VALIDATION OF DI-ELECTRIC HEATING AS A POTENTIAL FOOD SAFETY INTERVENTION

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NOVEMBER, 2014
DECLARATION

I hereby declare that this thesis submitted for the Masters Degree in Infectious Diseases, at the Kenyatta University, is my own original work and has not previously been submitted to any other institutions for the award of a degree or diploma. All quotes are indicated and acknowledged by means of a comprehensive list of references.

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DEDICATION

This thesis is dedicated to the Almighty God for enabling me to pursue my studies, my family and friends for their support as well as Dr. Lucy Mambo for her guidance and encouragement.
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TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>ABBREVIATIONS AND ACRONYMS</td>
<td>xii</td>
</tr>
<tr>
<td>OPERATIONAL DEFINITION OF TERMS</td>
<td>xiv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xv</td>
</tr>
</tbody>
</table>

CHAPTER ONE: INTRODUCTION ................................................. 1

1.1 Background of the study ........................................... 1
1.2 Problem statement and justification ............................ 4
1.3 Research questions ................................................ 5
1.4 Null hypothesis .................................................... 5
1.5 Objectives ............................................................ 6
  1.5.1 General objective ............................................... 6
  1.5.2 Specific objectives ............................................ 6
1.6 Significance of the study ........................................... 6

CHAPTER TWO: LITERATURE REVIEW .......................................... 7

2.1 Food borne diseases ................................................ 7
2.2 Microwaves ................................................................ 11
  2.2.1 Di-electric heating ............................................... 12
2.3 Food bacteria .......................................................... 14
  2.3.1 Escherichia coli .................................................... 14
2.3.1.1 Epidemiology of *Escherichia coli* ........................................... 15
2.3.1.2 Prevalence of *Escherichia coli* ........................................... 16
2.3.1.3 *Escherichia coli*’s resistance to antimicrobials .................... 17
2.3.2 *Salmonella enterica subspecies enterica serovar* Paratyphi ........... 18
   2.3.2.1 Epidemiology of *Salmonella* Paratyphi ............................ 18
   2.3.2.2 Prevalence of *Salmonella* Paratyphi ............................... 19
   2.3.2.3 *Salmonella* paratyphi’s resistance to antimicrobials ............ 20
2.3.3 *Shigella flexneri* ............................................................... 21
   2.3.3.1 Epidemiology of *Shigella flexneri* .................................. 21
   2.3.3.2 Prevalence of *Shigella flexneri* .................................... 22
   2.3.3.3 *Shigella flexneri*’s resistance to antimicrobials ............... 23
2.3.4 *Staphylococcus aureus* ....................................................... 25
   2.3.4.1 Epidemiology of *Staphylococcus aureus* ........................... 25
   2.3.4.2 Prevalence of *Staphylococcus aureus* ............................... 25
   2.3.4.3 *Staphylococcus aureus*’s resistance to antimicrobials .......... 26
2.4 Infective dose .............................................................................. 28
2.5 Antibiotics .................................................................................. 31

CHAPTER THREE: MATERIALS AND METHODS ..................................... 34

3.1 Location of the study ................................................................... 34
3.2 Research Design ........................................................................... 34
   3.2.1 Test organisms ..................................................................... 34
   3.2.2 Dependent and Independent variables .................................. 36
   3.2.3 Growth and maintenance of standard bacterial strains .......... 36
   3.2.4 Antimicrobial susceptibility test discs .................................. 36
   3.2.5 Properties of Mueller Hinton for Antimicrobial Susceptibility Testing .... 39
3.3 Physical properties of food ............................................................ 38
3.4 Sample size determination ............................................................. 39
LIST OF TABLES

Table 2.1: Guidance levels for determining the microbiological quality of ready-to-eat foods ........................................................................................................31

Table 3.1: Food–microorganism combination for testing ........................................35

Table 3.2: Table for interpreting sizes of zones of inhibition during AST ........48

Table 4.1: Biochemical tests ..................................................................................53

Table 4.2: AST results for *E. coli*, *S. paratyphi*, *S. flexneri* and *S. aureus* before and after exposure of samples to microwave radiation .................................................74

Table 4.3: Physical property changes that occurred in various food samples after exposure ........................................................................................................79
LIST OF FIGURES

Figure 2.1: Electromagnetic wave spectrum.................................11
Figure 4.1: Beef sample inoculated with Escherichia coli..................54
Figure 4.2: Beef sample inoculated with Salmonella Paratyphi........55
Figure 4.3: Beef sample inoculated with Staphylococcus aureus.........56
Figure 4.4: Chicken sample inoculated with Escherichia coli..........57
Figure 4.5: Eggs sample inoculated with Escherichia coli.............58
Figure 4.6: Eggs sample inoculated with Salmonella Paratyphi..........59
Figure 4.7: Fish sample inoculated with Escherichia coli............60
Figure 4.8: Fish sample inoculated with Salmonella Paratyphi.........61
Figure 4.9: Fish sample inoculated with Shigella flexneri.............62
Figure 4.10: Gravy sample inoculated with Escherichia coli.........63
Figure 4.11: Gravy sample inoculated with Salmonella Paratyphi.....64
Figure 4.12: Milk sample inoculated with Escherichia coli..........65
Figure 4.13: Milk sample inoculated with Salmonella Paratyphi.....66
Figure 4.14 Milk sample inoculated with Staphylococcus aureus.....67
Figure 4.15 Potato sample inoculated with Staphylococcus aureus...68
Figure 4.16 Vegetables sample inoculated with Salmonella Paratyphi..69
Figure 4.17 Vegetables sample inoculated with Shigella flexneri.....70
Figure 4.18 Chicken sample inoculated with Salmonella Paratyphi....71
Figure 4.19 Vegetables sample inoculated with Escherichia coli.....72
LIST OF PLATES

Plate 3.1: Food samples processed during the study.................................41

Plate 3.2: Food samples placed in the incubator before exposure to Microwave radiation.................................................................45

Plate 3.3: Milk samples cultured on both macconkey and nutrient agar before and after exposure to microwave radiation..........................46

Plate 3.4: Chicken samples cultured on macconkey agar before and after exposure to microwave radiation..............................................46

Plate 3.5: Antimicrobial susceptibility plates of bacteria acquired from chicken sample before and after exposure to microwave radiation........48

Plate 4.1: Plates showing similar antimicrobial susceptibility patterns........73
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>AST</td>
<td>Antibiotic Susceptibility Testing</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control and Prevention</td>
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<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
</tr>
<tr>
<td>CIA</td>
<td>Centre for Intelligence Agency</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical Laboratory Standards Institute</td>
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<tr>
<td>DEH</td>
<td>Dielectric Heating</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>FBD</td>
<td>Food borne Disease</td>
</tr>
<tr>
<td>FDA</td>
<td>United States Food and Drug Administration</td>
</tr>
<tr>
<td>H₂S</td>
<td>Hydrogen Sulphide gas</td>
</tr>
<tr>
<td>HACCP</td>
<td>Hazard Analysis Critical Control Points</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization of Standardization</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal Inhibitory Concentration</td>
</tr>
<tr>
<td>MoH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>MPHS (K)</td>
<td>Ministry of Public Health and Sanitation, Kenya</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin Resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MSSA</td>
<td>Methicillin Susceptible <em>Staphylococcus aureus</em></td>
</tr>
</tbody>
</table>
MW/MWs  Microwave/ Microwaves
NACMCF  National Advisory Committee on Microbiological Criteria for Food
NCCLS  National Committee for Clinical Laboratory Standards
NTS  Non Typhoidal Salmonella
RNA  Ribonucleic acid
SD  Standard Deviation
UV  Ultraviolet Radiation
WHO  World Health Organization
OPERATIONAL DEFINITION OF TERMS

Di-electric heating (DEH): In this study DEH refers to the mechanism by which MW oven generates heat in food and water.

Colony forming units (CFU): In this study CFU refer to the colonies formed on culture media capable of undergoing binary fission to produce more bacteria.

Bacterial contaminants: In this study bacterial contaminants refer to bacteria found in food that are capable of producing an infection (Pathogenic bacteria).

Inactivation: In this study inactivation refers to complete paralysis of growth and multiplication of bacteria.

Infective dose: In this study the infective dose refers to the minimum number of bacteria that are capable of causing food borne infection.

Physical Properties: In this study physical properties refer to colour, consistency, development of mould / slime and odour/smell of the food sample.

Effect size: Magnitude of treatment effect
ABSTRACT

Food and water borne diseases are leading causes of illness and death in lesser developed countries killing approximately 7.2 million people annually, 1.9 million of whom are children. WHO has estimated that each year 2.2 million people, including 1.9 million children, die because of diarrheal diseases. This study was intended to experimentally analyze if di-electric heating has any indirect impact on the control, management and prevention of food borne disease outbreaks in terms of reduction of bacteria infective doses and increase in antibiotic susceptibility. This included investigating the effects on bacterial colony forming unit counts before and after exposure to microwave radiation as well as investigating changes in antibiotic susceptibility patterns. Surviving colonies were also investigated for viability, growth and multiplication ability. The aim of the study was to determine if microwave radiation can be used as a food safety intervention; whereas other objectives included evaluating its effects on growth and multiplication of bacteria, reduction of bacterial infective doses, measuring appropriate time (in minutes) required for radiation to achieve the desired results, recording the most effective power at which radiation was most likely to achieve the desired results and lastly observing if there are any significant changes as a result of exposure to microwave radiation when it comes to antibiotic susceptibility patterns. Various food samples (n=73) were artificially contaminated by food borne disease pathogens (Escherichia coli, Staphylococcus aureus, Shigella flexneri and Salmonella paratyphi) and were exposed to microwave radiation at different powers (P-00, P-20, P-40, P-60, P-80) and time periods (2 min, 4 min, 6 min, 8 min). The degree of inactivation was estimated by measuring the colony forming units formed in culture before and after exposure of samples to radiation (Pre-test Post-test experimental design). The laboratory methods used were mainly culture techniques and biochemical tests. Data entry was done in Microsoft Excel after which data analysis was carried out using Statistical Package for Social Science (SPSS V13) software. The results showed that generally microwave radiation produced a 1-2 log reduction when artificially contaminated food samples were processed. Initial seeded bacterial numbers (>3.0 x 10^4) were reduced significantly (P<0.05) however, this was not the same case when it came to antibiotic susceptibility patterns. The most effective time-power combination for bacteria inactivation was 8 minutes at P-80 where bacteria numbers were very low (<1.0 x 10^3); whereas the least effective time-power combination for bacteria inactivation was 2 minutes at P-20 where bacteria numbers remained at (>3.0 x 10^4). The study shows that microwave radiation reduces infective doses of bacteria by 96.67% but does not affect their antibiotic susceptibility patterns. Indications of any other effects of microwave radiation on the microbial agents were also noted. This study will serve as a guide to policies implemented on management and control of food and water borne infections. Traditional methods for improvements in food safety are still being utilized however the number of food and water borne disease incidences and antibiotic-resistance cases continue to rise. This therefore means that prevention rather than cure is important now more than ever and so modern methods or practices such as the microwave radiation that promote food hygiene should be explored and encouraged.
CHAPTER ONE
INTRODUCTION

1.1 Background of the study

A microwave oven is a common kitchen appliance used most especially in urban areas for quick heating of cold food (Valsechi et al., 2004; Vollmer et al., 2004; Kango and Deshmukh, 2011), and is quite common in developed countries. At the same time its popularity is increasing at a very fast rate in developing countries (Vadivambal and Jayas, 2008). The food industry has been using the “quick wave”, otherwise known as microwaves (MWs) to heat food since the 1960s (Park et al., 2000). However, it was during the 1940s and the advent of radar development during World War II that microwave theory and technology began to receive substantial interest (Pozar, 2005; Shaheen et al., 2012). Some microwave ovens are used in industries; however they have higher power levels than the domestic ovens (Manickavasagan et al., 2007). Due to MW ovens being so convenient, simple, ‘safe’ and energy efficient as compared to conventional ovens, very few urban homes, offices and restaurants do not have them (Collins et al., 2005; Callebaut, 2007; Gedikli et al., 2008). Even in the food industry, it is a common practice to use microwave radiation for sterilization and pasteurization of food (Schlegel, 1992) and this is because studies show that there are no adverse effects of nutritional quality of food cooked by microwave radiation compared with those cooked conventionally (Jonker and Til, 1995).

In commercial/ domestic models, the oven has a power input of about 1000 watts of alternating current. The mechanism involved is such that as these MWs generated
from the magnetron bombard the food, they cause the polar molecules to rotate at the same frequency millions of times a second. All this agitation creates molecular friction which heats up the food. The friction also causes substantial damage to the surrounding molecules, often tearing them apart and forcefully causing deformation (Ashline et al., 2007).

Food and water borne diseases (FBDs and WBCs) are leading causes of illness and death in less developed countries killing approximately 7.2 million people annually, 1.9 million of whom are children (Ministry of Public Health and Sanitation, 2011). WHO has estimated that each year 2.2 million people, including 1.9 million children, die because of diarrheal diseases (Vandepitte et al., 2003; DeWaal et al., 2009). Two to four billion episodes of infectious diarrhoea have been estimated to occur annually in developing countries, resulting in 3 to 5 million deaths (Olaniran et al., 2011). Food borne diseases have been with us for centuries. However, since 1977 new or newly characterized food borne pathogens have been recognized at a rate of approximately one every two years (Tauxe et al., 2002). Therefore, the number of FBDs has increased globally, especially in developing countries where food safety interventions and sanitary control measures are not adequately implemented. Although the burden of FBDs is not well defined in many countries or regions on a global level (Flint et al., 2005), the CDC has identified more than 400 food-related illnesses. Some are rare while others occur frequently. The food safety community is eagerly awaiting the first results of pilot studies from the WHO’s ‘Initiative to estimate the Global Burden of FBDs’ (Angelika and Jenny., 2012; Hanson et al., 2012). The studies were conducted in recognition of the growing threat posed by FBDs worldwide to provide precise and comprehensive information on the magnitude of FBDs and to guide food safety
policy. Such data can then be used to estimate the burden associated with acute gastroenteritis of food borne origin, the burden caused by specific pathogens commonly transmitted by food and the burden caused by specific foods or food groups (Flint et al., 2005). Meanwhile, FBDs remain responsible for high levels of morbidity and mortality in the general population and particularly for certain risk-groups such as infants, the elderly and the immune-suppressed (Manson-Bahr et al., 1987; Mehata and Duan, 2011; Lampel et al., 2012).

Many studies show that MW heating can cause destruction of bacteria (Fujikawa and Ohta, 1994; Collins et al., 2005; Barnabas et al., 2011) with their bactericidal activity being dependent upon time and temperature (Lund et al., 1994; Celandroni et al., 2004). Other studies indicate opposite results attributed to uneven heating (Decareau, 1992). In addition, in many of these cases the results claimed could not be reproduced and they lacked an exact temperature distribution determination (Shaheen et al., 2012). Studies even report thermal resistance of strains of bacteria such as *Staphylococcus aureus* (Dewanti-Hariyadi et al., 2011). These effects of MW radiation on bacteria have been studied and debated for more than half a century (Shamis et al., 2011) with most of these studies exposing microorganisms to MW radiation in liquid suspensions and observing the effects. However, Jeng et al. (1987) disputed this method of analysis by pointing out that a liquid suspension containing microorganisms is not an efficient system for the purpose of studies because of its reduction of the local electric field strength.

More studies involving direct inoculation of microorganisms into foods, in a similar setting as the domestic situation are therefore required to test if the changes that occur
on bacteria due to di-electric heating (that is exposure to MW radiation) would have any significant effect on their growth and multiplication as well as their susceptibility to commonly prescribed antibiotics thus having an indirect impact on the control and prevention of FBD outbreaks.

1.2 Problem statement and justification

In 2009, 1219 single-etiology cases were reported by 51 states in the United States territories (1-122 outbreaks) resulting in 34,160 outbreak-associated illnesses, 999 hospitalizations and 48 deaths and the most common bacterial pathogens reported among all single-etiology outbreaks were *Shigella* spp. (72.6 %) and *Salmonella* spp. (57.5%) (Manikonda *et al.*, 2012). In France, it is estimated that these pathogens cause 10,200 - 17,800 hospitalizations yearly (Nyenje and Ndip, 2013). FBDs continue to represent a serious threat to the health of millions of people in the world (Angelika and Jenny., 2012) particularly in Africa, where the mortality rate is estimated to be at 700,000 for all ages in sub-Saharan Africa and mortality due to diarrhoea in children under five years of age is estimated at 15%. This has been reported to be the second cause of under-five mortality globally (Shapiro *et al.*, 2001; Mensah, 2012). Traditional methods for improvement in food safety, such as pasteurization of milk, safe canning, irradiation and disinfection of water supplies are still utilized but the the problem is that the number of FBD incidences and antibiotic-resistance cases continue to rise. Therefore, modern methods, intervention processes and new prevention technologies that promote food hygiene should be encouraged (Tauxe, 2001; Tauxe *et al.*, 2002; Sheen *et al.*, 2012). The effects of modern interventions such as UV radiation, gamma radiation and ionization radiation on bacteria have been extensively studied (Collins *et al.*, 2005; Trampuz *et al.*, 2006).
However, MW radiation continues to be used all over the world without significant data on the effect it has on the infective doses of food pathogens. The safety of MW cooking in relation to food borne pathogens continues to be questioned (Jamshidi et al., 2010). However, reports have shown that there are no ionizing radiations associated with domestic microwave radiation which means that the machine is safe (Krifi et al., 2014). This study was intended to measure the exact effect-size of microwave radiation on the overall burden of FBDs and thus validating the machines' contribution towards food safety.

