Phillips, M. (2003). *Analysis of Microbial Hazards related to time/temperature control of foods for safety; Comprehensive Review in Food Science and Food safety* 2: 33-35.

ISOLATION OF TOXIGENIC SPECIES OF *BACILLUS CEREUS* AND *STAPHYLOCOCCUS AUREUS* FROM MEAT PIE SOLD IN KANO METROPOLIS.

BY

NASIBA SANUSI USMAN

SPS/11/MBI/00039

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY BAYERO UNIVERSITY, KANO, IN PARTIAL FULFILLMENT OF REQUIREMENT FOR THE AWARD OF MASTER DEGREE IN MEDICAL MICROBIOLOGY (M.Sc)

AUGUST, 2015

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Food is any substance usually of plant or animal origin consumed to provide nutritional support for the body. It is composed of carbohydrate, fat, protein, vitamin, mineral and water (Weingartner, 2004). From the microbial perspective, food can be viewed as fertile ecosystem in which microorganisms vie for nutrients (Nester *et al.*, 2004). Street food has been defined by FAO (1989) as ready to eat foods and beverages prepared and/or sold by vendors especially in the street and other public places for immediate consumption. Different terms have been used to describe such foods; these include convenient, ready, instant, fast and junk foods. Ready to eat could be raw or cooked, hot or chilled and can be consumed without further treatment (Tsang, 2002). Examples of such foods include meat pie, burger, milk and milk products. In developing countries such as Nigeria, there are serious concerns about sanitation of ready to eat foods, particularly as potable water is seldom available at preparation venues and fast food stands and also most food handlers lack basic knowledge of proper personal and environmental hygiene (Bukar *et al*. 2009).

Microorganisms on foods are not always undesirable because sometimes their growth result in a more pleasant taste or texture. However, contamination and growth of pathogens such as *Staphylococcus aureus*, Bacillus species and Salmonella can result in perceptible changes in quality of a food. Such food can transmit a wide range of food borne diseases.

World Health Organization defined food borne diseases (FBD) as “disease of infectious or toxic nature caused by or thought to be caused by the consumption of food or water (Le loir *et al.*,

2003). There are over 200 types of bacteria, parasite and viruses that can cause food borne diseases (Bailey, 2013) and bacteria are the causative agents of two third of the disease outbreaks (Le loir *et al*., 2003). These microbes fit into three categories which include infectious invasive agents, toxigenic agents and toxico-infectious agents (Bailey, 2013). Infectious pathogens enter the body and invade or colonize the host tissue while toxigenic pathogens cause food poisoning (intoxication) by producing an enterotoxin in the food which produce symptoms shortly after the food is consumed because growth of the disease causing agent is not required (Behling *et al*., 2010) and Symptoms range from mild gastric discomfort to death (Bailey, 2013).

Incidence of food poisoning is probably underestimated for a number of reasons, which include misdiagnosis, unreported minor outbreaks, improper sample collection and improper laboratory examination (Bailey, 2013). The control of this disease is of social and economic importance. In fact, it represent a considerable burden in terms of loss of working days and productivity, hospital expenses and economic losses in food industries, catering companies and restaurants (Balaban and Rasooly, 2000; Le loir, 2003). United States center for disease control (CDC) estimated that around 80 million people a year in the U.S alone contact a food poisoning or other food borne disease (Bailey, 2013), resulting in 325,000 hospitalization and more than 5,000 deaths (Mead *et al*., 1999) and the economic lost for the disease may reach 35 million annually (Buzby and Robert, 1997). According to world health organization, death of about 2 million people in the world is due to food borne diarrheal disease (Buzby and Robert, 1997).

1.2 Justification

Food borne illness is a major international health problem with consequent economic reduction (Duff *et al*., 2003). Data on issues of food borne diseases are well documentated world wide (Hazariwala *et al*., 2002). Outbreak of food borne diseases is caused by foods that are contaminated during harvesting, processing or preparation (Torok *et al*., 1997). Enterotoxigenic strains of *Bacillus cereus* and *Staphylococcus aureus* have been isolated from foods implicated in illnesses. *Staphylococcus aureus* is a normal flora in human and animals; its presence in food is an indication of excessive human handling (Talaro and Talaro, 1996). Although *Bacillus cereus* has been isolated from stool of healthy people, its presence in food is more likely attributed to contamination of agricultural products (Rath and Patra, 2012).

