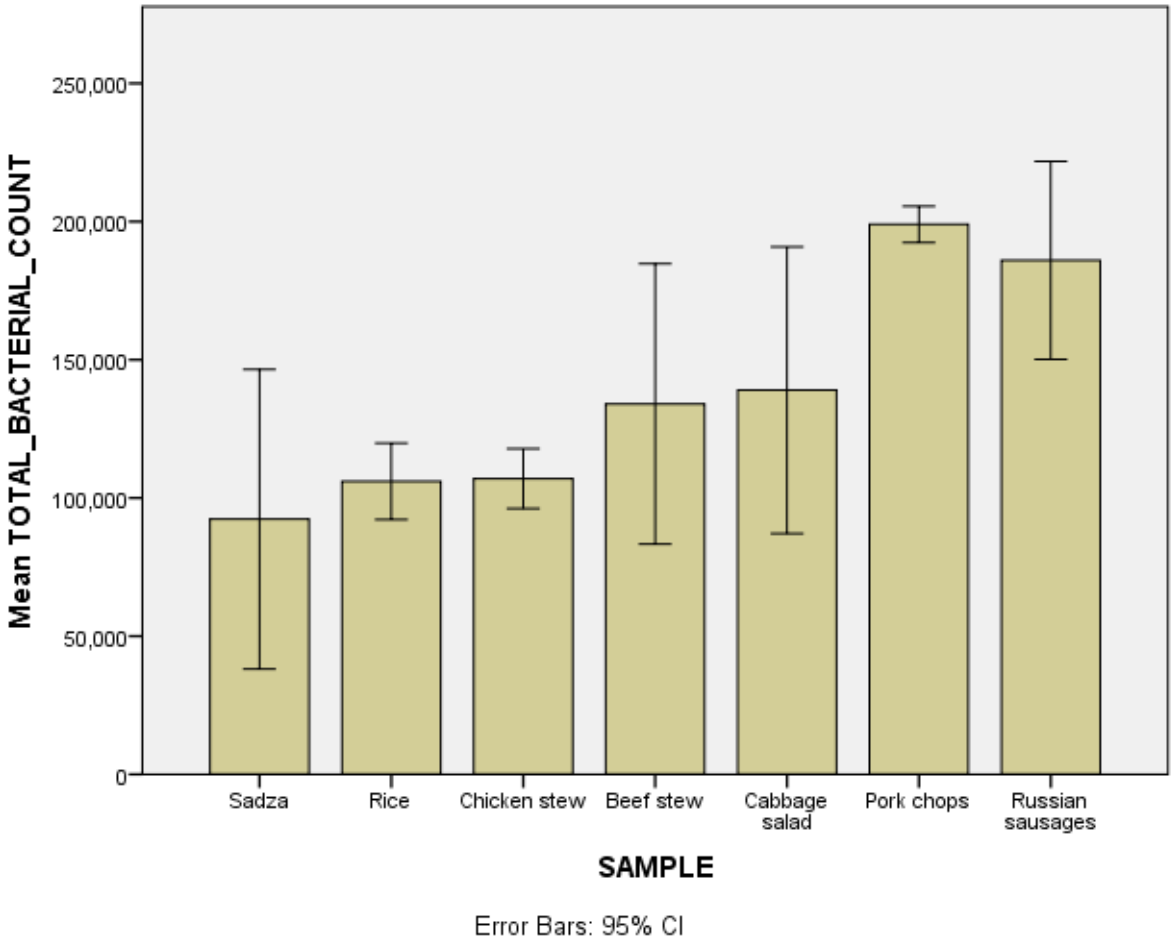

Figure 4.0 Mean Total Bacterial Counts (cfu/ml)