1.3 Research questions

i. What is the effect of microwave radiation on the CFU count of pathogenic bacteria?

ii. What is the effect of microwave radiation on bacterial infective doses?

iii. What are the effects of antibiotics on the survivors of MW exposed bacteria?

iv. What is the amount of time required to render food free of disease causing micro-organisms on exposure to microwave radiation?

1.4 Null hypothesis

i. Di-electric heating does not result in any significant changes on the infective doses, antibiotic susceptibility patterns, viability, growth and multiplication of bacteria capable of causing food borne diseases and well as the physical properties of food.
1.5 Objectives

1.5.1 General objective

To determine the use of microwave radiation as a potential food safety intervention.

1.5.2 Specific Objectives

i. To determine the effect of microwave radiation on the colony forming units of *E. coli*, *S. aureus*, *S. paratyphi* and *S. flexneri* in food.

ii. To determine susceptibility of *E. coli*, *S. aureus*, *S. paratyphi* and *S. flexneri* to antimicrobial agents after exposure to microwave radiation.

iii. To determine if there are any physical changes in the properties of contaminated food after exposure to microwave radiation.

1.6 Significance of the study

The purpose of this study is to determine the extent to which MW radiation can be used as a method of preventing/reducing the overall burden of FBDs. Specifically the study results should determine whether the use of MWs is to be encouraged and whether the public (especially those that are immune-suppressed) should be educated on the correct use of the MW oven and its importance. Thus the results of this study will benefit both the MW oven manufacturing companies and the general public. From the findings, the manufacturing companies can identify areas of improvement and the public will be better educated on the advantages or disadvantages of the MW oven when it comes to reduction of FBDs.
CHAPTER TWO
LITERATURE REVIEW

2.1 Food Borne Diseases

Food borne diseases are illnesses caused when people consume contaminated food or beverages (Tauxe et al., 2002). They are also defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of contaminated food (Le Loir et al., 2003; Ramanathan, 2010). They are caused by enteric pathogens which are commonly spread through the faecal-oral route (Sack et al., 2001). They are characterized by stomach upset, vomiting and diarrhoea which may lead to water and electrolyte imbalance in the body. Loss of water and electrolytes from the body can lead to severe dehydration which if untreated can be rapidly fatal (Cheesbrough, 2006). Although nausea and diarrhea are the most common symptoms, other consequences can include kidney and liver failure, brain and neural disorders, septicemia, stillbirths and death (Dewaal et al., 2009; Kuchenmuller et al., 2009).

The CDC defines FBD outbreaks as the occurrence of two or more cases of a similar food borne illness resulting from ingestion of a common food (Olsen et al., 2000). As highlighted by Bamaiyi (2011) and Dewanti-Hariyadi et al. (2011), the bulk of FBDs are caused by bacterial pathogens resulting in food borne infections such as salmonellosis, shigellosis, E. coli diarrhea, cholera, brucellosis, listeriosis and streptococcal infections (Ramanathan, 2010). Pathogens like E. coli 0157:H7 may even lead to development of serious complications such as severe bloody diarrhea, kidney failure, haemorrhagic colitis, haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura and blood clots in the brain (Pitout and Church, 2004;
Pacific Northwest Publications, 2009; Jamshidi et al., 2010). A national surveillance report confirmed *Salmonella* infections in China in 2010. It found that salmonellosis is a significant public health problem and 45% of the infections occur in patients <2 years old (Ran et al., 2010). The overall prevalence of antibiotic resistance was also high for conventional antibiotics such as ampicillin (49%), gentamycin (27%), and tetracycline (48%). However, resistance was relatively low for new antibiotics such as cefotaxime (7%), ceftazidime (6%) and ciprofloxacin (4%). In Japan, the leading causes of hospitalization rates due to FBDs are caused by *Salmonella* spp. (36%) (Toyofuku, 2012). Australia, with a population of 19.7 million is estimated to have 5.4 million cases of FBD gastroenteritis each year (DeWaal and Robert, 2005).

It is estimated that an annual burden of 76 million cases of food borne illness, 323,000 hospitalizations and 5000 deaths (Tauxe, 2002; DeWaal et al., 2009; Sharma et al., 2009) occur in the United States each year. This means that 1 in 4 Americans gets a food borne illness each year and more than 1 in 1000 is hospitalized. However, it must be recognized that not all FBD outbreaks are investigated, a definitively implicated food is not always identified and the causative agent is not always identified (NACMCF, 2008). The key pathogens isolated from diarrhoeal cases in Thailand are *Shigella* spp., *Salmonella* spp. and *E. coli* (DeWaal and Robert, 2005). In 2008, the largest numbers of outbreaks were reported in Africa, among three geographical regions included in the study but because of its less developed public health sector, the role of the mass media in outbreak reporting became more relevant and important for assessing the public health impacts of contaminated food or water (Dewaal et al., 2009). The government is unable to accurately assess the impact of food contamination problems on public health because there is inadequate
surveillance for food borne diseases (DeWaal and Robert, 2005). However, there were outbreak reports of diarrhoea in Congo, Kenya, Madagascar, Burundi, Comoros, Uganda, Botswana and Mozambique (Mensah et al., 2012). Fewer cases of food-related outbreaks occurred in 2009 except the key ones such as shigellosis which was reported in Malawi. Canada has a population of 32 million with 10,000 reported FBD cases and an estimated 2 million actual cases that go unreported (DeWaal and Robert, 2005).

The above information shows that there is a large global burden of FBDs; which means that there are still many food borne infections to be controlled, despite the successes of the 20th century. More food borne diseases appear to be emerging more frequently than ever before and the capacity of public health authorities to apply conventional control measures does not seem to be developing at the same rate (Kuchenmuller et al., 2009). Pathogens are most likely to be transmitted in foods that are inspected improperly, processed in an unsanitary manner, cooked improperly or refrigerated poorly (Black, 2008). Foods and other perishable products retain their quality longer when the growth of contaminating micro-organisms is prevented. The most common food handling error is keeping foods for more than four hours at a temperature between 4°C and 60°C which is the growth or so-called danger zone for most bacteria. Some microbes can grow in temperatures as low as 3°C, while it takes an internal temperature of 71°C for 15 seconds to kill E. coli O157:H7 in ground meat (FMI). Irradiation can be used to kill microbes without causing perceptible changes in the food but the FDA has approved this technology to treat only certain foods (Anderson et al., 2007). In addition to disease caused by direct infection, some FBDs are caused by the presence of a toxin in the food that was produced by a microbe in
the food. FBDs often occur together with food-poisoning which is caused by ingesting food contaminated with the pre-formed toxins (Black, 2008). For example, the bacterium *Staphylococcus aureus* can grow in some foods and produce a toxin that causes intense vomiting. This kind of FBD is known as food borne intoxication and it is commonly restricted to acute gastroenteritis due to bacterial contamination of food (Ramanathan, 2010).

However, the multiplication of micro-organisms in food is greatly influenced by the inherent characteristics (intrinsic factors) of that food (Onyenekwe et al., 2012). In general, microorganisms multiply most rapidly in moist, nutritionally rich, pH-neutral foods (Anderson et al., 2007). Food sold to the general public rightfully must meet reasonable public health standards for quality. Despite these precautions we continue to have periodic incidents that cause considerable human misery (Lory et al., 2002). Although everyone is susceptible, infants and young children, pregnant women, the immune-compromised and the elderly are more likely to experience food borne illness with severe consequences (DeWaal and Robert, 2005). Many FBD cases do not even visit a general practitioner or otherwise come to the attention of the medical system. It has been noted that for every one notified case of gastrointestinal disease of infectious etiology, there are 222 others occurring in the community (Campbell et al., 2012). The full extent of the burden and cost of unsafe food is currently still unknown but its impact on global health, trade and development is considered to be immense (Hanson et al., 2012).
2.2 Microwaves

Microwaves are electromagnetic waves (Vollmer, 2004; Anderson et al., 2007). They are part of the electromagnetic spectrum (Figure 2.1) and are considered to be that radiation ranging in frequency from 300 million cycles (300 MHz) to 300 billion cycles (300 GHz) per second, which correspond to a wavelength range of 1 m down to 1 mm (Vadivambal and Jayas, 2008; Al-Mayah and Ali, 2010; Dababneh, 2013). In the electromagnetic spectrum, the MW radiation region is located between infrared radiation and radio waves (Lidstrom et al., 2001; Kumar et al., 2007; Jamshidi et al., 2010). MWs are very short waves of electromagnetic energy that travel at a speed of light (299,791.89 km/s). Microwave heating involves conversion of electromagnetic energy into heat by selective absorption and dissipation (Barbosa – Canovas et al., 2002). Heating is accomplished by using di-electric heating (DEH) (Vadivambal and Jayas, 2008) to heat polarized molecules within the food. This is done through induced molecular vibration as a result of dipole rotation or ionic polarization (Ramaswamy and Tang, 2008).

![Electromagnetic wave spectrum](image)

The phenomenon is referred to as Dipolar Polarization mechanism which is one of the interactions of the electric field component of MW with the sample. A second major interaction of electric field component and sample is known as conduction mechanism (Lidstrom, 2001).

2.2.1 Di-electric heating

Foods are poor conductors of heat therefore they are referred to as di-electric materials or di-electrics. When this di-electric material is brought into a rapidly altering electrical field, heat is generated inside the material. This is known as heating by di-electric hysteresis or in short DEH (Callebaut, 2007). The development of di-electric heating applications in food industry started in the radio frequency range in the 1930's and thereafter the desired energy transfer rate enhancement led to an increased frequency of the microwaves (Shaheen et al., 2012). Contrary to convective heating with steam and hot-air or even radiation heating in general, DEH generates heat directly inside the exposed material. The conversion of electric energy to heat results from the di-electric losses of the electric non-conducting material, which is usually a poor thermal conductor (Pueschner, 1993). Therefore, interaction of microwaves with materials depends on their di-electric properties, which determine the extent of heating of a material when subjected to electromagnetic fields. Di-electric properties consist of di-electric constant and di-electric loss factor (Valsechi et al., 2004). Di-electric constant is a measure of the ability of a material to store electromagnetic energy, whereas di-electric loss factor is a measure of the ability of a material to convert electromagnetic energy to heat (Gunasekaran et al., 2005; Kumar et al., 2007).
In commercial models, the oven has a power input of about 1000 watts of alternating current. As these MWs generated from the magnetron bombard the food, they cause the polar molecules to rotate at the same frequency millions of times a second. All this agitation creates molecular friction which heats up the food (Ohlsson et al., 1993; Ashline et al., 2007). The friction also causes substantial damage to the surrounding molecules, often tearing them apart and forcefully deforming them (Feng, 2002). This volumetric heat generation results in rapid heating of foodstuffs (Ramaswamy and Tang, 2008).

The frequency at which domestic microwave apparatus is intended to operate is regulated to 12.2 cm, corresponding to a frequency of 2.450(+/-0.050) GHz (Lidstrom et al., 2001; Vollmer et al., 2004). This is different from industrial microwave ovens which normally operate at 915 MHz and are commonly used for frozen food tempering in food industries (TechCommentary, 1993; Ramaswamy and Tang, 2008). Microwaves that operate at 915 MHz generally have deeper penetration depths in food than domestic microwaves (Lau and Tang, 2002).

When food is placed in a MW oven, various food ingredients behave differently. The main ingredient that enables food to be heated by MWs is water. The higher the water content in food, the faster the heating rate. Water in molecular level behaves exactly like a magnet. Water has two oppositely charged ends due to presence of positively charged 2 hydrogen atoms and a negatively charged oxygen molecule. Therefore, water in food also behaves like a magnet. If a bar magnet is held above another bar magnet and you rotate the held magnet, the other one also rotates (Feng, 2002).
Due to two different poles in water, when MWs oscillate the water molecules rotate. This is because the negatively-charged end of water is attracted to positively charged end of MW, while the positively charged end of water is attracted to the negatively charged end of MWs. The MWs rotate at extremely high speed of 2450 times per second. This means for every second a MW rotates, the water molecule also rotates 2450 times. This extremely high rotation rate causes water molecules to collide with each other at a very fast rate. This creates friction between water molecules. This friction generates heat. The heat flows through the food by conduction, convection or radiation hence food warms up (Feng, 2002). The efficiency of microwave radiation is essentially a function of both the electromagnetic field strength and the exposure time. Whereby the electromagnetic energy is expressed in two forms: 1) the factors that depend on the dielectric properties of the dipole molecules of the irradiated materials in the form of heat [thermal effect] and (2) the factors that do not depend on the dipole molecules in the form of a direct effect of the radiofrequency [non-thermal effect] (Jeng et al., 1987).

2.3 Food bacteria

2.3.1 Escherichia coli

Based on clinical manifestation of disease, the types of virulence factors, epidemiology and phylogenetic profiles, strains causing intestinal infections are divided into six separate pathotypes viz. enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli /verotoxin-producing E. coli (EPEC/VTEC), enterohaemorrhagic E. coli/ shiga toxin producing E. coli (EHEC/STEC), diffuse adhering E. coli (DAEC) and enterotoxigenic E. coli (ETEC) (Crossman et al., 2010; Amaya et al., 2011; Konishi et al., 2011).
The EHEC were first described in 1977 (Werber et al., 2012). They have been classified into pathogroups A to E based on the severity of diseases they cause and their association with outbreaks (Olaniran et al., 2011). EHEC/STECs are so named because they produce one or more cytotoxins, called Shiga toxin 1 (stx1) and Shiga toxin 2 (stx2) (Farrokh et al., 2012). The Stx1 group consists of Stx1c, and Stx1d. The Stx2 group consists of Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g (Orth and Wurzner, 2006; Skinner et al., 2013). ETEC refers to those which specifically adhere to the microvilli of the small intestinal epithelial cells and which produce site-specific enterotoxins; that elaborate at least one member of two defined groups of enterotoxins ST and LT (Nataro and Kaper, 1998; Olaniran et al., 2011). EPEC is currently subdivided into typical and atypical subgroups. While typical EPEC carry the EPEC adherence factor plasmid (pEAF) that encodes the bundle-forming pilus (BFP) and a complex regulator of various virulence genes (Per), atypical EPEC is devoid of pEAF (or does not express a functional BFP). EAEC has recently been subgrouped into typical and atypical EAEC, which carry and lack AggR (a global regulator of EAEC virulence), respectively (Yingst et al., 2006). DAEC are characterized by the diffuse adherence pattern on cultured epithelial cells HeLa or HEp-2 and they appear to form a heterogeneous group (Servin, 2005; Mansan-Almeida, 2013).

2.3.1.1 Epidemiology of *Escherichia coli*

*Escherichia coli* are a common part of the normal facultative anaerobic microflora of the intestinal tracts of humans and warm-blooded animals (Ramanathan, 2010) and are commonly released into the environment through deposition of faecal material (Ibekwe et al., 2011). EHEC/STEC has been shown to be the most important group among the six characterized groups of diarrheagenic *E. coli* in developed countries and numerous outbreaks of patients developing life-threatening complications such as
haemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HC) have been reported
(Olaniran et al., 2011). The salient features of EHEC epidemiology include a
reservoir in the intestinal tract of animals; transmission by a wide variety of food
items, with beef being a major vehicle of infection and a very low infectious dose,
enabling high rates of attack and of person-to-person transmission (Nataro and Kaper,
1998). ETEC bacteria cause secretory diarrhoea in humans throughout the world
(Olaniran et al., 2011). Epidemiologic investigations have implicated faecal
contamination of water and food sources as the principal reason for the high incidence
of ETEC infection throughout the developing world (Nataro and Kaper, 1998). This
pathogen causes an estimated ≈400 million diarrheal episodes and 380,000 deaths in
children less than 5 years of age every year (Nicklasson et al., 2010). It is the most
common cause of diarrhea in travelers and in soldiers deployed to developing
countries and the most common E. coli in the region (Fleckenstein et al., 2000; Qadri
et al., 2007. EPEC is a leading cause of infantile diarrhea in developing countries
(Trabulsi et al., 2002; Ramanathan, 2010). The reason(s) for the relative resistance of
adults and older children is not known, but loss of specific receptors with age is one
possibility (Nataro and Kaper, 1998). In industrialized countries, the frequency of
these organisms has decreased, but they continue to be an important cause of diarrhea
(Trabulsi et al., 2002).