Meat pie is one of the most popular ready to eat foods commonly consumed by all levels of people and is well appreciated by consumers because of its taste, low cost, nutritional value and availability for immediate consumption. In developing countries like Nigeria, there are serious concerns about the sanitation of ready to eat foods, particularly as portable water is seldom available at preparation venues and fast food standards and also most food handlers lack basic knowledge of proper personal and environmental hygiene.

1.3 Aim

The aim of this study is to isolate toxigenic species of *Bacillus cereus* and *Staphylococcus aureus* in meat pie sold around Kano metropolis.

1.4 Objectives

i. To isolate and identify *Bacillus cereus* and *Staphylococcus aureus* from meat pie.

ii. To determine the antimicrobial susceptibility profile of *Bacillus cereus* and *Staphylococcus aureus* to some commonly used antibiotics.

iii. To detect enterotoxins from *Bacillus cereus* and *Staphylococcus aureus* using Reverse Passive Latex Agglutination test kits (Oxoid BCET-RPLA and SET-RPLA).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Bacillus cereus* Group

The genus bacillus is a heterogeneous group of Gram-positive, spore forming rods that belong to the phylum Firmicutes (Holt *et al.*, 1994). Bacillus sub group 1 (*Bacillus cereus sensulato* group) comprises the species *Bacillus anthrasis*, *Bacillus cereus sensustricto*, *Bacillus mycoids* and the recently described *Bacillus weihenstephanensis* (Lechner *et a*l., 1998) and *Bacillus Pseudomycoides* (Nakamura, 1998). A close genetic relation was observed between all *Bacillus cereus* group members (Helgason *et al*., 2000). Several characteristics have been suggested for differentiation of *B. cereus* group. The main diagnostic features of *B. cereus sensu lato* group are their inability to ferment mannitol and the ability to hydrolyze lecithin (Jaaskelain, 2008). Some Bacillus species have applications in industry in enzyme production or environmental as insecticide and some are pathogenic to man and/or animals (Wijnand *et al*., 2003). The most important species with respect to food is *B. cereus*.

2.1.1 The Specie *Bacillus cereus*

Bacillus cereus was first isolated in 1887 from cowshed air by Frankland and Frankland (Roberts *et al*., 1996; Forsythe, 2000; Griffiths and schraft, 2002). At least 12 *B. cereus* strains have been

fully genome sequenced (Jaaskelain, 2008). The cells are Gram-positive although positive staining is often difficult to obtain in older cultures (Vamman and Evans, 1991), rod-shaped. Spore forming (Positioned centrally or pericentrally), facultative anaerobic bacterium (Griffith and schraft, 2002). Most strains of *B. cereus* are motile with pertrichous flagella (Vamman and Evans, 1991), while 4% are found non motile (Jaaskelain, 2008). Growth may occur from PH 4.5 to 9.3, temperature 4^0C – 55^0C (Robert *et al*., 1996) and water activity most be higher than 0.92 (Wijnand *et al*., 2003).

Bacillus cereus is naturally found in soil, as harmless microflora in the intestinal tract of many soil dwelling invertebrates (Jaaskelain, 2008). *B. cereus* has also been isolated from stool of healthy people (human carriage range from 15 – 40% of the population), probably due to dietary intake, though human carriage is not considered to be of any significance in food borne disease (Wijnand *et al*., 2003).

2.1.2 Virulence and Pathogenicity

The key virulent factor of *B. cereus* is the ability to form endospores and toxins. The endospore is a dormant, tough and non -reproductive structure. The primary function of endospores is to ensure the survival of the bacterium through periods of environmental stress. The spores are highly resistant to heat, drying, toxic chemicals, UV radiation, gamma radiation and other adverse environmental factors. *Bacillus* spores are among the life forms most resistant to inactivation, with examples of spores being revived from amber 25-40 million years old or from brine inclusions dated at 250 -million years (Todar, 2008). The vegetative form starts to grow when cooked food is exposed to warm temperature for a prolonged period of time (Jaaskelain, 2008). *B. cereus* produces many types of toxins, two of which are most frequently associated

with food poisoning. The thermo-stable enterotoxins that are not detoxified by heat and the thermo-labile enterotoxins that are likely to be detoxified by heat (Jaaskelein, 2008).