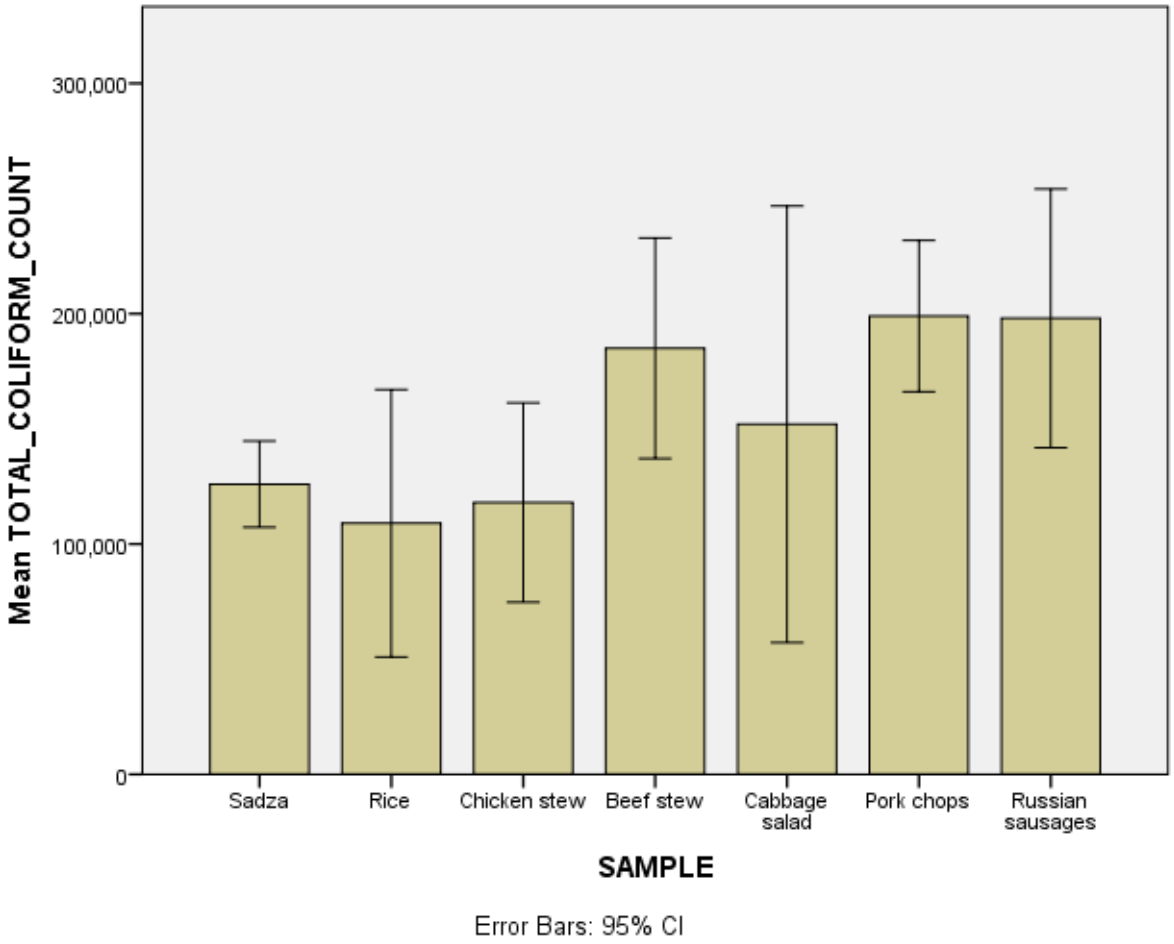

Figure 4.1 Mean Total Coliform Counts (cfu/ml)

At 0.05 significant level, there was enough evidence to suggest that the type of food had no significant effect on the total bacterial count, ANOVA (P< 0.05). The mean total bacterial count ANOVA (P< 0.05), Welch's Test, (P< 0.05) (Appendix 3). The mean total coliform count ANOVA (P< 0.05), Welch's Test, (P= 0.004) (Appendix 4). Tukey post hoc test, revealed no significant differences of means between sadza, rice and chicken stew. Also no significant differences of means between beef stew and cabbage salads (ANOVA: p>0.05, Appendix 8). There was no significant differences of means between pork chops and Russian sausages only (ANOVA: p>0.05, Appendix 8). The mean total coliform counts for all four food samples were above the recommended threshold of 1 x 10^6 cfu/ml (Appendix 6).

4.3 Antibiotic susceptibility tests

Clear zones of inhibition were checked against the zone size interactive chart (Appendix 1). Clear zones of inhibition which were more than the recommended threshold diameter on either side of the antibiotic disk were recorded as sensitive, while zones less than recommended threshold diameter were considered to be resistant. The clear zones of inhibition measured, showed that all the coliforms displayed a general resistance to ampicillin and penicillin. All coliforms were sensitive to ciprofloxacin and amikacin. The antibiotic susceptibility tests also showed that all *S. aureus* were resistant to all antibiotics, ampicillin, penicillin, ciprofloxacin and amikacin (Appendix 2).