2.3.1.2 Prevalence of Escherichia coli

The burden of disease due to E. coli infections is enormous (Garau, 2008).
Diarrhoeagenic Escherichia coli strains are among the bacteria most frequently
associated with diarrhoea in children from developing countries. The ETEC pathovar
represents a significant global health problem. ETEC is the cause of nearly one billion
cases of diarrheal disease annually, resulting in the deaths of 300,000 to 500,000 children under the age of five in low- and middle-income nations (Sahl et al., 2011). It is responsible for the majority of *E. coli*-mediated cases of human diarrhea worldwide (Crossman et al., 2010).

2.3.1.3 *Escherichia coli’s* resistance to antimicrobials

Diarrhea caused by multidrug-resistant bacteria has been recognized as an important public health problem among children in developing countries and is a research priority of the Diarrheal Disease Control Program (DDCP) of the World Health Organization and among these bacteria, strains of the different diarrheagenic categories of *Escherichia coli*, such as ETEC, EHEC, EPEC, EIEC and EAEC are among the most important causes of acute enteritis and subsequent morbidity and mortality in children in developing countries (Vila, 1999). The chromosomal multiple antibiotic resistance (mar) locus of *Escherichia coli* and other members of the *Enterobacteriaceae* controls resistance to multiple, structurally unrelated compounds including antibiotics, household disinfectants, organic solvents and other toxic chemicals (Mehata and Duan, 2011). *Escherichia coli* strains are particularly adept at disseminating resistance genes within bacterial population (Coe et al., 2008). Among other reasons, antibiotic resistance normally occurs due to uncontrolled antibiotic prophylaxis normally recommended for high risk individuals for example immunocompromised (Yates, 2005).

Probiotics colonize the gastrointestinal tract and theoretically prevent pathogenic organisms from infecting the gut (Yates, 2005). Resistance in commensal *Escherichia coli* isolates is emerging and it is well known that commensal bacteria are reservoirs
for antibiotic resistance genes in both the community and hospital settings (Amaya, 2011). Several uncontrolled studies suggest that antibiotics may increase the risk of Haemorrhagic Uraemic Syndrome in patients with hemorrhagic colitis (CDC/MMWR, 2004; Pitout and Church, 2004). Therapy with fluoroquinololones shortens the length of bouts of diarrhea in patients with AIDS and in international travellers with EAEC diarrhea (Adachi et al., 2001).

2.3.2 *Salmonella* Paratyphi

Two *Salmonella* species exist; *Salmonella enterica* and *Salmonella bongori*. All medically important *salmonellae* are included in the former and are divided into more than 2500 serotypes on the basis of surface antigens (O, H and Vi) by use of a scheme initially devised by Kauffman (Feasey et al., 2012). For convenience the serovars are written in an abbreviated form for example the accepted abbreviation for *Salmonella enterica subsp. enterica* serovar Typhi is S. Typhi (Cheesbrough, 2006). *Salmonella* species include *Salmonella Typhi*, *Salmonella Paratyphi*, *Salmonella Enteritidis*, *Salmonella Typhimurium*, and *Salmonella Choleraesius* (Vandepitte et al., 2003).

2.3.2.1 Epidemiology of *Salmonella* Paratyphi

FBDs caused by zoonotic *Salmonella enterica* species represent an important public health problem worldwide (Weill et al., 2006; Diana et al., 2012). Infections are mostly in the middle- and low-income countries where sanitation and water supplies are poor. These include India, South-East Asia, Africa, Central and South America and the Mediterranean region (Effa and Bukirwa, 2008). Salmonellosis is the most important FBD both in terms of the disease intensity, ease of spread and difficulty in prevention and control (Ramanathan, 2010). It is estimated that only 3% of *Salmonella*-related food borne disease is confirmed by microbiology and reported to
surveillance systems (Niehaus et al., 2011). Salmonella serotypes are associated with three distinct human disease syndromes, bacteremia, typhoid fever and enterocolitis (Zhang et al., 2003). Enteric fever occurs mainly in young people between 5-19 years and in some areas it is common among children less than 5 years old (Effa and Bukirwa, 2008). In the United States, there are an estimated 1.4 million Salmonella infections per year and approximately 600 are fatal (Weill et al., 2006). In Europe, deaths were reported in 14% of FBD outbreaks and half of these deaths were linked to Salmonella (Dewaal et al., 2009). Mortality rate is about 10% to 15% if untreated and is highest among children aged less than one year and the elderly (Effa and Bukirwa, 2008). The total burden of invasive disease attributable to invasive non-typhoidal salmonella (NTS) in Africa has not been measured due to underreporting but is probably substantial, with an estimated annual incidence of 175–388 cases per 100,000 children aged 3–5 years and 2000–7500 cases per 100,000 HIV-infected adults (Feasey et al., 2012).

2.3.2.2 Prevalence of Salmonella Paratyphi

Animals are the principal reservoir of this pathogen (Jamshidi et al., 2009). Fish and shellfish appear to be passive carriers of Salmonella spp., demonstrating no clinical disease and can excrete Salmonella spp. without apparent trouble. The contamination of this organism derives from terrestrial sources and fish may serve as a vector (Novotny et al., 2004). It is estimated that Salmonella spp. are responsible for approximately 1.4 million illnesses and 600 deaths annually in the United States alone (Pitout and Church, 2004; Galanis et al., 2006). Although once a substantial public health problem in developed countries, improvements in sanitation have led to near eradication and most cases are imported. A large burden of disease remains in
developing countries, particularly in Asia (Faesey et al., 2012). Some infected individuals recovering from this infection become temporary or permanent carriers harboring the organisms in the gall bladder, biliary tract and rarely in the intestines. Chronic carriers are generally over 50 years old and are commonly women, food handlers or people working in supply and distribution of food stuffs (Yousefi-Mashouf et al., 2003). Published accounts of invasive salmonella in Africa show that the disease is highly seasonal. Peaks of infection during the rainy season in both adults and children coincide with increased incidences of malaria and malnutrition (Faesey et al., 2012).

2.3.2.3 Salmonella Paratyphi resistance to antimicobials

Before 1990, antibiotic resistance in NTS species was rare (Pitout and Church, 2004) therefore fluoroquinolones such as ciprofloxacin and extended-spectrum cephalosporins were the drugs of choice for these severe infections (le Hello et al., 2011). As a consequence of the extensive use of antibiotics, the incidence and severity of human diseases related to Salmonella caused by antibiotic resistant Salmonella is rising in many countries (Breuil et al., 2000). These illnesses can be very difficult to control (Oliveira et al., 2006). In fact, when severe extra-intestinal infections occur and treatment is indicated that can be life-saving, it is difficult when the organism is resistant to the antimicrobials used (Tauxe, 1986).

Infection with drug-resistant strains of Salmonella Typhi or Paratyphi increases morbidity and mortality (Effa and Bukirwa, 2008). With the emergence of resistance to ampicillin, chloramphenicol and cotrimoxazole, first choice of empiric treatment of typhoid fever has changed to ciprofloxacin, ceftriaxone (Shadia et al., 2011),
azithromycin and cephalosporins (Effa and Bukirwa, 2008). An increase in antibiotic resistance was observed during the 1990s due to the emergence of an epidemic multidrug resistant (MDR) strain of serotype Typhimurium (Weill et al., 2006). Antibiotic therapy is generally encouraged because it may decrease the inoculum required to establish infection by these microorganisms (Paton et al., 1991). It also reduces the risk of invasive Salmonellosis as in the case of immunocompromised individuals (Pitout and Church, 2004). However, recent experience with multidrug-resistant S. Typhimurium DT 104 demonstrates the potential for global spread of resistant Salmonella infection (le Hello et al., 2011). Some studies argue that antimicrobial therapy does not hasten clinical recovery and may lengthen the convalescence and asymptomatic carrier state therefore therapy should be recommended only for complicated cases (Vandepitte et al., 2003).

2.3.3 Shigella flexneri

Shigella dysenteriae is among 40 serotypes of Shigella spp. which are divided into four groups: [AHW, 2011] Group A - Shigella dysenteriae, Group B - Shigella flexneri, Group C - Shigella boydii and Group D - Shigella sonnei. This division is based on biochemical and serological differences. With the exception of S. sonnei, each of the Shigella species is further divided into serotypes on the basis of reactivity with hyperimmune serum: S. dysenteriae (15 serotypes), S. flexneri (6 serotypes and 2 variants) and S. boydii (20 serotypes) (van der Ploeg, et al., 2010).

2.3.3.1 Epidemiology of Shigella flexneri

Of the estimated 164.7 million Shigella diarrhoeal episodes occurring globally every year, most occur in developing countries (99%) and mainly in children (69%)
Shigellosis or bacillary dysentery, an acute bloody diarrhoea, is a major public health burden in developing countries (Nato et al., 2007) which occasionally causes illness and death worldwide (Christopher et al., 2008). The genus *Shigella* is specific host-adapted to humans and higher primates and its presence in the environment is associated with faecal contamination (Novotny et al., 2004; Ramanathan, 2010). *S. flexneri* and *S. dysentriae* are the most commonly isolated *Shigella* species in the developing world and the most frequent cause of bacterial dysentery. In countries with improved water supply and sanitation, the predominant species is *S. sonnei* (Madiyarov et al., 2010). *S. dysenteriae* type 1 was the first described and stands out for causing deadly epidemics in the most impoverished areas. This bacterium was responsible for large dysentery epidemics in Guatemala and other parts of Zaire, Central America, Bangladesh, Kenya and recently West Africa and India (Taneja et al., 2011).

### 2.3.3.2 Prevalence of *Shigella flexneri*

About 1.1 million people around the world are estimated to die from *Shigella* infection each year, with 60% of the deaths occurring in children under 5 years of age (Christopher et al., 2008). More recent estimates fix the *Shigella* disease burden at 90 million episodes and 108,000 deaths per year and about 500,000 cases of shigellosis are reported each year among military personnel and travelers from industrialized countries (WHO, 2009). The species distribution varies globally; for example, *S. flexneri* was reported to be the most prevalent in India (58%) and Rwanda (68%) while *S. sonnei* was the most frequently detected species in Thailand (85%), Israel (48.8%) and the USA (75%) (Christopher et al., 2008). In China, *Shigella* spp. is the most frequently isolated gastrointestinal pathogen accounting for up to 1.7 million
episodes of bacillary dysentery annually, with up to 200,000 patients admitted to hospitals (Chen et al., 2010) with *Shigella flexneri* being the major pathogen implicated (Ying et al., 2005; Qiu et al., 2012). This pathogen was also the most frequently isolated when a prospective, multicentre, population-based study was carried out in six Asian countries to determine the disease burden, clinical manifestations and microbiological properties of *Shigella* spp. (von Seidlein et al., 2006) with a majority of the *S. flexneri* isolates being resistant to amoxicillin and cotrimoxazole. In a study carried out at the National Institute for Medical Research in Mwanza city, Tanzania among the *Shigella* spp. isolated, 90% was *Shigella flexneri* and 10% *Shigella dysentriae* (Temu et al., 2007). In contrast to many other enteric infections, shigellosis is clearly not confined to childhood. On the contrary, the incidence of shigellosis not only increases steadily after age 40 years, but the bacterial load of shigellosis patients increases after 40 years of age, suggesting that older people as well as very young children shed the highest bacterial load and may contribute disproportionally to the transmission of shigellosis (von Seidlein et al., 2006).

### 2.3.3.3 *Shigella flexneri* resistance to antimicrobials

Mild symptoms of shigellosis are self-limiting but antibiotics are recommended for more severe cases, to speed up recovery, reduce the length of time patients are infective and for preventing relapse (Christopher et al., 2008). A range of different antimicrobials are effective for treatment of shigellosis, although options are becoming limited due to the emergence of multidrug resistance in different species and serotypes (von Seidlein et al., 2006). Thus, sulfonamides, tetracycline, ampicillin, and cotrimoxazole are no longer recommended for empirical treatment (Niyogi,
2005). This is quite unfortunate considering that antibiotics are the primary treatment for shigellosis (Sack et al., 2001). Antibiotic therapy for shigellosis reduces the duration and severity of the disease, reduce shedding of organisms and can also prevent potentially lethal complications (Orett, 2008). However, over the past few decades *Shigella* spp. have become resistant to most of the widely used antimicrobials (Mehata and Duan, 2011) *S. dysenteriae* 1 (Shiga’s bacillus) is generally the first to develop resistance to a new antibiotic, but then the other *Shigella* species follow (Sack et al., 2001). The shift in the prevalence of serogroups and the changing patterns in antibiotic susceptibilities among *Shigella* isolates pose a major difficulty in the determination of an appropriate drug for the treatment of shigellosis (Bhattacharya et al., 2005). The primary factor for increase in resistance to antibiotics is over-use of antibiotics (Sack et al., 2001). This is the main reason why this study encourages the use of modern interventions that decrease overall rates of morbidity and mortality (by targeting the overall burden of the disease) and will also decrease the spread of resistant organisms. Ciprofloxacin resistant *Shigella flexneri* isolates were identified in China, Pakistan and Vietnam (von Seidlein et al., 2006). Epidemic *Shigella dysenteriae* 1 strains resistance to ampicillin, chloramphenicol, nalidixic acid, tetracycline, trimethoprim - sulfamethoxazole, and only moderate susceptibility to ciprofloxacin have been observed with increasing frequency in Africa and Asia, while strains resistant to ciprofloxacin have recently been isolated in India and Bangladesh, thus reducing the availability of effective oral therapy (Taneja et al., 2011). According to World Health Organization report, antibiotic resistance pattern for *shigella* varies in different parts of the world and with the time (Savadkoohi and Ahmadpour-kacho, 2007).
2.3.4 *Staphylococcus aureus*

Ribosomal RNA (rRNA) hybridization and comparative oligonucleotide analysis of 16S rRNA has demonstrated that Staphylococci form a coherent group at the genus level. This group occurs within the broad *Bacillus-Lactobacillus-Streptococcus* cluster defining Gram-positive bacteria with a low G + C content of DNA. At least 30 species of staphylococci have been recognized by biochemical analysis and in particular by DNA-DNA hybridization. Eleven of these can be isolated from humans as commensals (Baron, 1996).

2.3.4.1 Epidemiology of *Staphylococcus aureus*

*Staphylococcus aureus* has been a food safety concern for decades because it is widespread in the environment and often detected in air, dust, water, raw milk, other foods and on environmental surfaces (Doyle *et al.*, 2011). The epidemiology of Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently being studied however, it should be noted that in most hospitals and geographic areas, MRSA are responsible for a greater number of infections and are often also resistant to multiple classes of antibiotics (Doyle *et al.*, 2011). MRSA have spread world-wide and are now the most commonly identified antibiotic-resistant bacteria in hospitals in Europe, the Americas, North Africa and the Middle and Far-East (Doyle *et al.*, 2011).

2.3.4.2 Prevalence of *Staphylococcus aureus*

*Staphylococcus aureus* are important pathogens contributing to the FBDs worldwide because they are natural microbiota that live in human body and could contaminate food due to poor sanitation and hygienic practices (Dewanti-Hariyadi *et al.*, 2011). The bacteria are found particularly in the skin and mucous membranes of animals,
including humans (Friendship and Wesse, 2009). These organisms are present in nasal passages or skin of about 50% of people and in the intestines of 20% of people in the general population (Doyle et al., 2011). Staphylococcal food poisoning was estimated to account for 185,000 food borne illnesses per year in the United States in 2002 with most of the cases going unreported (Jones et al., 2002). It is ranked as the most prevalent cause of gastroenteritis world-wide (Ramanathan, 2010) and continues to be among the top five most reported food-borne illnesses to the CDC (Carmo et al., 2004). MRSA strains were first reported in 1961 and the major mode of transmission from patient to patient is on the contaminated hands of healthcare workers (Brown et al., 2005; Friendship and Wesse, 2009). They are also community acquired however some argue that this could be an example of second-generation spread of a healthcare-associated pathogen in the community (Jones et al., 2002). Initially MRSA infections were primarily a problem of hospitals and nursing homes (Jones et al., 2002). There have been only two reported outbreaks associated with MRSA contaminated foods; one was a community outbreak of food borne illness in US in 2000 and another occurred in a Dutch hospital and affected 27 patients and 14 health workers from 1992-1993 resulting in 5 deaths (Jones et al., 2002; Doyle et al., 2011).