2.1.3 *Bacillus cereus* Food Poisoning

Bacillus cereus was first proven to cause food borne disease in 1950. The food was highly contaminated vanilla sauce and consumption resulted in diarrhoeal illness (Jay *et al*., 2005). About 20 years later, *B. cereus* was also recognized to cause emetic gastro-intestinal disease. In 1971, many cases associated with *B. cereus* in fried rice from Chinese restaurant were reported (Mortimer and McCann, 1974). Subsequently, *B. cereus* was recognized as an important cause of food poisoning worldwide. In 2005 five epidemics involving 64 persons were caused by *B. cereus* in Finland. Similar developments has continues in 2006 (Niskanen *et al*., 2007).

The reported food-borne outbreaks and cases attributed to *B. cereus* in North America, Europe and Japan range from 1% - 22% of outbreak. Netherland and Norway were reported to have the most extensive problem due to *B. cereus* (Griffith and Schraft, 2002). *B. cereus* may have been involved in outbreaks involving heated foods in which no viable bacteria could be isolated. In 2005 – 2006 *B. cereus* was the most common cause of food-borne disease in Finland (Jaaskelein, 2008).

The type of food-borne illness caused by *B. cereus* varies among countries. In Japan the emetic syndrome is about 10 times more frequent than the diarrhoeal illness, while in Europe the diarrhoeal illness is more frequently reported. This difference is presumably due to the difference in foods and cooking tradition among these areas (Jaaskelein, 2008).

2.1.4 *Bacillus cereus* diarrhoeal food-borne intoxication

Bacillus cereus diarrhoeal intoxication occurs due to ingestion of spores that survive proteolysis enzymes and pH of the gastrointestinal tract. The spores survive digestion and may germinate in the intestine (Jensen *et al*., 2003) and the vegetative cell may produce toxin. The diarrhoeal syndrome is characterized by abdominal pain and diarrhea. Incubation period is 8 – 16 hours and symptoms last for about 12 – 24 hours (Jaaskelein, 2008).

Bacillus cereus diarrhoeal syndrome is mediated by one or the three diarrhoeal enterotoxins. The tripartite toxins haemolysin BL (HBL) and non-haemolytic enterotoxin (NHE), Cytotoxin K (CytK), enterotoxin T (Guinebretiere *et a*l., 2002; Moravek *et al*., 2006). HBL, NHE and CytK have been reported to be related to food-borne outbreaks, whereas enterotoxin T is not (Agata *et al*., 1995; Lund *et al*., 2000).

Haemolysin BL was the first toxin – complex to be identified. It consists of 3 proteins, L1, L2 and B. All 3 proteins are necessary to form an active enterotoxin within a cell. Each of these proteins is encoded by a single gene (Granum, 1994). NHE was first described in 1996 (Lund and Granum, 1996). It also consists of 3 proteins; A, B and C. Here too, all 3 proteins are necessary to form an active enterotoxin within the cell and each protein is encoded by a single gene (Granum, 1994). CytK was first discovered during the investigation of a French outbreak (Lund *et al*., 2000). CytK has been associated with severe symptom of *B. cereus* food-borne disease which is characterized by bloody diarrhea (Popoff, 2011). CytK is of 2 forms; CytK-1 and CytK – 2 (Fagerlund, 2004). It consists of single protein which structurally resembles the α-haemolysin of *Staphylococcus aureus* and the β-toxin of *Clostridium perfringens* (Wijnand *et al*., 2003).

These toxins are secreted as water soluble monomers, bind to the cell membrane and associated in oligomeric prepores which subsequently insert into lipid bilayer, forming transmembrane pores (Stenfor *et al*., 2008; Geny and Popoff, 2006).

Diarrhoeal enterotoxins are only effective when produced in the gut. There is no documentation on the illness-causing effect of the toxins ingested in food. Probably the ingested toxin proteins are inactivated by proteolytic enzymes in the gastro-intestinal tract. Since the spores of *B. cereus* are always present in heated foods, the actual illness will only occur if the spores germinate in the gut.

2.1.5 *Bacillus cereus* emetic food-borne intoxication

Bacillus cereus emetic food-borne illness is caused by an emetic enterotoxin (Cereulide) which is a ring-structured dodecadepsipeptide (Popoff, 2011) that is synthesized by a non ribosomal peptide synthetase. Cereulide structurally resembles Valinomycin produced by Streptomyces (Jaaskelein, 2008; Popoff, 2011).