CHAPTER 5: DISCUSSION.

5.1 Total Bacterial Counts and Total Coliform Counts

The mean total bacterial counts were below the recommended threshold of the permissible bacterial load for food sold to consumers in Zimbabwe ($\leq 1 \times 10^5$) . According to this study, there was enough evidence 95% confidence level to suggest that the type of food sample had no significant effect on the total bacterial count, ANOVA ($P<0.05$) (Appendix 3). This means that, the foods themselves did not harbour that much bacteria originally, in their unprocessed state but during preparation, site of sale and handling by the vendors, may have introduced a sizeable amount of bacterial contamination. The total coliform counts for all food samples were above the recommended threshold of 1×10^5 cfu/ml, ANOVA, ($P< 0.05$) (Appendix 4). On multiple comparisons, Tukey post hoc test revealed significant differences of means on different food samples (ANOVA: $p<0.05$).

The pork chops and russian sausages had the highest number of TBC than all other samples. This is because pork needs to be cooked thoroughly before consumption and it needs more hygienic conditions since it get contaminated easily. Pork meat can keep bacterial toxins in its fat and they can be only eliminated by thorough cooking. In cabbage salads, contamination by faecal coliforms may have risen due to inadequate washing of the raw cabbage and also washing with contaminated water (Shamilla, 2011). Sadza and rice samples had significantly low TCC and TBC than other samples, because after being cooked they can keep heat for much longer periods than other samples in this study. Therefore, bacterial growth is be slowed down. The vendors do not subject their products to any quality body that would subject their products to any rigorous testing like the cold storage commission (CSC) in Zimbabwe, which inspects all meats before they are consumed. Meat products and cabbage salads pose a greater risk MSU students, staff members and the surrounding community as shown in this study.

5.2 Bacterial Isolation

The identification of the three bacterial pathogens (*E. coli, Klebsiella* spp, and *S. aureus)* food samples from MSU bus terminus, was based on morphological characteristics of bacteria on different media and biochemical tests. This study showed that all the mean total coliform counts of all the food samples exceeded the required threshold value of 1 x 10^5 cfu/ml that is according to the Centre for Food Safety Food and Environmental Hygiene Department, (2014). Therefore the food is not recommended for any human consumption (Appendix 5). The presence of coliform bacteria (*E. coli and Klebsiella* spp) in food vended at MSU bus terminus as shown in this study, shows that this food poses a significant risk to university students, staff members as well as the surrounding community. *E. coli* is a coliform and its isolation in any food sample reflects faecal contamination (Bhowick , 2005). The concurrence of the same type of bacterial could be explained by the close proximity, that the vendors are to each other at the same site.

Most vendors who sell their food at bus terminuses, do not cover their foods from dust and smoke from vehicles, and the same results were observed by the study conducted on street foods in Nigeria (Chukuezi, 2010). The presence of coliforms (*E. coli* and *klebisiella*) may be caused by negligence of the vendors who do not wash their hands and utensils properly before food handling (Drapper, 1996). The terminus has no toilets and most individuals would usually use the bush at the rear of the terminus as the toilets, hence by the presence of dust which rise up carrying the bacterial coliforms into the open foods being sold leads to contamination. Above all the causes of food contamination, personal hygiene is very important (Okojie and Issa, 2014).

Most street vendors do not wear aprons or caps, and they handled food with bare hands. Cooked street food should not be handled with bare hands (Burt *et al.*, 2003). According to revised guidelines for the design of control measures for street foods in Africa, clean tongs, forks,

spoons or disposable gloves should be used when handling, serving and selling food (Gordon, 2011). Handling food with bare hands may result in cross contamination, hence introduction of microbes on safe food. The person handling money should not handle food. This is because money is dirty and can contaminate safe food (Gordon, 2011).Proper methods of storing left over food may have not been used, hence this could promote the sale of stale food by vendors.

The over use of the beta-lactam antimicrobial drug to treat foodborne disease leads to selective pressure on the emerging of drug resistance. Overuse of antimicrobial drugs is the major driving force behind the emergence and spread of drug-resistance traits among pathogenic and commensal bacteria (Luzzaro *et al*., 2006). Antimicrobial drugs which were used in the study have played an important role in decreasing fatal illness associated with the bacterial coliforms and *S.eureus* in the society. The variation in antibiotic susceptibility patterns in this study may help in the clinical case of a disease outbreak caused by one of the isolated pathogens (Tayebi *et al*., 2016).