2.3.4.3 Staphylococcus aureus’ resistance to antimicrobials
Most Staphylococcus aureus isolates are multiple-resistant to various antibiotics. Resistance to penicillin among Staphylococcus aureus strains appeared a few years after the introduction of penicillin therapy (Ombui et al., 2000; Chakraborty, 2011). This resistance is mediated by penicillinase (a form of β-lactamase) production: an enzyme which breaks down the β-lactam ring of the penicillin molecule (Islam et al., 2008). It is estimated that now >80% of Staphylococcus aureus produce penicillinase
Introduction of other antibiotics such as streptomycin, tetracycline, chloramphenicol and the macrolides was similarly followed by emergence of resistant organisms. The appearance of antibiotic-resistant staphylococci over the years was regarded as an inevitable genetic response to the selective pressure imposed by antibiotic use. Indeed, resistance within the staphylococci to several therapeutically useful antibiotics is thought to be derived from chromosomal mutation (Ombui et al., 2000).

The first clinical isolates of macrolide-resistant staphylococci were described in reports from France, England, Japan, and the United States shortly after the introduction of erythromycin into clinical practice in 1953 (Weisblum, 1995). Methicillin (Meticillin) is a β-lactam antibiotic which was introduced in the 1950s and is not inactivated by penicillinase but by 1961 there were reports that there was resistance in a hospital in the United Kingdom (Doyle et al., 2011). The MRSA is a specific strain of the Staphylococcus aureus bacterium that has developed antibiotic resistance to all penicillins, including methicillin and other narrow-spectrum β-lactamase-resistant penicillin antibiotics. MRSA may also be known as oxacillin-resistant Staphylococcus aureus (ORSA) and multiple-resistant Staphylococcus aureus (Islam et al., 2008). Many Staphylococcus aureus isolates obtained as part of outbreak investigations may not be tested for antibiotic susceptibility and therefore methicillin-resistant strains may go unrecognized as the cause of food borne outbreaks of acute gastroenteritis (Jones et al., 2002). Most resistance to oxacillin in staphylococci is mediated by the meca gene, which directs the production of a supplemental penicillin-binding protein, PBP 2a and is expressed either homogeneously or heterogeneously. Homogeneous resistance is easily detected with
standard testing methods, whereas heterogeneous expression may be more difficult to
detect with some methods because only a fraction of the population (for example, 1 in
100 000 cells) expresses the resistance phenotype. In the past, the presence of
resistance to other classes of agents was an indication of oxacillin resistance.
However, some MRSA such as those found in community-associated infections are
not multiple resistant (CLSI, 2006). The first occurrence of a strain of *S. aureus* with
reduced susceptibility to vancomycin (MICs 4 to 16 μg/mL) was reported from Japan
in 1997, followed by reports from the United States and France. The exact
mechanisms of resistance that result in elevated MICs are unknown, although they are
likely to involve alterations in the cell wall and changes in several metabolic
pathways. To date, most vancomycin intermediate *Staphylococcus aureus* strains
appear to have developed from MRSA (CLSI, 2006).

### 2.4 Infective dose

Risk assessment for any food borne pathogens requires animal and/or human data that
deal with the pathogen dose and severity of the disease (Kothary and Babu, 2001).
This is why knowledge of a pathogen’s infective dose is important. The term
‘infectious’ is defined as ‘capable of being transmitted by infection’ and ‘denoting a
disease due to action of a microorganism’. The definition of ‘infectious dose’, also
called ‘infective dose’, is hard to come by in medical texts, however in day to day
layman’s terms it is defined as the number of organisms necessary to cause disease
(Johnson, 2003). Legget *et al.* (2012) defined it as number of pathogenic cells
required to successfully infect a host. These number required to infect a host vary
dramatically across pathogen species. Therefore, the Occupational Safety and Health
Administration (OSHA) suggested that a more practical definition would be ‘a dose at
which an organism can reproduce in the host and produce a measurable effect’ (Johnson 2003).

Some pathogens can begin an infection with only a small number of cells in the initial inoculums, for example, enterohemorrhagic strains of *E. coli* and *Shigella* spp. require an infective dose of only about ten cells. By contrast, other pathogens such as *S. aureus* require a large number of cells (10³ to 10⁸ cells) in the inoculum to successfully infect a host (Schmid-Hempel and Frank, 2007). The infectious dose of *Salmonella* spp. is food-dependent as well as dependent on the type of infection, for instance, infectious dose for the enteric fever *salmonellas* is about 10²–10³ organisms per inoculum, whereas the infectious dose for the gastroenteritis *salmonellas* is about 10⁶–10⁸ organisms per inoculum, mainly because of their susceptibility to gastric acid (WHO, 2003).

Infectious dose is inversely related to the incubation period. Generally the incubation period ranges between 5-72 hours/12-36 hours depending on the infectious dose and the immune status of the individual (Ramanathan, 2010). Schmid-Hempel and Frank (2007) postulated that local pathogenic action requires only a small number of molecules and thus relatively few cells are needed to start an infection, compared to distantly acting mechanisms where a large number of diffusible molecules need to accumulate in order to overwhelm the host’s immune clearance. This statement was proven to be true by a study done in 2012 which concluded that pathogens with immune modulators that act distantly within the host have significantly higher infective doses than pathogens with locally acting molecules (Legget et al., 2012). The human infective dose of *Salmonella* varies depending on the serovar of the
organism. Results from the volunteer studies indicated that the infective dose for various serovars was $10^5-10^{10}$ organisms per inoculum and epithelial penetration was the major mechanism involved in pathogenesis of *S. dysenteriae* infection and that the infectious dose for the highly virulent strain was less than 10 organisms per inoculum (Kothary and Babu, 2001). Overall, *S. flexneri* 2a appeared to have an infectious dose of 140 organisms or less per inoculum. This dose might have varied depending on the age and/or physical condition of the individuals. The infective dose of *S. sonnei* was found to be $<500$ organisms. The study suggested that in order for *E. coli* to induce infection, the number of organisms should be exceeding $10^{10}$ (Kothary and Babu, 2001). Table 2.1 provides guidance on the status of food with regard to number of colony forming units present (New South Wales Food Authority, 2009).

A pathogen's infectious dose is just one of the many factors that are considered when a biological hazard analysis is performed. Other factors include: virulence, pathogenicity, environmental stability, route of transmission, quantity, communicability, operations, availability of vaccine or treatment and gene product effects such as toxicity, allergenicity and physiological activity (Johnson, 2003). Information of bacterial infective dose is however very useful in assessing the risk of consuming certain foods contaminated with these pathogens (Kothary and Babu, 2001).
Table 2.1: Guidance levels for determining the microbiological quality of ready-to-eat foods (acceptable limits). These limits are based on the infective doses mentioned on section 2.4.

<table>
<thead>
<tr>
<th>Organism</th>
<th>CFU/g in 25 g of food</th>
<th>Acceptable</th>
<th>Unacceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>&lt;10² to &lt;10⁴</td>
<td>&lt;10⁴</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>&lt;3 to 10³</td>
<td>≥10²</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>&lt;10² to &lt;10³</td>
<td>10³ to &gt;10⁴</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Should not be detected</td>
<td>Detected</td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>Should not be detected</td>
<td>Detected</td>
<td></td>
</tr>
</tbody>
</table>

(Risk Assessment Section, 2007)

2.5 Antibiotics

Antimicrobial agents may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function or blocking metabolic pathways through inhibition of key enzymes (Prescott *et al.*, 2002; Tenover, 2006). Numerous antimicrobials including most structural classes of antibiotics were discovered during 1920-1970. Chemical modification of many of these compounds led to new entities with superior activities (IFT, 2006). There are different classes of antibiotics whose inhibitory and cidal activities vary in terms of their pharmacodynamic properties (Jenkins and Jerris, 2011).

Many pathogenic organisms have developed resistance to well-established and newer antimicrobials; therefore the threat to human health is of growing concern (Sack *et al.*, 2001; Onyango *et al.*, 2008). Some of these strains are multi-resistant and the agents...
available to treat infections caused by them are few and dwindling (Finch and Hunter, 2006). Widespread in vitro resistance to traditional antibiotics has been reported since the 1980s (Gomi et al., 2001). This resistance to antibiotics in bacteria may occur due to a spontaneous mutation or may be acquired through transmission from other resistant bacteria (Ombui et al., 2000). Bacterial species have become capable of transferring virulence genes not only between members of a particular species but also between different bacterial species (Shapiro et al., 2001; Olaniran et al., 2011). Resistant pathogens are emerging and spreading more rapidly than in previous decades (Finch and Hunter, 2006) and therefore there is no doubt that emerging antibiotic resistance is a serious global problem (ICMR Bulletin, 2009). It results in ineffective treatment which has multiple potential consequences including delaying the time until effective treatment is offered to patients when antimicrobial agents are indicated, creating a false sense of security for both patients and health care providers. It adds significantly to the cost of treating diarrhoeal diseases and contributes to the development of further resistance of bacterial pathogens (Shapiro et al., 2001).

According to the British National Formulary 59 (2010) and Cheesbrough (2006), the best antibiotics recommended for Shigella spp. are norfloxacin, cloramphenical, amoxicillin, ampicillin and trimethoprim; with ciprofloxacin and azithromycin being recommended most especially for resistant organisms. In fact, ciprofloxacin is currently the first-line antibiotic recommended by WHO for treatment of Shigellosis (Qiu et al., 2012). Smith et al. (2012) also recommend ceftriaxone as an effective drug for Shigella spp. When it comes to E. coli the drugs recommended are aminoglycosides, paromomycins, erythromycin, ciprofloxacin, penicillin, ampicillin, gentamycin and vancomycin. As for S. aureus, cephalosporin, cephalexin, cephadroxil, nitrofurantoin, vancomycin (for MRSA), penicillin and oxacillin are
recommended; with tilmacosin, erythromycin and tetracycline being the most recommended. Several antibiotics have been used in combination as empiric antibiotic therapy for some of the staphylococcal infections, especially aminoglycosides, penicillins, cephalosporins and vancomycin as a consequence of constantly changing antibiotic susceptibility patterns (Gakuu, 1997; Weinstein, 1998; Ayliffe, 1999). The drugs recommended for *Salmonella* include cephalexin, nalidixic acid, gentamycin, norfloxacin, cefotaxime, azithromycin, ceftriaxone, ciprofloxacin, sulfadiazine, neomycin, tetracycline, streptomycin, kanamycin, cotrimoxazole and amoxicillin/clavulanic acid i.e. augmentin. Onyango *et al.* (2008) also recommended ampicillin, chloramphenical and ciprofloxacin. Most antimicrobials are often chosen on the basis of availability and expense (Mwansa *et al.*, 2002). In this study, the drugs selected are not to be recommended for treatment but serve as a phenotypic characteristic to study any mutation of the pathogens due to exposure to MW radiation.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of the study

The study was carried out at an accredited laboratory located in the Microbiology Department of Xi’an Jiaotong University in Xi’an, Shaanxi district, People’s Republic of China.

3.2 Research design

This was an experimental study where the pretest - posttest research design was applied. The mean counts of bacterial colonies formed before exposure to MW radiation were compared to the mean counts after exposure to MW radiation. In addition, antibiotic susceptibility testing (Kirby- Bauer disc diffusion technique) was performed on organisms before and after exposure to MW radiation.

3.2.1 Test organisms

Microwave radiation was applied to food artificially contaminated with standard bacteria strains procured from American Type Culture Collection (ATCC) and Chinese Medical Culture Collection (CMCC). The bacteria used included Staphylococcus aureus ATCC 25922 (ID $= >10^4$ CFU/mL), Escherichia coli ATCC 25923 (ID $= >10^2$ CFU/mL), Salmonella enterica subsp. enterica serovar Paratyphi CMCC 50319 (Similar to ATCC 19430) (ID = $10^1$ - should not be detected in 25 g) and Shigella flexneri CMCC 51285 (Similar to ATCC 29903) (ID $= <500$ - should not be detected in 25 g) (Roberts and Greenwood, 2003; Greig et al., 2010). These
bacteria were selected because they are among the most common causative agents of food borne diseases.

Table 3.1: Food-Microorganism combination for testing (criteria for inclusion)

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Microorganisms tested</th>
<th>Methods from which food – microorganism combinations were based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td><em>Salmonella</em> Paratyphi, <em>Staphylococcus aureus</em>, <em>Escherichia coli</em></td>
<td>EN ISO16140:2008(E), AOAC, 1998 International Methods Committee Guideline for Validation,</td>
</tr>
<tr>
<td>Fish</td>
<td><em>Salmonella</em> Paratyphi, <em>Escherichia coli</em></td>
<td>Classification of food categories for validation studies, ICMSF vol. 6 (1998).</td>
</tr>
<tr>
<td>Vegetables</td>
<td><em>Salmonella</em> Paratyphi, <em>Escherichia coli</em></td>
<td>Microbial ecology of food commodities</td>
</tr>
<tr>
<td>Milk</td>
<td><em>Salmonella</em> Paratyphi, <em>Staphylococcus aureus</em>, <em>Escherichia coli</em></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td><em>Salmonella</em> Paratyphi, <em>Escherichia coli</em></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td><em>Staphylococcus aureus</em>,</td>
<td></td>
</tr>
<tr>
<td>Gravy / Sauce</td>
<td><em>Salmonella</em> Paratyphi, <em>Escherichia coli</em></td>
<td></td>
</tr>
</tbody>
</table>
3.2.2 Dependent and independent variable

The Independent variable (Exploratory/Predictor Variable) \([X]\) refers to the MW radiation which was manipulated in terms of time and degree of exposure. The dependent variables (outcome/response variables) \([Y]\) include number of CFU formed, effective exposure time, antibiotic susceptibility and viability (i.e. growth and multiplication of the bacteria).

3.2.3 Growth and maintenance of standard bacterial strains

Each reference culture was checked thoroughly upon receipt. Gram stain and biochemical testing were performed to confirm bacteria identities. Pure cultures were stored at a temperature of -80°C in nutrient broth supplemented with 20% (v/v) of glycerol. The bacteria were daily cultivated for 24 hours on nutrient agar at 37°C. Working bacterial suspensions were freshly prepared for each independent experiment. Medium and incubation conditions were used as specified in the ATCC / CMCC catalogue when first reviving strains to ensure optimal conditions for recovery. Drops (100 ul) of bacteria suspension were also transferred to an agar slant. They were also stored at a temperature of 5°C. Cultures were incubated under the appropriate conditions and were sub-cultured twice prior to testing each week.

3.2.4 Antibiotic susceptibility testing discs

When received, jars with discs (9 mm diameter) containing antimicrobial agents were placed directly into a freezer (-20°C) where they were stored until needed. All discs were used before their labelled expiration dates. To prevent condensation, the jars and disc dispensers were allowed to warm to room temperature before being opened. The unused portion of the discs was put back into the refrigerator to minimize the exposure to room temperature and humidity (WHO, 2003). The antibiotics used in
this study were purchased from Beijing Tiantan Biological Products Company Limited (600161. SH). They included; nitrofurantoin (300 μg), oxacillin (1 μg), ampicillin (10 μg), gentamycin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), vancomycin (30 μg), azithromycin (15 μg) and cefotaxime (30 μg). They were used for semi-quantitative in vitro susceptibility testing by the Kirby Bauer agar disc diffusion test procedure. These discs were applied to the surface of Mueller-Hinton Agar plates (refer to section 3.4.3) that were inoculated with pure cultures of clinical isolates. After overnight incubation, the zones of inhibition surrounding the discs were measured (CLSI, 2006) as described in section 3.5.9. The guidelines and interpretive criteria of the Clinical Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) were applied. Multi-drug resistance was defined as resistance to three or more antimicrobials (Orett, 2008; CDC/National Antimicrobial Resistance Monitoring System for Enteric Bacteria, 2012).

The suggested battery of antibiotics for susceptibility testing was applied as advised by Clinical Laboratory Standards Institute (2006) and ICMR Bulletin (2009). For E. coli, S. Paratyphi and S. flexneri, ampicillin, gentamycin, ciprofloxacin and cefotaxime were used and the antibiotics which were tested against S. aureus included nitrofurantoin, oxacillin, vancomycin, erythromycin and azithromycin.

3.2.5 Preparation of Mueller Hinton Media for Antimicrobial Susceptibility Testing

Mueller Hinton agar is considered to be the best for routine susceptibility testing of non-fastidious bacteria for the following reasons: It shows acceptable batch-to-batch reproducibility, it is low in sulphonamide, trimethoprim and tetracycline inhibitors, it gives satisfactory growth of most non-fastidious pathogens and a large body of data
and experience has been collected concerning susceptibility tests performed with this medium (CLSI, 2006; ICMR Bulletin, 2009). International collaborative studies have confirmed the value of this agar in terms of reproducibility and simplicity of its formula (Bauer et al., 1966; Ericsson and Sherris, 1971).