Cereulide is a potassium ionophore (Andersson *et al*., 2007; Popoff, 2011) it promotes potassium ion uptake by mitochondria, efflux of hydrogen ion and in the inner membrane potential, leading to mitochondrial swelling, arrest of respiratory function, inhibition of ATP synthesis and release of necrotic factors (Popoff, 2011). Cereulide is resistant to heat, extreme PH and proteolytic activities of pepsin and trypsin (Jaaskelein, 2008). Emetic strains of *B. cereus* are unable to hydrolyze starch and starchy foods will look and taste good (Pirttijarvi, 2000). The contaminated food remains intact and become absorbed in the gut in its active form. Symptoms may occur 0.5-5 hours after ingestion of contaminated food and illness is characterized by nausea and vomiting lasting for 6 – 24 hours (Jaaskelein, 2008).

Cereulide and Cytotoxin K are regarded as the most dangerous to human health of the toxins produced by *B. cereus* because of its responsibility in death of young healthy persons (Lund *et al*, 2000).

2.2 *Staphylococcus aureus*

Staphylococcus aureus was first isolated by Alexander Ogston in 1882 from pus (Ogston, 1883). *Staphylococcus aureus* is ubiquitos (Pinchuk *et al*., 2010), non-spore forming (Forster, 1996), non motile, facultative anaerobic, catalase and coagulase positive, Gram positive cocci that appears singly, paired or grape-like cluster (Le loir *et al*., 2003). *S. aureus* is found in the nostril, skin and hair of warm blooded animals. Up to 30 – 50% of the human population are carriers (Le loir *et al;* 2003). Human carriage of *S. aureus* is considered as an increase risk of infection when host immune system is compromised. Non carriers acquire infection through contaminated food or when food handlers contaminate the food during preparation (Pinchuk *et al*., 2010). *S. aureus* grow at wide range of temperature (7^0C - 48^0C with an optimum of 30^0C - 37^0C (Schmid *et al*., 1990)) pH of 4.3 - 9.3 with an optimum of 7 to 7.5, Sodium chloride concentration of up to 15% and relatively low water activity (a_w 0.86) (Le loir *et al*., 2003). These characteristics enable *S. aureus* to grow on wide variety of foods.

2.2.1 Virulence and Pathogenisity

Staphylococcus aureus express many virulent factors, these include; Surface proteins that promotes colonization of host tissue, Factors that probably inhibit phagocytosis (Capsule, immunoglobin binding proteins), Toxins that damage host tissue and cause disease symptoms (Foster, 1996).

All strains of *S. aureus* can cause furulent infection. In addition, some strains produce exotoxins that can result in several unique diseases. Strains that carries the Toxic Shock Syndrome toxin 1 (TSST-1), a superantigen causes toxic shock syndrome (Public Health Agency Canada, 2001). Strains that produce exfoliative toxin A and B can cause Scalded Skin Syndrome (Durand *et al.,* 2006). Strains of *S. aureus* that are able to produce staphylococcal enterotoxins (SEs) are the

causative agents of staphylococcal food poisoning (Popoff, 2011). The enterotoxins are also superantigens and can cause Toxic Shock Syndrome if they are release systemically (Durand *et al.,* 2006). Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via manual contact or through respiratory secretions. Since *S. aureus* does not compete well with indigenous microbiota in raw foods, contamination is mainly associated with improper handling of cooked or processed food and storage under conditions which allow the growth of *S. aureus* and production of enterotoxins (Argudin, 2010). The bacterium multiply in foods and produces toxins even at refrigrirator temperatures and the toxin may be present in dangerous amounts in foods that have no sign of spoilage (Stoppler, 2011).

2.2.2 Staphylococcal Food Poisoning

Staphylococcal food poisoning is caused by ingestion of preformed enterotoxins secreted in foods (Awny *et al*., 2010). It has been estimated by the center for Disease Control (CDC) on food-borne disease that SEs affects approximately 80 million individuals in the US alone, resulting in 325,000 hospitalization and more than 5,000 deaths. Staphylococcal food-borne diseases acquired from ingestion of pre-formed toxins in food are the second most common reported type of food-borne disease (Pinchuk *et al*., 2010). The high incidence of staphylococcal food poisoning is due to insufficient decontamination of originally contaminated product source or its contamination during preparation and handling by individuals who are carriers (Pinchuk *et al*., 2010).