5.2.1 Escherichia *Coli*.

E. coli was identified in eleven food samples of this study, thus shows that food samples were contaminated. *E.coli* was the most prevalent isolate in the prospective food samples, with a prevalence rate of 52.38% (Table 4.3). This may be due to handling of food with bare hands and uncovering of food for long time of serving customers. Lack of adequate sanitary places at the bus terminus lead to coliform contamination of food to be easy as it is a congested area. Vendors usually have a tendency to populate in overcrowded areas where there are high numbers of potential customers (Abdussalam and Kaferstein, 1993). This may mean that, vendors may end up with limited access to basic sanitary facilities like water and toilets (Dawson, 1991). In Zimbabwe, no business is allowed to operate where they are no adequate sanitary facilities like toilets and running tap water as this pose risk to the society. The

bacteriological quality of the water used by most street vendors have been found to be contaminated with faecal coliforms. When the street foods in Trinidad and Tobago were analysed, it was reported that 35 % of foods were contaminated by *E. coli* while 57.5 % of water used by vendors was contaminated by coliforms (Rampersad, 1999).

In this study, *E. coli* was resistant to penicillin and ampicillin, sensitive to ciprofloxacin and amikacin. The resistance of *E.coli* to ampicillin and penicillin May be due to the over use of beta-lactam antimicrobial agents for treating infections caused by *E. coli* (Kazemian *et al.*, 2016). Also the wide range usage of antibiotics which a led to an increase and emergence of antimicrobial resistant straits. Resistance in *E. coli* is consistently highest for antibiotic agents that have been in use the longest time in human and veterinary medicine (NARMS, 2008).

5.2.2 *Klebsiella* spp

Klebsiella spp was identified in eight food samples and it had a prevalence of 38.1% (Table 4.3). *Klebsiella* spp coliforms are obtained from a dirty environment and unhygienic food handling. The MSU bus terminus has a garbage place which is just merely a dumb place near the vendors thus makes contamination easy as pathogens are carried by flies into the food. Also however, *Klebsiella* spp coliform are often resistant to multiple antibiotics and plasmids acts as the primary source of the resistance genes. The identification *Klebsiella* spp in the food samples also pose a risk to consumers. *Klebsiella spp* causes stomach pains diarrhoea and may cause mild migraine headaches.

In this study *Klebsiella* spp was resistant to penicillin and ampicillin, and sensitive to Ciproflaxin and Amikacin. Mutations of genes may also be a contributing factor on the resistances to antibiotics (Tayebi *et al.*, 2016). This is because nowadays antimicrobial resistance is serious public health problem worldwide, since the infections caused by resistant

strains have been shown to be more commonly related to increased morbidity and motility than the susceptible ones (WHO, 2013).

5.2.3 Staphylococcus aureus

In this study *Staphylococcus aureus* prevalence was 42.85% in the sampled food (Table 4.3). *S.aureus* is part of the normal microbiota present in the upper respiratory tract, and on skin and in the gut mucosa (WHO, 2009). *S. aureus* usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population. Personal hygiene is important because according to Center for Disease Control and Prevention, (2011), humans are the largest contamination sources of food.

S. aureus infections can spread through contact with pus from skin infection, contact with an infected person, and contact with objects used by an infected person such as utensils, towels and clothing (WHO, 2011). *S. aureus* is able grows in a wide temperature range between 6^{o}C to 48°C. The vendors at MSU who sell ready to eat cooked food, usually cook their food at home and sell at MSU bus terminus. The time they transport food to their vending site it will be already cold such that gives optimum temperatures for growth of bacterial pathogens. *S. aureus* was resistant to all the antibiotics in the study and this is because of the evolution of the overuse of antibiotics which leads to mushrooming of resistant strains (Appendix 2). Also the mutation of genes which led to the emergence resistant strains may have contributed to its resistance to antibiotics.