**Composition:** Casein hydrolysate -17.5 g, Starch- 1.5 g, Beef extract – 5.0 g, Agar – 12.5 g

36.5 g of the powder was added to 1000 ml of dH₂O, boiled to dissolve and autoclaved at 121°C for 15 minutes as instructed by manufacturer. As advised by CLSI (2006), the pH of M.H. agar was checked at room temperature; that is after gelling. The pH was between 7.2 - 7.4 at room temperature for each batch of culture plates made. After solidification, the plates were dried for immediate use for 20 minutes at 35°C in the incubator (Vandepitte et al., 2003)

### 3.3 Physical properties of Foods

Physical examination of foods was also carried out to ensure they were in good condition when they arrived in the laboratory. The appearance of food in terms of change in colour, growth of mould, consistency or development of slime as well as the food odor/aroma was taken into consideration (Lattuada and Dey, 1998). Changes in physical properties of the food samples were evaluated before and after treatment with microwave radiation.
3.4 Sample size determination

The Fishers et al., 1998 formula was applied to determine sample size because it is valid for all sample sizes plus it is one of those exact tests where the significance of the deviation from the null hypothesis can be calculated exactly for example p-value, rather than relying on an approximation. The sample size, in this case refers to the number of food samples that were processed in the laboratory. This was determined largely by three factors:

i. The estimated prevalence of food borne illnesses in Kenya. This is determined by calculating the average number of food borne diseases outbreak incidences divided by the population of Kenya.

ii. The desired level of confidence

iii. The acceptable margin of error

This study was based on a simple random sample; therefore the sample size required was calculated using the Fischer’s exact formula (Fischer’s et al., 1998)

\[ N = Z^2 p Q / d^2 \]

Where \( N \) = required sample size

\[ Q = 1 - p \]

\( Z \) = confidence interval at 95 % (standard value of 1.96)

\( p \) = estimated prevalence of food borne illnesses in Kenya

\( d \) = margin of error at 5 % (standard value of 0.05)

In Kenya it has been estimated that roughly 2.2 million people suffer from FBDs every year (FAO/WHO, 2005). The CIA recorded that by July-2011 the total population of Kenya had increased to approximately 41,070,934. Therefore the value of \( p = 2,200,000 / 41,070,934 \), which is 0.053\%.
Calculations:

\[ N = \frac{1.96^2 \times 0.05(1-0.05)}{0.05^2} \]
\[ = \frac{3.84 \times 0.05(0.95)}{0.0025} \]
\[ = \frac{3.84 \times 0.0475}{0.0025} \]
\[ = 0.1824/0.0025 \]
\[ = 72.96 = 73 \text{ samples.} \]

3.5 Sampling techniques

The sampling technique used in this study was purposive sampling where food samples were purchased depending on the type of organism to be tested as highlighted in section 3.92 and convenient sampling where samples were purchased based on ease of access from nearby supermarkets.

3.5.1 Food samples

The food samples, as shown on figure 3.1, were obtained from a commercial source. They included cooked beef \((n=12)\), milk \((n=10)\), cooked chicken \((n=10)\), eggs \((n=8)\), gravy and sauce \((n=8)\), potatoes \((n=4)\), fish \((n=11)\) and vegetables \((n=10)\). Therefore in total \(n=73\). These particular foods were selected because they are very popular foods and most sensitive foods that can easily get spoiled due to their high protein and water content. They are therefore classified as high risk foods (Kenya Food, Drugs and Chemical Substances Act, CAP 254, 1992; Bekker, 2003; Kitagwa et al., 2005). All samples were transferred to the laboratory within 1-2 h in insulated boxes and stored at 4°C until use within 24 h after purchase.
3.5.2 Microwave radiation and temperature

The microwave apparatus used had a fixed frequency of 2.45 GHz; however radiation of samples were done at several powers (P-80, P-60, P-40, P-20) as well as various time periods and compared for efficiency in bactericidal activity (Fujikawa, et al., 1992). The time range of food exposure to microwave radiation was between 1-8 minutes at 2 minute intervals. This time range was selected based on previous studies (Banik et al., 2003) which indicated that it is the best range required to kill microorganisms without necessarily destroying the nutritional content and palatability of food.

Irradiation was performed using a domestic microwave oven, Galanz type: G702011YSL-V1, equipped with a rotating glass plate with maximum power output of 1180 watts at 2450 MHz. Five (5) Power levels (with each higher power having a 20% increment), Product Dimension: 295mm x 458mm x 445mm, European design. Manufacturer: Guangdong Galanz Enterprise Group Company Limited. The following treatments were evaluated: Power-80 (1180 W), Power-60 (885 W), Power-
40 (590 W) and Power-20 (295 W) at 2, 4, 6 and 8 minutes at each power. Treatments were applied by choosing the selected combination of power and time, using the control panel of the microwave oven.

The temperature and radiation frequency was monitored using a thermometer and radiometer during the whole process of exposing foods to MW radiation. The permitted radiation leakage was <5Mw/cm².

### 3.5.3 Microwave radiation of food and isolation of *Salmonella enterica subsp. enterica serovar Paratyphi*

Food samples (25 g) were weighed then autoclaved at a temperature of 121°C for 15 minutes to ensure destruction of other bacteria. They were inoculated with *Salmonella enterica subsp. enterica serovar Paratyphi* CMCC 50319 corresponding to 1 ml of 0.5 McFarland standard (this is in excess of their infectious dose) and incubated for 6 hours at a temperature of 37°C (as shown on figure 3.2). Each sample was then exposed to MW radiation at 0, 2, 4, 6 or 8 minutes and exposed to different power levels (P-80, P-60, P-40, P-20 and P-00). The temperature of each sample was measured immediately after removal from microwave oven, after which each of the samples was stored at a temperature of 3°C overnight to ensure favorable outgrowth of any surviving colonies; they were then aseptically homogenized. The sample-to-broth ratio of 1:9 was maintained by enriching 9 ml of Selenite Cysteine Broth with 1 ml of sample and incubated at a temperature of 37°C for 24 hours. The enrichment media were then plated on selective agar media (Desoxycholate Lactose Agar) using the spread plate method. The plates were incubated at 37°C for 24 hours and then examined for non-lactose fermenting pale-coloured colonies where the colony forming units were enumerated. Bacteria identity was reconfirmed using gram stain
and biochemical testing (Okolie et al., 2011; Kiiyukia, 2003; Roberts and Greenwood, 2003; Feng et al., 2002).

3.5.4 Microwave radiation of food and isolation of *Escherichia coli*

Food samples (25 g) were weighed then autoclaved at a temperature of 121°C for 15 minutes to ensure destruction of other bacteria. They were inoculated with *E. coli* ATCC 25923 corresponding to 1 ml of 0.5 McFarland standard (this is in excess of their infectious dose) and incubated for 6 hours at a temperature of 37°C. Each sample was then exposed to MW radiation at 0, 2, 4, 6 or 8 minutes and exposed to different power levels (P-80, P-60, P-40, P-20 and P-00). The temperature of each sample was measured immediately after removal from microwave oven, after which each of the samples was stored at a temperature of 37°C overnight to ensure favourable outgrowth of any surviving colonies; they were then aseptically homogenized. The sample-to-broth ratio of 1:9 was maintained by enriching 9 ml of Nutrient Broth with 1 ml of sample and incubated at a temperature of 37°C for 24 hours. The enrichment media were then plated on selective agar media (MacConkey Agar) using the spread plate method (as shown on figure 3.4). The plates were incubated at a temperature of 37°C for 24 hours and then examined for lactose fermenting smooth pink colonies where the colony forming units were enumerated. Bacteria identity was reconfirmed using gram stain and biochemical testing (Okolie et al., 2011; Kiiyukia, 2003; Roberts and Greenwood, 2003; Feng et al., 2002).

3.5.5 Microwave radiation of food and isolation of *Shigella flexneri*

Food samples (25 g) were weighed then autoclaved at a temperature of 121°C for 15 minutes to ensure destruction of other bacteria. They were inoculated with *Shigella flexneri* CMCC 51285 corresponding to 1 ml of 0.5 McFarland standard (this is in
excess of their infectious dose) and incubated for 6 hours at a temperature of 37°C. Each sample was then exposed to MW radiation at 0, 2, 4, 6 or 8 minutes and exposed to different power levels (P-80, P-60, P-40, P-20 and P-00). The temperature of each sample was measured immediately after removal from microwave oven, after which each of the samples was stored at a temperature of 37°C overnight to ensure favorable outgrowth of any surviving colonies; they were then aseptically homogenized. The sample-to-broth ratio of 1:9 was maintained by enriching 9 ml of Selenite Cysteine Broth with 1 ml of sample and incubated at a temperature of 37°C for 24 hours. The enrichment media were then plated on selective agar media (Desoxocholate Lactose Agar) using the spread plate method. The plates were incubated at a temperature of 37°C for 24 hours and then examined for non-lactose fermenting pale-coloured 1-2mm diameter colonies where the colony forming units were enumerated. Bacteria identity was reconfirmed using gram stain and biochemical testing (Okolie et al., 2011; Kiiyukia, 2003; Roberts and Greenwood, 2003; Feng et al., 2002).

3.5.6 Microwave radiation of food and isolation of Staphylococcus aureus

Food samples (25 g) were weighed then autoclaved at a temperature of 121°C for 15 minutes to ensure destruction of other bacteria. They were inoculated with S. aureus ATCC 25922 corresponding to 1 ml of 0.5 McFarland standard (this is in excess of their infectious dose) and incubated for 6 hours at a temperature of 37°C. Each sample was then exposed to MW radiation at 0, 2, 4, 6 or 8 minutes and exposed to different power levels (P-80, P-60, P-40, P-20 and P-00). The temperature of each sample was measured immediately after removal from microwave oven, after which each of the samples was stored at a temperature of 37°C overnight to ensure favorable outgrowth of any surviving colonies; they were then aseptically homogenized. The sample-to-broth ratio of 1:9 was maintained by enriching 9 ml of Nutrient Broth with 1 ml of
sample and incubated at a temperature of 37°C for 24 hours. The enrichment media were then plated on selective agar media (Salt Agar) using the spread plate method. The plates were incubated at a temperature of 37°C for 24 hours and then examined for lactose fermenting 0.1-0.5mm diameter colonies where the colony forming units were enumerated. Bacteria identity was reconfirmed using gram stain and biochemical testing (Okolie et al., 2011; Kiiyukia, 2003; Roberts and Greenwood, 2003; Feng et al., 2002).

Plate 3.2: Food samples placed in the incubator before exposure to microwave radiation
Plate 3.3: Milk samples cultured on both Mackonkey and Nutrient agar before and after exposure to microwave radiation

Plate 3.4: Chicken samples cultured on Mackonkey agar before and after exposure to microwave radiation
3.5.7 Susceptibility testing procedure

This procedure was carried out according to guidelines as set forth by the Clinical Laboratory Standards Institute (CLSI, 2006). Agar plates and antibiotic discs were brought to room temperature before use and gram stain was performed before starting the test to confirm culture purity. Five colonies from agar plate were transferred using a sterile wire-loop into 4-5 ml of diluent and incubated for 2-4 hours until it achieved or exceeded the turbidity of the 0.5 McFarland standard (Lund and Hawkinson, 1983). This resulted in a suspension containing approximately $1 - 2 \times 10^8$ CFU / mL. The turbidity of the standard and the test inoculum was compared by holding the tubes against a dark background. After 10 minutes a sterile cotton swab was immersed into the properly diluted inoculum and rotated firmly several times against the upper inside wall of the tube to express excess fluid. The entire agar surface of the tube was then inoculated by streaking the swab three times and rotating the plate 60 degrees each time. The lid of the plate was replaced allowing the inoculum to be absorbed for about 3 minutes before applying the antibiotic susceptibility test discs. Using a sterile forceps, the appropriate discs were placed onto the respective cultures. The plates were then immediately incubated aerobically at a temperature of $37^\circ$C and examined after 24 hours where the zones diameters of complete zones of inhibition were measured to the nearest mm. These measurements were then compared to those in the table below of the CLSI Document M100 - S16 (M2). Results were then reported as susceptible, intermediate or resistant.
Table 3.2: Table for interpreting sizes of zones of inhibition during antibiotic susceptibility testing

<table>
<thead>
<tr>
<th>ANTIBIOTIC</th>
<th>Disc Concentration</th>
<th>ZONE OF INHIBITION (nearest mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resistance</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>300µg</td>
<td>&lt;=14</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15µg</td>
<td>&lt;=13</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30µg</td>
<td>&lt;=14</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30µg</td>
<td>&lt;=14</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5µg</td>
<td>&lt;=15</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10µg</td>
<td>&lt;=12</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>15µg</td>
<td>&lt;=13</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10µg</td>
<td>&lt;=13</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>1µg</td>
<td>&lt;=10</td>
</tr>
</tbody>
</table>

Plate 3.5: Antimicrobial susceptibility testing plates of bacteria acquired from chicken sample before and after exposure to microwave radiation
3.6 Construction and research instruments

3.6.1 Observation check list

An observation checklist was used to record observations made during performance of the experiment. Details on power at which food sample was exposed to MW radiation, time-period of exposure, CFU numbers, physical properties and antibiotic susceptibility patterns were recorded on this list.

3.7 Quality Control

3.7.1 Internal validity

The methodology applied in this study was well defined and developed based on the Laboratory manual of food microbiology for Ethiopian health and nutrition research institute (Kiiyukia, 2003), bacteriological analytic manual (Feng et al., 2002) and Practical Food Microbiology (Roberts and Greenwood, 2003). The contaminated food samples that were not exposed to microwave radiation served as the control. Evaluation of results was based on the International Commission on Microbiological Specifications for Foods as well as CLSI, 2006. Un-inoculated culture media representative plates were aerobically incubated at a temperature of 37°C for 72 hours to examine for contamination.

3.7.2 Instrument validity

This was established by ensuring frequent calibration and troubleshooting of instruments used in the study such as microwave, incubator, refrigerator, biosafety cabinet and micropipettes.
3.7.3 Construct validity

This was established by ensuring that the treatment (microwave radiation) actually affected the target variable (that is the variable that was intended to be measured: colony forming units). This was carried out by counting the CFU before and after treatment. For AST testing, test discs were deposited so that the centers are at least 24 mm apart, the end point during antimicrobial susceptibility was taken as the area showing no obvious visible growth and discs were always placed at a temperature of -20°C or below in a non-frost free freezer, in containers with tight fitting lids and always under anhydrous conditions.

3.7.4 Reliability

This was established by administering the same treatment twice over a period of time to the same food - microorganism combination sample. Scores from Time 1 and Time 2 (before and after exposure) were then compared and correlated in order to evaluate the test stability.

3.8 Data collection techniques

The data collection technique used in this study was experimental. Where the laboratory techniques incorporated the HACCP principles as well as the ISO protocol: 6579: 2002. These included obtaining food samples, inoculating them with the specific bacteria in doses capable of causing infection, exposure of some food samples to MW radiation, direct culturing of now contaminated food samples, performing of the required physical and biochemical tests for isolation and identification and observation for changes in antibiotic susceptibility patterns using AST.
3.9 Data analysis

The data was analyzed using Statistical Package for the Social Sciences (SPSS) computer software version 13. The variables tested included colony forming unit numbers, antimicrobial susceptibility patterns, physical properties of food, time and power. Raw data was first cleaned, screened and explored thus the following summary statistics were calculated for every sample; mean, median and standard deviation. The table in Appendix VI was used to present the findings in a visual manner. This was done before understating quantitative data analysis. As shown on the table, the mean colony forming unit numbers before sample exposure to microwave radiation were at $\geq 3.0 \times 10^4$ which reduced for most samples after exposure. The *Paired samples t-test* (Appendix III) was then used and its non-parametric alternative *Wilcoxon Signed Ranks test* (Appendix IV) to the general sample statistics to address the question whether the average colony forming units for the two groups differ (exposed [$y_1$] and unexposed [$y_2$]). Relationship analysis between the two groups was done by calculating the number of correlation coefficients (refer to section 3.9.1).

3.9.1 Correlations

The correlations between CFUA and CFUB were statistically significant at the $P = 0.01$ level for all food-microorganism combinations except for milk sample inoculated with *S. aureus* where it was at the $P = 0.05$ level (2-tailed). However, there was no statistically significant correlation between vegetables sample inoculated with *S. Paratyphi* and time-power combinations ($P = 0.350$). There was a negative correlation observed between the dependent and independent variables. Correlations
could not be computed for beef inoculated with *E. coli* and *S. Paratyphi* because the CFUA variable was constant.

### 3.10 Logistical and Ethical Considerations

There were no breaches of integrity executed while conducting this study. The informed consent of food vendors was not necessary for the study. However, their anonymity was protected at all times by ensuring their identities remained private. Therefore, this project conforms to ethical guidelines regarding risk and benefits, consent, anonymity and confidentiality. There were no conflicts of interest declared.
CHAPTER FOUR

RESULTS

4.1 Biochemical Tests

Biochemical test results for standard microorganisms used in the study were as shown in Table 4.1. The tests were used to confirm the identities of the bacteria included in the study. The results were obtained when the test organisms were subjected to a range of conventional biochemical tests included in the study.