5.3 Conclusion

This study enlightened on the risk to public health and safety of the ready to eat cooked foods sold by vendors at MSU bus terminus. Contaminated and spoiled foods are being sold by these vendors and consumed by students, staff and the general public. The mean coliform counts were above the required threshold of the permissible bacterial load for food sold to consumers in Zimbabwe ($\leq 1 \times 10^5$). The study also confirmed the presence of harmful bacterial faecal coliforms (*E. coli and Klebisiella* spp) and *S. eureus* vendor sold food at MSU bus terminus. Isolation of faecal coliforms (*E. coli and Klebsiella* spp) from the food samples may be due to contamination by dust which arise in the unhygienic environment. Identification of faecal coliforms in food which is consumed by people indicates poor quality of the food and a high degree of spoilage it have undergone. Isolation of *Staphylococcus aureus* showed poor personal hygiene of vendors and lack of knowledge on food safety.

5.4 Recommendations

The study findings can be used by the university to establish awareness campaigns on the dangers of buying food from unauthorised sources. The information of this study can be used to educate the vendors on the importance of hygiene on food preparation and handling. Vending stall or building should be designed solely for that purpose and constructed so that they are easily cleaned and maintained. The importance of training among food vendors is to ensure perpetuation of best practices in the street food vending business thereby protecting public health. There is a need to construct toilets at MSU bus terminus and put clean taped running water. More rubbish bins need to be put at bus terminus to prevent littering of dirty.

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APPENDICES

Appendix 1. Zone size interactive chart.

Antibiotic	Disk Potency	Resistance	Intermediate	Sensitive
Ampicillin	10 mcg	<26 mm	26-30 mm	>30 mm
Ciproflaxin	5 mcg	<24 mm	22-26 mm	>26 mm
Amikacin	30 mcg	<20 mm	20-25 mm	>25 mm
Penicillin	10 mcg	<16 mm	16-24 mm	>24 mm

Key: Micrograms (mcg), Millimetres (mm)

Appendix 2. Antibiotic susceptibility tests on coliforms and staphylococcus isolated.

Sample I.D	Isolate	Penicillin	Ampicillin	Amikacin	Ciprofloxacin
A	*Ecoli*	R	R	S	S
	S. Aureus	R	R	R	R
B	*S. Aureus*	R	R	R	R
	Klebisiella	R	R	S	S
C	*Ecoli*	R	R	S	S
D	*Ecoli*	R	R	S	S
	S. Aureus	R	R	R	R
	Klebisiella	R	R	S	S
E	*Ecoli*	R	R	S	S
	S. Aureus	R	R	R	R
	Klebisiella	R	R	S	S
F	*Ecoli*	R	R	S	S
	S. Aureus	R	R	R	R
	Klebisiella	R	R	S	S
G	*Ecoli*	R	R	S	S
	S. Aureus	R	R	R	R
	Klebisiella	R	R	S	S

Key: S-Sensitive, R-Resistant, T-Tetracycline, A(sadza), B(rice), C(Chicken Stew), D(Beef Stew), E(Russian Sausage), F(Cabbage Salad), G(Pork Chops)

Appendix 3. SPSS output of Total Bacterial Counts ANOVA

ANOVA

TOTAL BACTERIAL COUNT

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	30334285714.286	6	5055714285.714	22.197	.000
Within Groups	3188666666.667	14	227761904.762		
Total	33522952380.952	20			

Appendix 4. SPSS output of Total Bacterial Counts on equality of means

Robust Tests of Equality of Means

TOTAL BACTERIAL COUNT

	Statistic[a]	df1	df2	Sig.
Welch	150.552	6	5.900	.000

a. Asymptotically F distributed.

Appendix 5. SPSS output of Total Coliform Counts ANOVA

ANOVA

TOTAL COLIFORM COUNT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	27058285714.286	6	4509714285.714	9.225	.000
Within Groups	6844000000.000	14	488857142.857		
Total	33902285714.286	20			

Appendix 6. SPSS output of Total Coliform Counts on equality of means

Robust Tests of Equality of Means
TOTAL COLIFORM COUNT

	Statistic[a]	df1	df2	Sig.
Welch	12.080	6	6.013	.004

a. Asymptotically F distributed.