Table 4.1: Biochemical tests

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>Urea</th>
<th>Citrate</th>
<th>Kligler Iron Agar</th>
<th>Gas</th>
<th>Catalase</th>
<th>Coagulase</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella flexneri</em> CMCC 51285</td>
<td>-ve</td>
<td>-ve</td>
<td>R/Y acid butt/ alkaline slant</td>
<td>No gas produced</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Salmonella Paratyphi</em> CMCC 50319</td>
<td>-ve</td>
<td>-ve</td>
<td>R/Y acid butt/ alkaline slant</td>
<td>H₂S present</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25923</td>
<td>-ve</td>
<td>-ve</td>
<td>Y/Y Acid butt/acid slant</td>
<td>Cracks observed (gas produced)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25922</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

**KEY**

-ve: negative  
+ve: positive  

N/A: the test is not applicable to that microorganism
4.2 Effects of microwave radiation before and after exposure of food samples inoculated with various test organisms at different time-power combinations

4.2.1 Beef sample inoculated with *E. coli*

Microwave radiation had no effect on the colony forming unit numbers in the beef sample artificially contaminated with *E. coli*. The y-axis represents the CFU numbers where as the x-axis represents the food samples.

![Figure 4.1: Beef sample inoculated with *E. coli*](image-url)
4.2.2 Beef sample inoculated with *S. Paratyphi*

Microwave radiation had no effect on the colony forming unit numbers in the beef sample artificially contaminated with *S. Paratyphi*. The y-axis represents the CFU numbers whereas the x-axis represents the food samples.

![Figure 4.2 Beef sample inoculated with *S. Paratyphi*](image-url)
4.2.3 Beef sample inoculated with *S. aureus*

Microwave radiation had no effect on the colony forming unit numbers in the beef sample artificially contaminated with *S. aureus* after exposure for 2 minutes at power 20. However, an observable effect was noted after exposure at all the other time-power combinations. The y-axis represents the CFU numbers whereas the x-axis represents the food samples.

![Figure 4.3: Beef sample inoculated with *S. aureus*](image)
4.2.4 Chicken sample inoculated with *E. coli*

Microwave radiation had no effect on the colony forming unit numbers in the chicken sample artificially contaminated with *E. coli* at all time - power combinations except after exposure for 8 minutes at power 60. The y-axis represents the CFU numbers where as the x-axis represents the food samples.

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**Figure 4.4:** Chicken sample inoculated with *E. coli*
4.2.5 Egg sample inoculated with \textit{E. coli}

Microwave radiation had no effect on the colony forming unit numbers in the egg sample artificially contaminated with \textit{E. coli} after exposure for 2 minutes at power 20 and 40. However an observable effect was noted at all the other time - power combinations. The y-axis represents the CFU numbers whereas the x-axis represents the food samples.

![Figure 4.5: Eggs sample inoculated with \textit{E. coli}]
4.2.6 Egg sample inoculated with *S. Paratyphi*

Microwave radiation had no effect on the colony forming unit numbers in the egg sample artificially contaminated with *S. Paratyphi* after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time-power combinations. The y-axis represents the CFU numbers whereas the x-axis represents the food samples.

![Time-power combinations during microwave radiation](image)

Figure 4.6: Eggs sample inoculated with *S. Paratyphi*
4.2.7 Fish sample inoculated with *E.coli*

Microwave radiation had no effect on the colony forming unit numbers in the fish sample artificially contaminated with *E. coli* after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time - power combinations. The y-axis represents the CFU numbers whereas the x-axis represents the food samples.

*Figure 4.7: Fish sample inoculated with *E. coli***
4.2.8 Fish sample inoculated with S. Paratyphi

Microwave radiation had no effect on the colony forming unit numbers in the fish sample artificially contaminated with S. Paratyphi at these time-power combinations (2.20, 4.20, 2.40, and 4.40). It had some effect at 6.20 and 8.20. However, the largest effect was observed at all the other time - power combinations. The y-axis represents the CFU numbers where as the x-axis represents the food samples.

Figure 4.8: Fish sample inoculated with S. Paratyphi
4.2.9 Fish sample inoculated with *S. flexneri*

Microwave radiation had no effect on the colony forming unit numbers in the fish sample artificially contaminated with *S. flexneri* at the following time - power combinations: 2.20, 4.20, 6.20, 2.40 and 2.60. However, an observable effect was noted at all the other time - power combinations. The y-axis represents the CFU numbers where as the x-axis represents the food samples.

![Figure 4.9: Fish inoculated with *S. flexneri*](image)
4.2.10 Gravy sample inoculated with *E. coli*

Microwave radiation had no effect on the colony forming unit numbers in the gravy sample artificially contaminated with *E. coli* at the following time - power combinations 2.20, 2.40, 6.40 and 8.40. However, an observable effect was noted at all the other time - power combinations. The y-axis represents the CFU numbers where as the x-axis represents the food samples.

Figure 4.10: Gravy inoculated with *E. coli*
**4.2.11 Gravy sample inoculated with *S. Paratyphi***

Microwave radiation had no effect on the colony forming unit numbers in the gravy sample artificially contaminated with *S. Paratyphi* after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time - power combinations. The y-axis represents the CFU numbers whereas the x-axis represents the food samples.

![Time-power combinations during microwave radiation](image)

*Figure 4.11: Gravy sample inoculated with *S. Paratyphi*
4.2.12 Milk sample inoculated with *E. coli*

Microwave radiation had no effect on the colony forming unit numbers in the milk sample artificially contaminated with *E. coli* after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time-power combinations. As seen on Appendix VI, the observed effect was very minute when compared to other food samples processed in the study. The y-axis represents the CFU numbers where as the x-axis represents the food samples.

![Figure 4.12: Milk inoculated with *E. coli*](image)
4.2.13 Milk sample inoculated with \textit{S. Paratyphi}

Microwave radiation had no effect on the colony forming unit numbers in the milk sample artificially contaminated with \textit{S. Paratyphi} after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time - power combinations. As seen on Appendix VI, in this case the observed effect was also very minute when compared to other food samples processed in the study. The y-axis represents the CFU numbers while the x-axis represents the food samples.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.13.png}
\caption*{Figure 4.13: Milk sample inoculated with \textit{S. Paratyphi}}
\end{figure}
4.2.14 Milk sample inoculated with *S. aureus*

Microwave radiation had no effect on the colony forming unit numbers in the milk sample artificially contaminated with *S. aureus* after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time - power combinations. In this case also, the observed effect was very minute when compared to other food samples processed in the study. The y-axis represents the CFU numbers where as the x-axis represents the food samples.

![Figure 4.14: Milk sample inoculated with *S. aureus*](image)
4.2.15 Potato sample inoculated with *S. aureus*

Microwave radiation had no effect on the colony forming unit numbers in the potato sample artificially contaminated with *S. aureus* after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time-power combinations. The y-axis represents the CFU numbers whereas the x-axis represents the food samples.

![Time-power combinations during microwave radiation](image)

*Figure 4.15: Potato sample inoculated with *S. aureus*
4.2.16 Vegetable sample inoculated with *S. Paratyphi*

Microwave radiation had no effect on the colony forming unit numbers in the vegetable (VEG) sample artificially contaminated with *S. Paratyphi* after exposure at the following time-power combinations 2.20, 2.40 and 2.60. However, an observable effect was noted at all the other time-power combinations.

**Figure 4.16:** Vegetable sample inoculated with *S. Paratyphi*
4.2.17 Vegetable sample inoculated with *S. flexneri*

Microwave radiation had no effect on the colony forming unit numbers in the vegetable (VEG) sample artificially contaminated with *S. flexneri* after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time-power combinations.

![Figure 4.17: Vegetable sample inoculated with *S. flexneri*](image-url)
4.2.18 Chicken sample inoculated with *S. Paratyphi*

Microwave radiation had no effect on the colony forming unit numbers in the chicken (Chic..) sample artificially contaminated by *S. Paratyphi* after exposure at the following time - power combinations 2.20 and 4.20. However, an observable effect was noted at all the other time - power combinations.

![Time - power combinations during microwave radiation](image)

*Figure 4.18: Chicken sample inoculated with *S. Paratyphi*
4.2.19 Vegetable sample inoculated with \textit{E. coli}

Microwave radiation had no effect on the colony forming unit numbers of the vegetable (VEG) sample artificially contaminated with \textit{E. coli} after exposure for 2 minutes at power 20. However, an observable effect was noted at all the other time-power combinations.

\textbf{Figure 4.19: Vegetable sample inoculated with \textit{E. coli}}
4.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done to address the second objective. However, there were no observable changes in antimicrobial susceptibility patterns between the control and all the other time-period combinations as shown on 3.5, Plate 4.1 and Appendix VIII). This showed that for all the bacteria included in this study, microwave radiation had no effect on their ability to resist or remain susceptible to various antibiotics used against pathogenic food bacteria.

Plate 4.1: Antimicrobial susceptibility testing plates
4.4 Effects of microwave radiation on specific food - microorganism combinations (reduction in colony forming unit numbers and change in physical properties highlighted)

The colony forming units were counted and physical property changes (colour, odour, consistency, growth of mould / development of slime) were observed before and after exposure to microwave radiations to address the first and third objective. The following observations were made for each food – microorganism combination.

The initial populations of viable *Shigella flexneri* microorganisms in fish samples (mean CFU before radiation= 300) decreased by a 1.5 log unit at power 40 for 4, 6 and 8 minutes, at power 60 for 4, 6 and 8 minutes and at power 80 for all time-periods. After running the paired samples t-test statistic a P value of \(<0.05\) was generated which indicated significance. The mean CFU after exposure to microwave radiation was 112.35. The physical properties for this sample were not affected by exposure to microwave radiation.

For *Salmonella* Paratyphi microorganisms which were artificially introduced into vegetables samples (mean CFU before radiation= 300), the initial populations decreased by 1.5 log unit at power 20 for 8 minutes, power 40 for 8 minutes and at power 80 for 4, 6 and 8 minutes. Although the magnitude of the effect was much less than most of the other samples the paired samples t-test statistic still indicated significance. The mean CFU after exposure to microwave radiation was 209.17. The physical properties for this sample were also not affected by exposure to microwave radiation.

*Salmonella* Paratyphi microorganisms in gravy sample’s initial populations decreased by a 1.5 log unit at power 20 for 4, 6 and 8 minutes. The paired samples t-test statistic generated a P value of \(<0.05\). The mean CFU after exposure to microwave radiation was 133.44. The physical properties for this sample (that is the colour, smell and
consistency) were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80. The initial populations of viable *Shigella flexneri* microorganisms in vegetable samples decreased by a 1.5 log unit at power 20 for 4 minutes as well as power 40, power 60 and power 80 for all time-periods. The mean CFU after exposure to microwave radiation was 44.12 and after running the paired samples t-test statistic a P value of <0.05 was generated. The physical properties for this sample were also not affected by exposure to microwave radiation.

Viable *Salmonella Paratyphi* microorganisms initially inoculated into beef samples were not decreased at any time-power combinationations and the paired samples t-test statistic could not be run because the standard error of the difference was 0. The physical properties for this sample were also not affected by exposure to microwave radiation. The initial populations of viable *Escherichia coli* microorganisms in beef samples were also not decreased at any time-power combinations. The mean CFU after exposure to microwave radiation remained at 300 and the paired samples t-test statistic could not be run. The physical properties for this sample were also not affected.

*Escherichia coli* colony forming unit numbers in chicken samples were also not decreased at any time-power combinations. The mean CFU count after exposure to microwave radiation remained at 300 and the paired samples t-test statistic could not be run. The physical properties for this sample (that is the colour and consistency) were affected by exposure at power 80 for 8 minutes. The initial populations of viable *Salmonella Paratyphi* microorganisms in eggs samples (mean CFU before radiation=300) decreased by a 1.5 log unit at all time-power combinations except 2 minutes at power 20. A P value of <0.05 was generated and the mean CFU after exposure to
microwave radiation was 74.44. The physical properties for this sample (that is the colour, smell and consistency) were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80.

Salmonella Paratyphi microorganisms in fish samples (mean CFU before radiation=300) decreased by a 1.5 log unit at 6 minutes at power 40 all the way to 8 minutes at power 80. A P value of <0.05 was generated and the mean CFU after exposure was 125.47. The physical properties for this sample were not affected by exposure to microwave radiation. Salmonella Paratyphi microorganisms in gravy samples decreased by a 1.5 log unit at all time-power combinations except 2 minutes at power 20. The mean CFU after exposure to microwave radiation was 74.44 and a P value of <0.05 was generated which indicated significance. The physical properties for this sample (that is the colour, smell and consistency) were affected by exposure at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80.

The initial population of Staphylococcus aureus in beef samples decreased by a 1.5 log unit at all time-power combinations except 2 minutes at power 20. After running the paired sample t-test statistic a P value of <0.05 was generated. The mean CFU after exposure was 44.14 and the physical properties for this sample were not affected. Salmonella Paratyphi microorganisms in chicken samples (mean CFU before radiation= 300) decreased by a 1.5 log unit at all time-power combinations except 2 minutes and 4 minutes at power 20. A P value of <0.05 was generated and the mean CFU after exposure to microwave radiation was 61.18. The physical properties for this sample (that is the colour and consistency) were affected by exposure to microwave radiation at power 80 for 8 minutes.
Viable *Escherichia coli* microorganisms in eggs samples decreased by a 1.5 log unit at all time-power combinations except 2 minutes at power 20 and power 40. After running the paired sample t-test statistic a P value of <0.05 was generated. The mean CFU after exposure to microwave radiation was 82.50 and the physical properties for this sample (that is the colour, smell and consistency) were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80. Viable *Escherichia coli* microorganisms in fish samples (mean CFU before radiation= 300) decreased by a 1.5 log unit at all time-power combinations except 2 minutes at power 20 and 6 minutes at power 60. The paired sample t-test statistic generated a P value of <0.05. The mean CFU after exposure to microwave radiation was 52.65. The physical properties for this sample were not affected by exposure to microwave radiation.

The initial populations of viable *Escherichia coli* microorganisms in vegetables samples were reduced by a 1.5 log unit at all time-power combinations except 2 minutes at power 20. Significance was indicated by a P value of <0.05. The mean CFU after exposure was 44.12 and the physical properties for this sample were also not affected. *Salmonella* Paratyphi microorganisms in milk samples (mean CFU before radiation= 300) decreased by a 1.5 log unit at power 40 for 6 and 8 minutes. Significance was indicated with a P value of <0.05. The mean CFU after exposure to microwave radiation was 173.50. The physical properties for this sample (that is the colour, smell and consistency) were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80.

The initial populations of viable *Escherichia coli* microorganisms in milk samples were not reduced by 1.5 log unit at any time-power combinations. However, there was a slight decrease at all time-power combinations except power 20 for 2 minutes and
after running the paired sample t-test statistic a P value of <0.05 was generated. After exposure to microwave radiation the mean CFU was 268.22. The physical properties for this sample (that is the colour, smell and consistency) were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80. The initial populations of viable *Staphylococcus aureus* microorganisms in milk samples (mean CFU before radiation = 300) decreased by 1.5 log unit at power 20 for 4 and 8 minutes and power 40 for 6 and 8 minutes. However, there was a slight decrease in CFU numbers at other time-power combinations such as power 20 for 6 minutes and at power 40 for 6 and 8 minutes. After running the paired sample t-test statistic a P value of <0.05 was generated which indicated significance. The mean CFU after exposure to microwave radiation was 158.72. The physical properties for this sample (that is the colour, smell and consistency) were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80.

Lastly, the initial population of *Staphylococcus aureus* microorganisms in potato samples was decreased by 1.5 log unit at various time-power combinations. However, the time-power combinations were not consistent for all the duplicate experiments. After running the paired sample t-test statistic a P value of <0.05 was generated. The mean CFU after exposure to microwave radiation was 117.76. The physical properties for this sample were not affected by exposure.

All samples showed bacterial numbers at $3.0 \times 10^4$ before exposure to microwave radiation (Initial seed) and in some cases only showed a decrease in numbers after exposure. The average exact effect size (Cohen’s d) of the reduction of food pathogens by microwave radiation was $r = 2.82$. The specific effect sizes for each sample processed is highlighted in the last column of Appendix VI where beef
artificially contaminated with *S. aureus* and vegetables contaminated with *S. flexneri* show the largest effect size and beef contaminated with *E. coli* and *S. Paratyphi* as well as chicken contaminated with *E. coli* showed the smallest effect size.

Table 4.3: Physical property changes that occurred in various food samples

<table>
<thead>
<tr>
<th>Type of Food</th>
<th>Bacteria Inoculated</th>
<th>Change in physical property of food</th>
<th>Time-power combinations where changes occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td><em>Shigella flexneri</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Fish</td>
<td><em>Salmonella Paratyphi</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Fish</td>
<td><em>Escherichia coli</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Vegetable</td>
<td><em>Salmonella Paratyphi</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Vegetable</td>
<td><em>Shigella flexneri</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Vegetable</td>
<td><em>Escherichia coli</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Gravy</td>
<td><em>Salmonella Paratyphi</em></td>
<td>Yes [ colour changed from red to brown, consistency changed from semi-solid to solid and there was presence of an unpleasant odour after exposure]</td>
<td>2.60, 4.60, 8.60, 2.80, 4.80, 6.80, 8.80</td>
</tr>
<tr>
<td>Gravy</td>
<td><em>Escherichia coli</em></td>
<td>Yes [ colour changed from red to brown, consistency changed]</td>
<td>2.60, 4.20, 4.40, 4.60, 8.60, 2.80, 4.80, 6.20, 6.60,</td>
</tr>
<tr>
<td>Item</td>
<td>Microorganism</td>
<td>Effect</td>
<td>Notes</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Beef</td>
<td><em>Salmonella</em> Paratyphi</td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Beef</td>
<td><em>Escherichia coli</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Beef</td>
<td><em>Staphylococcus aureus</em></td>
<td>No change observed</td>
<td>n/a</td>
</tr>
<tr>
<td>Chicken</td>
<td><em>Escherichia coli</em></td>
<td>Yes [colour changed from light brown to dark brown, consistency changed from soft-moisturized solid to hard solid without any moisture]</td>
<td>8.80</td>
</tr>
<tr>
<td>Chicken</td>
<td><em>Salmonella</em> Paratyphi</td>
<td>Yes [colour changed from light brown to dark brown, consistency changed from soft-moisturized solid to hard solid without any moisture]</td>
<td>8.80</td>
</tr>
<tr>
<td>Eggs</td>
<td><em>Escherichia coli</em></td>
<td>Yes [colour changed from yellow to brown, consistency changed from liquid to solid and there was presence of an unpleasant odour after exposure]</td>
<td>2.60, 4.60, 8.60, 2.80, 4.80, 6.80, 8.80</td>
</tr>
<tr>
<td>Eggs</td>
<td><em>Salmonella</em> Paratyphi</td>
<td>Yes [colour changed from yellow to brown, consistency changed from liquid to solid and there was presence of an unpleasant odour after exposure]</td>
<td>2.60, 4.60, 8.60, 2.80, 4.80, 6.80, 8.80</td>
</tr>
</tbody>
</table>
Milk | *Salmonella Paratyphi* | Yes [colour changed from white to brown, consistency changed from liquid to solid and there was presence of an unpleasant odour after exposure] | 2.60, 4.60, 8.60, 2.80, 4.80, 6.80, 8.80 |
---|---|---|---|
Milk | *Escherichia coli* | Yes [colour changed from white to brown, consistency changed from liquid to solid and there was presence of an unpleasant odour after exposure] | 2.60, 4.60, 8.60, 2.80, 4.80, 6.80, 8.80 |
Milk | *Staphylococcus aureus* | Yes [colour changed from white to brown, consistency changed from liquid to solid and there was presence of an unpleasant odour after exposure] | 2.60, 4.60, 8.60, 2.80, 4.80, 6.80, 8.80 |
Potato | *Staphylococcus aureus* | No change observed | n/a |

**KEY**

n/a – not applicable; 2.60 – 2 minutes at power 60; 4.60 – 4 minutes at power 60
8.60 – 8 minutes at power 60; 2.80 – 2 minutes at power 80; 4.80 – 4 minutes at power 80; 6.80 – 6 minutes at power 80; 8.80 – 8 minutes at power 80
5.1 DISCUSSION

The exact effect size of MW radiations on reducing the infective dose numbers of food bacteria as well as determining their effect on antimicrobial susceptibility patterns has not been addressed by previous studies. The effect size is essential because it is used to interpret the magnitude of treatment effect.

During this study, when the artificially contaminated food samples were irradiated for 2 minutes at power 20 (295W) the bacteria survival rate was 100% (bacteria numbers remained at $>$3.0 x $10^4$) whereas when artificially contaminated food samples were irradiated for 8 minutes at power 80 (1180W), the bacteria survival rate was 3.33% (bacteria numbers were very low $<$1.0 x $10^3$). This huge difference in reduction of seed numbers at higher time-power combinations is quite significant ($P < 0.05$) and strongly indicates that there can be total destruction of bacteria in food samples and thus 100% reduction of their infective doses. When time-periods were kept constant, higher powers were shown to be very effective at reducing bacterial numbers and when the radiation power was kept constant, longer time-periods were shown to be more effective in the destruction of bacteria. The contaminated food samples that were not exposed to radiation served as the control and they all showed that the bacterial numbers remained at $>$3.0 x $10^4$. This showed that the inocula of test organisms used to infect food samples before exposure were sufficient to produce growth under the conditions used in the experiment similar to the report by Sheen et al., (2012) where if no colonies were observed from a plate, the bacterial counts in the sample were treated as $<$1.0x$10^3$. 
When confirming the identities of the test organisms after exposure of samples to radiation, the results corresponded to those highlighted by Vandepitte et al. (2003); Roberts and Greenwood., 2003) and Cheesbrough. (2006). The highest mean CFU count after exposure of sample to microwave radiation was detected in beef sample processed for E. coli and S. Paratyphi (>3.0 x 10^4), whereas the lowest mean colony forming unit count after exposure of sample to microwave radiation was detected in vegetable sample processed for S. flexneri and E. coli as well as beef sample processed for S. aureus. The above results were consistent with studies carried out by Gedikli et al. (2008); Gomolka-Pawlicka et al. (2013) and Sheen et al. (2012) which showed that exposure of bacteria at higher powers during microwave radiation of food resulted in higher destruction than at lower powers. Sheen et al. (2012) demonstrated that after 1 min with full power, approximately 1.5 to 2 log-reductions in the bacterial counts of Salmonella, L. monocytogenes, and E. coli O157:H7 were achieved.

Microwave power is a factor greatly influencing the rate of microwave heating. If a high value of power is applied, a high rate of temperature elevation in the heated body can be expected (Housova and Hoke, 2002). Therefore, the conceivable reason as to why the bacteria survived better at lower time-power combinations would be that enough heat was not generated during radiation whereas when it came to higher time-period combinations there was sufficient heat generated that may have been lethal to the microorganisms. Other factors that could have affected the outcome after exposure to MWs may have been power fluctuation, probe location, varying dielectric properties in each food tested, sample placement within the microwave cavity, probe error or product density, ionic nature and thermal conductivity (Heddleson et al.,
1994; Gunasekaran et al., 2005; Dumuta-Codre et al., 2010). Another factor may be presence of an uneven electric field distribution inside the microwave cavity (Shamis et al., 2011).

The cavity in a domestic microwave oven is designed to have typically 3-6 different modes intended to provide a uniform heating pattern for general food items (Lidstrom, 2001). Heating uniformity has been a controversial topic when it comes to microwave radiation with some studies suggesting a non-uniform temperature distribution resulting in hot and cold spots in the heated product (Vadivambal and Jayas, 2008) and others suggesting that the temperature uniformity obtained by microwave heating is more than that of conventional heating (Valsechi et al., 2004). Reproducibility was ensured in the study by placing samples in the exact same position inside the microwave during radiation treatment.

The results were also consistent with a study by Dababneh, (2013); Morey et al. (2012) and Sheen et al. (2012) which showed that MW radiation produced a 1-2 log reduction when contaminated samples were processed. According to Park et al. (2000) there was a 5 - log reduction of viable cells of E. coli and B. subtilis after exposure to microwave radiation. The large difference with the findings can be attributed to the use of contaminated food samples unlike Park et al. (2000) in which saline suspensions were utilized. Therefore, there was a difference in the local electric field strength (Jeng et al., 1987). These results may also apply to other food pathogens that were not included in the study for example Listeria monocytogens, Vibrio cholera, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Brucella abortus, Brucella suis, Streptococcus pyogens, Yersinia enterocolytica et cetera.
Although spore-formers may require additional treatment, further studies have to be conducted to ascertain this.

Microbial death during exposure to microwave radiation may be attributed to a combination of microwave treatment and heat (Sharma et al., 2009; Sheen et al., 2012). If a high value of power is applied, a high rate of temperature elevation in the heated body can be expected. However, a difference can exist between the rated power output and the power actually absorbed during the microwave heating in the heated substance of certain parameters (Housova and Hoke, 2002).

By using the Mcfarland standard to standardize the initial number of bacteria seeded into the food samples, it is assumed that the initial seed range was $1-2 \times 10^8$ CFU/MI (CLSI, 2006). This was in excess of the known infective doses of each specific bacteria included in the study (refer to section 2.4 and table 2.2). Reduction of each of the specific bacterial infective doses by 97.67% would theoretically result in the following numbers ($E. \ coli < = 0.333 \times 10^1$, $S. \ flexneri < = 1.665 \times 10^1$, $S. \ aureus < = 3.33 \times 10^2$, $S. \ Paratyphi < = 3.33 \times 10^1$). These numbers are lower than their infective doses and are therefore unlikely to cause food borne disease.

As highlighted by Morey et al., 2012 in their study, exposure of food samples to microwave radiation at longer periods of time leads to reduction of microbial populations at significant levels ($p<0.05$) but also leads to the product quality being severely compromised. This unwanted effect of MW radiation was also highlighted by Sheen et al. (2012) who stated that MW heating induces texture damage, poor yield due to loss of moisture and poor appearance. It was also seen in the present
study that treatment at higher power and longer periods of time also resulted in alterations in the physical properties of the samples and therefore the affected samples were disqualified from the experiment. Samples such as eggs, milk and gravy were the most affected by changes in physical properties. They could not withstand exposure at both power 60 and power 80 at any time-period. In all foods (especially those with little moisture) moderate power and time combinations would appear to be required to reduce populations of contaminating micro-organisms while avoiding the undesirable alterations in physical properties.

Antimicrobial resistance has been a major challenge in the management of FBDs (Nyenje and Ndip, 2013). Studies have also shown that antimicrobial drugs are not the only selective pressure that results in emergence of resistant organisms (Chao et al., 2007) this is why this study also included assays to determine if exposure to microwave radiation had any effect on the antimicrobial susceptibility patterns of the test microorganisms. Susceptibility or resistance of staphylococci to a wide array of beta-lactam antibiotics can be observed from testing only penicillin and oxacillin (CLSI, 2006). In the present study there were no observable differences between antibiotic susceptibility patterns before and after exposure of contaminated food samples to microwave radiation (refer to Appendix VIII). The results were similar at the 95% Confidence Interval. However, Al-Mayah and Ali (2010) demonstrated that exposure of *Staphylococcus aureus* to mobile microwave radiation for a long period of time affected their antimicrobial susceptibility patterns tremendously. All strains showed more antibiotic resistance development after long period of exposure which they speculated may have occurred due to slight changes in the DNA structure of the microorganism. Japoni et al. (2010) proposed that to preserve the effectiveness of
antibiotics, rational prescription and concomitant application of preventive measures such as these against the spread of bacteria are recommended.

The destruction mechanisms of microwaves required in a system to affect microbial cells has been proved to be due to heat generated in a medium; in a solution and/or a solid substrate but it's not until recently when it was otherwise thought that microwaves have a direct destructive effect on the microbial cells with minimal contribution being from the substrate heat generated (Barnabus et al., 2011). In addition to reducing the living cell numbers of bacteria, microwave radiation has also been shown to lower acid resistance and verocytotoxin productivity of enterohaemorrhagic E. coli 0157: H7 (Tsuji and Yokoigawa, 2011). The decrease in the verocytotoxin productivity suggests that the bacteria that may have survived exposure to microwave radiation may not be as virulent/pathogenic as the unexposed bacteria.

With all the parameters kept constant, the current study would thus concur with Rodríguez-Marval et al., 2009 in suggesting that when reheating instructions should be formulated for microwave radiation of food, these instructions must be designed specifically for each type of food product, and consider variations in microwave appliance maximum output power, amount of food to be reheated, age of the product, and the presence of antimicrobial compounds in the formulation.

5.2 CONCLUSION

As indicated by numerous studies, the possibility of survival of various pathogens in food products subjected to microwave heating exists; therefore, undertaking studies concerning the widely understood influence of microwaves on microorganisms
(particularly the bacteria whose presence in food has direct influence on food safety) seems fully justified (Gomolka-Pawlicka et al., 2013). From the results in the present study, it can be concluded that the least effective time-period when it comes to killing food pathogens in food samples is 2 minutes and the least effective power is P-20 (295W). Whereas, the most effective time-period when it comes to killing food pathogens in food samples is 8 minutes and the most effective power is 80 (1180W – full power). However, the latter power-time combination is detrimental to the physical properties of most foods thus making them inedible. The reduction of bacteria infective doses is dependent on the power-time combinations with longer time periods and exposure to higher powers being more effective. However, it seems likely combining moderate powers (P-40 – 590W, P-60 – 885W) with moderate time (4 min, 6 min) is effective in decreasing the number of microorganisms while also avoiding unwanted effects on physical properties of the foods.

These findings indicate that exposure of foods to higher time-power combinations in a domestic microwave oven (2450 MHz) plays a role in reducing the spread of food borne diseases. However, the same radiation does not play any role in the increase/decrease of bacterial antibiotic susceptibility. Another concern is that colonies that survive exposure to microwave radiation seem to maintain their ability to grow, multiply and remain viable. The major parameters included in this study were infective doses, time, power, physical property of food and antibiotic susceptibility. It is not guaranteed that the recommended microwaving power-time combinations in this study will always be effective in eliminating all the pathogenic bacteria in food. Its’ efficacy will always depend upon factors such as product type and the level of bacterial contamination.
5.3 RECOMMENDATIONS

i. There is need that the data obtained in this study should be validated in other types of food samples, with other types of microwaves and against a larger number of microorganisms (more bacteria, fungi, parasites and even viruses).

ii. As shown in this study, effectiveness of microwave radiation differs by type of foods therefore microwave companies should conduct studies where suspected foods are tested to determine best cooking time in terms of sufficient pathogen destruction.

iii. Further studies should be carried out to determine effect of microwave radiation on toxin production and virulence of various bacteria.
REFERENCES


Doyle, M., Hartmann, F. and Wong, A. (2011). White paper on sources of Methicillin- resistant *Staphylococcus aureus* (MRSA) and other methicillin resistant Staphylococci: Implications for our food supply? *Food Research Institute: Food Safety Reviews, 1*-25.


and prevalence of class 1 and class 2 integrons in *S. flexneri* and *S. sonnei* isolated in Uzbekistan. *Gut Pathogens, 2*, 18.


Risk Assessment Section. (2007). Microbiological guidelines for ready-to-eat food. Centre for food safety, food and environmental hygiene department, 66 Queensway, Hong Kong.