Appendix 7. Total Bacterial Counts and Total Coliform Counts of all food samples

Sample I.D	TBC (cfu/ml)	TCC (cfu/ml)
A1	0.88×10^5	1.33×10^5
B1	1.01×10^5	0.82×10^5
C1	1.04×10^5	1.10×10^5
D1	1.57×10^5	1.63×10^5
E1	1.98×10^5	2.09×10^5
F1	1.63×10^5	1.76×10^5
G1	2.02×10^5	2.14×10^5
A2	1.16×10^5	1.18×10^5
B2	1.12×10^5	1.23×10^5
C2	1.12×10^5	1.38×10^5
D2	1.18×10^5	1.99×10^5
E2	1.90×10^5	1.72×10^5
F2	1.29×10^5	1.08×10^5
G2	1.97×10^5	1.89×10^5
A3	0.73×10^5	1.27×10^5
B3	1.05×10^5	1.22×10^5
C3	1.05×10^5	1.06×10^5
D3	1.27×10^5	1.93×10^5
E3	1.70×10^5	2.13×10^5
F3	1.25×10^5	2.72×10^5
G3	1.98×10^5	1.94×10^5
Recommended TBC/TCC	$\leq 1 \times 10^6$	$\leq 1 \times 10^5$

Key: A(sadza), B(rice), C(Chicken Stew), D(Beef Stew), E(Russian Sausage), F (Cabbage Salad), G(Pork Chops)

Appendix 8. Multiple comparisons

Multiple Comparisons

Dependent Variable: TOTAL BACTERIAL COUNT

Tukey HSD

(I) SAMPLE	(J) SAMPLE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Sadza	Rice	-13.667	12.322	.915	-55.74	28.41
	Chicken stew	-14.667	12.322	.887	-56.74	27.41
	Beef stew	-41.667	12.322	.053	-83.74	.41
	Cabbage salad	-46.667*	12.322	.026	-88.74	-4.59
	Pork chops	-106.667*	12.322	.000	-148.74	-64.59
	Russian sausages	-93.667*	12.322	.000	-135.74	-51.59
Rice	Sadza	13.667	12.322	.915	-28.41	55.74
	Chicken stew	-1.000	12.322	1.000	-43.08	41.08
	Beef stew	-28.000	12.322	.320	-70.08	14.08
	Cabbage salad	-33.000	12.322	.175	-75.08	9.08
	Pork chops	-93.000*	12.322	.000	-135.08	-50.92
	Russian sausages	-80.000*	12.322	.000	-122.08	-37.92
Chicken stew	Sadza	14.667	12.322	.887	-27.41	56.74
	Rice	1.000	12.322	1.000	-41.08	43.08
	Beef stew	-27.000	12.322	.358	-69.08	15.08
	Cabbage salad	-32.000	12.322	.199	-74.08	10.08
	Pork chops	-92.000*	12.322	.000	-134.08	-49.92
	Russian sausages	-79.000*	12.322	.000	-121.08	-36.92
Beef stew	Sadza	41.667	12.322	.053	-.41	83.74
	Rice	28.000	12.322	.320	-14.08	70.08
	Chicken stew	27.000	12.322	.358	-15.08	69.08
	Cabbage salad	-5.000	12.322	1.000	-47.08	37.08
	Pork chops	-65.000*	12.322	.002	-107.08	-22.92
	Russian sausages	-52.000*	12.322	.012	-94.08	-9.92
Cabbage salad	Sadza	46.667*	12.322	.026	4.59	88.74
	Rice	33.000	12.322	.175	-9.08	75.08
	Chicken stew	32.000	12.322	.199	-10.08	74.08
	Beef stew	5.000	12.322	1.000	-37.08	47.08
	Pork chops	-60.000*	12.322	.004	-102.08	-17.92
	Russian sausages	-47.000*	12.322	.024	-89.08	-4.92
Pork chops	Sadza	106.667*	12.322	.000	64.59	148.74
	Rice	93.000*	12.322	.000	50.92	135.08
	Chicken stew	92.000*	12.322	.000	49.92	134.08
	Beef stew	65.000*	12.322	.002	22.92	107.08
	Cabbage salad	60.000*	12.322	.004	17.92	102.08
	Russian sausages	13.000	12.322	.931	-29.08	55.08

Russian sausages	Sadza	93.667*	12.322	.000	51.59	135.74
	Rice	80.000*	12.322	.000	37.92	122.08
	Chicken stew	79.000*	12.322	.000	36.92	121.08
	Beef stew	52.000*	12.322	.012	9.92	94.08
	Cabbage salad	47.000*	12.322	.024	4.92	89.08
	Pork chops	-13.000	12.322	.931	-55.08	29.08

*. The mean difference is significant at the 0.05 level.