The study reported here looked at the survival of microorganisms from different types of food in a consumer-type microwave oven. These foods (milk, beef, chicken, vegetables, eggs, gravy, potatoes and fish) were exposed to microwave radiation at different power and time combinations and the bacterial survival, physical properties as well as antibiotic susceptibility patterns after exposure were recorded. The idea was to find out the proper processing time for reduction of bacterial numbers in contaminated foods as well as explore the possibility of effects of microwave radiation on antibiotic susceptibility patterns. \textit{Staphylococcus aureus} (ATCC 25922), \textit{Escherichia coli} (ATCC 25923), \textit{Shigella flexneri} (CMCC 51285) and \textit{Salmonella enterica subsp. enterica} serovar Paratyphi (CMCC 50319) were the target microorganisms.
APPENDIX II
DETAILS OF SPECIFIC ANTIBIOTICS INCLUDED IN THE CURRENT STUDY

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nitrofurantoin</strong></td>
<td>Nitrofurantoin (1-[5-nitro-2-furanyl] methylene) amino-2, 4-imidazolidinedione) is a bactericidal nitrofuran antibiotic (Cetti et al., 2008). It is absorbed by the oral route, mainly excreted in urine, well-tolerated, active against putative pathogens (bearing in mind local resistance patterns) and is also inexpensive (Brumfitt and Hamilton-Miller, 1998). This, coupled with improvements in the formulation, to minimize gastrointestinal side-effects, has led to its recommendation as a first-line agent (Cetti et al., 2008). It has a broad-spectrum activity against most gram-negative organisms and many gram-positive organisms (Bains et al., 2009). The drug works by damaging bacterial DNA; this is made possible by the rapid reduction of nitrofurantoin inside the bacterial cell by flavoproteins to many reactive intermediates that attack ribosomal proteins, DNA and other macromolecules within the cell (Cetti et al., 2008).</td>
</tr>
<tr>
<td><strong>Oxacillin</strong></td>
<td>Oxacillin belongs to a group of drugs known as penicillins. These were the first antibiotics to be widely used in medicine. However, due to increase in antibiotic resistant pathogens, this drug is frequently employed against bacterial pathogens that are penicillin-resistant (Prescott et al., 2002; ICMR, 2009). It is active against predominantly gram-positive bacteria including penicillinase-producing staphylococci. Oxacillin susceptibility test results can be applied to the other penicillinase-stable penicillins, i.e., cloxacillin, dicloxacillin, flucloxacillin, methicillin, and nafcillin (CLSI, 2006). This group of antibiotics function by inhibiting cell wall synthesis through inhibition of peptidoglycan polymerization (Baron, 1996).</td>
</tr>
<tr>
<td><strong>Ampicillin</strong></td>
<td>Ampicillin is a beta-lactamase antibiotic, a part of the amino-penicillin family, they are amino acids and peptide antibiotics. It is a derivative of 6-aminopenicillanic acid, stable in acid medium (Udeze et al., 2012). Its primary effect is bactericidal and broad-spectrum and its side effects include allergic responses for example diarrhoea and anaemia (Prescott et al., 2002). It can be administered orally as well as injected (Udeze et al., 2012). Enterococci with lower levels of ampicillin resistance may be susceptible to synergistic killing by this penicillin in combination with gentamicin or streptomycin (in the absence of high-level resistance to gentamicin or streptomycin) if high doses of the penicillin are used, whereas strains with higher levels of ampicillin resistance may not be susceptible to the synergistic effect (CLSI, 2006).</td>
</tr>
<tr>
<td><strong>Gentamycin</strong></td>
<td>This is an aminoglycoside antibiotic synthesized by the bacterium Micromonospora purpurea (Prescott et al., 2002). Its advantages include rapid onset of action, synergy with β-lactam antibiotics and low cost (Safa et al., 2010).Its primary effect is bactericidal and narrow-spectrum (targets only gram-negative microorganisms such as Proteus spp., Escherichia spp., Klebsiella spp., and Serratia spp.) and its side effects include allergic responses, nausea, hearing loss and renal damage (Prescott et al., 2002). Toxic effects include kidney failure; nephrotoxicity, usually reversible, is</td>
</tr>
</tbody>
</table>
manifested by oliguria and azotemia. Neurotoxicity can appear as numbness or skin tingling, ataxia, muscle twitching and even convulsions (Baselt and Cravey, 2005). High-level resistance to gentamycin and/or streptomycin indicates that an enterococcal isolate will not be killed by the synergistic action of a penicillin or glycopeptide combined with that aminoglycoside. Other aminoglycosides need not be tested, because their activities against enterococci are not superior to gentamycin or streptomycin (CLSI, 2006)

**Ciprofloxacin**

This drug belongs to group of synthetic antibiotic agents known as Quinolones - more specifically Fluoroquinolones (Prescott et al., 2002). The fluoroquinolones are a group of new drugs which were derived from nalidixic acid; chemical modification has resulted in enhanced in vitro activity and better absorption after oral administration (Fass, 1987). Its primary effect is bactericidal and broad-spectrum; its side-effects include gastrointestinal upsets and allergic responses (Prescott et al., 2002). It is commonly used to treat severe Salmonella infections and has been categorized by the World Health Organization as one of the critically important antimicrobials (CDC/NARMS, 2012).

**Erythromycin**

Erythromycin, the first macrolide antibiotic discovered, has been used since the early 1950s (Zuckerman, 2004). It is synthesized by *Streptomyces erythraeus* (Prescott et al., 2002). It is often used in children with minor bacterial infections, especially in those with an allergy to penicillin (Li and Fok, 2000). Erythromycin inhibits protein synthesis in bacteria by its effect on ribosome function (Weisblum, 1995). Its primary effect is bacteria-static and narrow spectrum (targets gram-positive microorganisms and mycoplasma) and its side-effects include gastrointestinal upset and hepatic injury (Prescott et al., 2002). Advanced macrolide antimicrobials have been synthesized by altering the erythromycin base resulting in compounds with extended spectrum of activity, favourable pharmacodynamics, once-a-day administration, and good tolerability (Zuckerman, 2004).

**Vancomycin**

Vancomycin is a glycopeptide antibiotic produced by *Streptomyces orientalis* (Jones, 2006). Its primary effect is bactericidal and narrow spectrum (targets only aerobic gram-positive microorganisms such as *Staphylococcus* spp., *Clostridium* spp., *Bacillus* spp., *Streptococcus* spp. and *Enterococcus* spp. and its side-effects include hypotension, neutropenia, kidney damage and allergic reactions (Prescott et al., 2002). It is an accepted agent for treatment of a gram-positive bacterial infection in the penicillin-allergic patient, and it is useful for therapy of infections due to β-lactam-resistant, gram-positive bacterial strains, for example, methicillin-resistant *Staphylococcus aureus* (MRSA) and some enterococci (CLSI, 2006).

**Azithromycin**

Azithromycin is an azalide antibacterial agent, with a long half-life therefore enabling once-daily dosing regimens (Foulds et al., 1996). Azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) is formed by inserting a methyl-substituted nitrogen in place of the carbonyl group at the 9a position of the aglycone ring. The resulting dibasic 15- membered ring macrolide derivative is more appropriately referred to as an “azalide” (Kanatani and Guglielmo, 1994). This structural change makes the compound more stable in acid, significantly increases the serum half-life and tissue penetration and results in increased activity against gram-negative organisms and decreased activity against some gram-positive organisms when compared with erythromycin (Amacher et al., 1991; Lode, 1991; Zuckerman, 2004).
Azithromycin has activity against enteric pathogens including *Escherichia coli*, *Salmonella* spp, *Yersinia enterocolitica*, and *Shigella* spp. It has several distinct advantages over erythromycin including the following: improved oral bioavailability [approximately 37%] (Foulds *et al.*, 1996), longer half-life allowing once- or twice-daily administration, higher tissue concentrations, enhanced antibiotic activity and less gastrointestinal adverse effects (Zuckerman, 2004). Overall, the microbiologic spectrum of the activity of azithromycin mirrors that of erythromycin, with increased potency against certain pathogens (Kanatani and Guglielmo, 1994). Adverse effects of the drug include allergic reactions, liver damage, nausea, diarrhoea, abdominal pains, rashes and arrhythmias (Effa and Bukirwa, 2008).

**Cefotaxime**

Cefotaxime is a third-generation cephalosporin which has been reported to have a broad antimicrobial spectrum and to be a potent inhibitor of β-lactamase and stable in the presence of the enzyme (Masuyoshi *et al.*, 1980). Its chemical name is sodium (6R, 7R)-3-[(acet-yloxy) methyl]-7-{{[(Z)-2-(2-aminothiazol-4-yl)-2(methoxyimino) acetyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate (Hamood, 2011). Common adverse reactions include rash, pruritus, diarrhoea, nausea, vomiting, and pain at injection site (Babu and Sharmila, 2011). Cefotaxime primarily acts by inhibiting bacterial cell wall synthesis. This is brought about by binding with penicillin-binding proteins which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell wall. This arrest in cell wall assembly leads to bacterial lysis by cell wall autolytic enzymes (Babu and Sharmila, 2011). The presence of methoxyimino group in cefotaxime molecule is very important for its chemical and electrochemical behaviour (Hamood, 2011).
APPENDIX III
THE PAIRED SAMPLES T-TEST

Paired samples T-test

The difference between the two groups (CFUA or y1 and CFUB or y2) was assumed to have a normal distribution

\[ D = y1 - y2 \]

The null hypothesis here was that the two means difference is equal to zero.

Paired t-test statistic is shown below:

\[ t = \frac{d}{s_{d}\sqrt{n}} \]

Where \( d \) is equal to mean difference between paired groups, \( s_{d} \) is equal to standard deviation of the differences and \( n \) is equal to the number of pairs.

Under the null hypothesis the test statistic has a t-distribution with \( n-1 \) degrees of freedom. A 100 (1 - \( \alpha \)) % confidence interval was constructed as follows:

\[ d \pm t_{\alpha} s_{d}/\sqrt{n} \]

where \( t_{\alpha} \) is the critical value for the 2-sided test with \( n-1 \) degrees of freedom (Everitt and Landau, 2004).

Paired samples statistics

<table>
<thead>
<tr>
<th>Pair 1</th>
<th>Mean colony forming units counted after exposure</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean colony forming units counted before exposure</td>
<td>144.6750</td>
<td>18</td>
<td>92.15352</td>
<td>21.72079</td>
</tr>
<tr>
<td></td>
<td>300.0000</td>
<td>18</td>
<td>.00000</td>
<td>.00000</td>
<td></td>
</tr>
</tbody>
</table>
## Paired samples test

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Std. Error Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% Confidence Interval of the Difference</td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pair</th>
<th>Mean colony forming units counted after exposure</th>
<th>Mean colony forming units counted before exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>155.32500</td>
<td>155.32500</td>
</tr>
<tr>
<td></td>
<td>92.15352</td>
<td>21.72079</td>
</tr>
<tr>
<td></td>
<td>201.15187</td>
<td>109.49813</td>
</tr>
<tr>
<td></td>
<td>7.151</td>
<td>17</td>
</tr>
</tbody>
</table>

0.00
Wilcoxon signed ranks test

Two observations were made on each of the subjects in the sample $y_1$ and $y_2$ i.e. before and after treatment. The difference between each pair of observation was made $= z$. To compute the Wilcoxon signed rank statistic $T^+$, the absolute values of the differences was formed and then ordered from least to greatest. If ties among the calculated differences were found, each of the observations in the tied group was assigned the average of the integer ranks that were associated with the tied group. After, a positive or negative sign was assigned to the ranks of the differences which were positive or negative. Zero values were discarded and the $n$ value changed accordingly. The statistic \( T^+ \) is the sum of the positive ranks. A large sample approximation involved testing the statistic $z$ as a standard normal (Hollander and Wolfe, 1999). P-values of less than 0.05 were considered statistically significant (Mehata and Duan, 2011). Statistical analyses were performed by the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Illinois, USA).

Wilcoxon Signed Ranks Test

<table>
<thead>
<tr>
<th>Test Statistics(b,c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean colony forming units counted before exposure - Mean colony forming units counted after exposure</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Lower Bound</td>
</tr>
<tr>
<td>Upper Bound</td>
</tr>
</tbody>
</table>

-3.519(a)

a Based on negative ranks.
b Wilcoxon Signed Ranks Test
c Based on 73 sampled tables with starting seed 2000000.
APPENDIX V

EXAMPLE OF AN OBSERVATION CHECK-LIST USED IN THE STUDY FOR DATA COLLECTION

<table>
<thead>
<tr>
<th>FOOD SAMPLE</th>
<th>MICROWAVE RADIATION</th>
<th>PHYSICALS</th>
<th>CFU NUMBERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POWER</td>
<td>TIME</td>
<td>BEFORE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
### Appendix VI

**General Statistics for Each Food Sample Processed**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Bacteria</th>
<th>Mean CFUA</th>
<th>Mean CFUB</th>
<th>SDA</th>
<th>SDB</th>
<th>P-Value</th>
<th>D-CFUACFUB</th>
<th>PoOLED SD</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Escherichia coli</td>
<td>$3.0 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beef</td>
<td>Salmonella Paratyphi</td>
<td>$3.0 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beef</td>
<td>Staphylococcus aureus</td>
<td>$4.41 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>94.13</td>
<td>0</td>
<td>0</td>
<td>255.88</td>
<td>47.065</td>
<td>5.44</td>
</tr>
<tr>
<td>Chicken</td>
<td>Escherichia coli</td>
<td>$2.89 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>55.443</td>
<td>0</td>
<td>0.083</td>
<td>10.87</td>
<td>27.722</td>
<td>0.39</td>
</tr>
<tr>
<td>Eggs</td>
<td>Escherichia coli</td>
<td>$82.5 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>127.355</td>
<td>0</td>
<td>0</td>
<td>217.5</td>
<td>63.678</td>
<td>3.42</td>
</tr>
<tr>
<td>Eggs</td>
<td>Salmonella Paratyphi</td>
<td>$7.44 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>122.275</td>
<td>0</td>
<td>0</td>
<td>225.56</td>
<td>61.138</td>
<td>3.69</td>
</tr>
<tr>
<td>Fish</td>
<td>Escherichia coli</td>
<td>$52.65 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>103.471</td>
<td>0</td>
<td>0</td>
<td>247.35</td>
<td>51.736</td>
<td>4.78</td>
</tr>
<tr>
<td>Fish</td>
<td>Salmonella Paratyphi</td>
<td>$1.25 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>140.999</td>
<td>0</td>
<td>0</td>
<td>174.53</td>
<td>70.5</td>
<td>2.48</td>
</tr>
<tr>
<td>Fish</td>
<td>Shigella flexneri</td>
<td>$1.12 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>139.617</td>
<td>0</td>
<td>0</td>
<td>187.65</td>
<td>69.809</td>
<td>2.69</td>
</tr>
<tr>
<td>Gravy</td>
<td>Escherichia coli</td>
<td>$1.33 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>143.327</td>
<td>0</td>
<td>0</td>
<td>166.56</td>
<td>71.664</td>
<td>2.32</td>
</tr>
<tr>
<td>Gravy</td>
<td>Salmonella Paratyphi</td>
<td>$7.44 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>121.927</td>
<td>0</td>
<td>0</td>
<td>225.56</td>
<td>60.964</td>
<td>3.7</td>
</tr>
<tr>
<td>Milk</td>
<td>Escherichia coli</td>
<td>$2.68 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>29.852</td>
<td>0</td>
<td>0</td>
<td>31.78</td>
<td>14.926</td>
<td>2.13</td>
</tr>
<tr>
<td>Milk</td>
<td>Salmonella Paratyphi</td>
<td>$1.74 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>117.817</td>
<td>0</td>
<td>0</td>
<td>126.5</td>
<td>58.909</td>
<td>2.15</td>
</tr>
<tr>
<td>Milk</td>
<td>Staphylococcus aureus</td>
<td>$1.59 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>128.873</td>
<td>0</td>
<td>0</td>
<td>141.28</td>
<td>64.437</td>
<td>2.19</td>
</tr>
<tr>
<td>Potato</td>
<td>Staphylococcus aureus</td>
<td>$1.18 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>123.577</td>
<td>0</td>
<td>0</td>
<td>182.24</td>
<td>61.789</td>
<td>2.95</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Salmonella Paratyphi</td>
<td>$2.09 \times 10^4$</td>
<td>$3.0 \times 10^4$</td>
<td>120.155</td>
<td>0</td>
<td>0</td>
<td>90.83</td>
<td>60.078</td>
<td>1.51</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Shigella flexneri</td>
<td>$4.41 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>94.13</td>
<td>0</td>
<td>0</td>
<td>255.88</td>
<td>47.065</td>
<td>5.44</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Escherichia coli</td>
<td>$4.41 \times 10^3$</td>
<td>$3.0 \times 10^4$</td>
<td>94.365</td>
<td>0</td>
<td>0</td>
<td>255.88</td>
<td>47.183</td>
<td>5.42</td>
</tr>
</tbody>
</table>

**Key**

CFUA - Colony forming unit numbers after exposure to microwave radiation

CFUB - Colony forming unit numbers before exposure to microwave radiation

SDA - Standard Deviation after exposure to microwave radiation

SDB - Standard Deviation before exposure to microwave radiation

SD - Standard Deviation

ES - Effect Size
APPENDIX VII

THE LABORATORY IN WHICH THE STUDY WAS CARRIED OUT
### Antibiotic susceptibility testing for E, S¹, S² and S³ before and after exposure of sample to microwave radiation

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>AMPICILLIN</th>
<th>GENTAMYCIN</th>
<th>CIPROFLOXACIN</th>
<th>CEFOTAXIME</th>
<th>NITROFURANTOIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHICKEN (E)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>GRAVY (E)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>MILK (E)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>VEGETABLES(E)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>BEEF (E)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>EGGS (E)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>FISH(E)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>MILK (S¹)</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>n/a</td>
</tr>
<tr>
<td>EGGS (S¹)</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>n/a</td>
</tr>
<tr>
<td>GRAVY (S¹)</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>n/a</td>
</tr>
<tr>
<td>VEGETABLES(S¹)</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>n/a</td>
</tr>
<tr>
<td>BEEF (S¹)</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>n/a</td>
</tr>
<tr>
<td>CHICKEN (S¹)</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>n/a</td>
</tr>
<tr>
<td>FISH (S¹)</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>n/a</td>
</tr>
<tr>
<td>FISH (S²)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>n/a</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>VEGETABLES(S²)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>n/a</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>OXACILLIN</th>
<th>VANCOMYCIN</th>
<th>ERYTHROMYCIN</th>
<th>AZITHROMYCIN</th>
<th>NITROFURANTOIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEEF (S³)</td>
<td>sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>resistant</td>
</tr>
<tr>
<td>MILK (S³)</td>
<td>sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>resistant</td>
</tr>
<tr>
<td>POTATOES(S³)</td>
<td>sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>resistant</td>
</tr>
</tbody>
</table>

**KEY:**

E – *Escherichia coli*

S¹ – *Salmonella Paratyphi*

S² – *Shigella flexneri*

S³ – *Staphylococcus aureus*