2.2.3 Staphylococcal Enterotoxins

Staphylococcal enterotoxins belong to a large family of Staphylococcal and Streptococcal pyrogenic exotoxins (PT), sharing common phylogenetic relationship, structure,function and sequence homoly (Balaban and Rasooly, 2000). The enterotoxins are single chain polypeptide and have the molecular weight of about 26-28kDa and 228-239 aminoacid residue (Panneerseelan, 2008). Staphylococcal enterotoxins possess superantigenic activity. Superantigens interaction with major histocompatibility complex (MHC) class II molecules on the surface of antigen presenting cells, and with T-cell receptors (TCR) on specific T-cell subsets. Interaction typically occurs to the variable region of the TCR β chain (Vβ) but binding to the TCR Vα domain has been reported (Larkin, 2009). This leads to activation of a large number of T-cells followed by proliferation and massive release of chemokines and proinflammatory cytokines that may led to potentially lethal toxic shock syndrome (Balaban and Rasooly, 2009))

Staphylococcus aureus enterotoxins (SEs) are synthesized by *S. aureus* throughout the logarithmic phase of growth or during the transition from the exponential to the stationary phase (Betley *et al*., 1992; Deizelle *et al*., 2009). There are 17 major types of SEs, SEA to SER with no SEF which is now designated as TSST-1 and it is not considered as enterotoxin as it lack emetic activity which is characteristic feature of enterotoxins. Most genes coding for SEs are located on mobile elements such as plasmids, bacteriophages or Pathogenility Island (Zhang *et al*., 1998). Thus, horizontal transfer between strains is not rare (Wijnand *et al*., 2007). SEB, SEC, SEG, SEI, SEM, SEN, SEO, SEK, SEL, SEQ and TSS are encoded by pathogenicity islands (Becker *et al.*, 2003). SEA, SEE and SEP are encoded by prophage (Holtfreter *et al.,* 2004), whereas SED, SEE and SER are encoded by a plasmid called plB485 (Becker *et al.*, 2004). The association with mobile elements implies a horizontal transfer of the PTSAg genes between Staphylococcal strains and an important role in evolution of *S. aureus* as a pathogen. Genes encoding several of enterotoxins are physically clustered in the Staphylococcal genome (Belkum *et al.,* 2006). Atleast

three different egc subtypes were suggested. Enterotoxin cluster gene 1 haboring seo, sem, sei,Φent1, Φent2, sen and seg, egc2 containing seu instead of Φent1 and Φent2, egc 3 containing sei, seu, sen and seg variant (biotyping). Although this cluster among S. aureus strains in general, antibodies are rarely raised against egc enterotoxins, which is a unique feature of this group.SEA is the most common toxin in staphylococcal related food poisoning. SEB is associated with food poisoning and it has been studied for potential use as inhaled bioweapon (Ler *et al*., 2006). SEE has also been demonstrated in some cases of food poisoning. SEG, SEH and SEI are not well studied as the other SES, but were associated with one of the food poisoning outbreaks in Taiwan (Chen *et al*., 2004). SEH has also been identified as one of the causes of massive food poisoning associated with reconstituted milk consumption in Osaka, Japan in 2000 (Ikeda *et al*., 2005).

2.3 Overview of Antibiotics

2.3.1 Antibiotics

Antibiotics are a type of antimicrobial used in the treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria. Sometimes the term antibiotic is used to refer to any substance used against microbes, synonymous to antimicrobial. Some sources distinguish between antibacterial and antibiotic; antibacterials used in soaps and cleaners etc., but not as medicine. The terms are treated synonymous and according to the most widespread definition of antibiotics being a substance used against bacteria (Finberg *et al.,* 2004). Antibacterial antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity (Willey *et al.,* 2008).

Amoxycillin

Amoxycillin ($C_{16}H_{19}N_3O_5S$) is a broad spectrum semisynthetic β-lactam antibiotic similar to penicillin except that it is resistant to gastric acid and permit higher serum levels with oral administration. Amoxycillin is bactericidal, it binds to and inactivates penicillin-binding proteins (PBPs) located on the inner membrane of the bacterial cell wall. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This interrupts bacterial cell wall synthesis and results in the weakening of the bacterial cell wall and causes cell lysis (NIH, 2004).

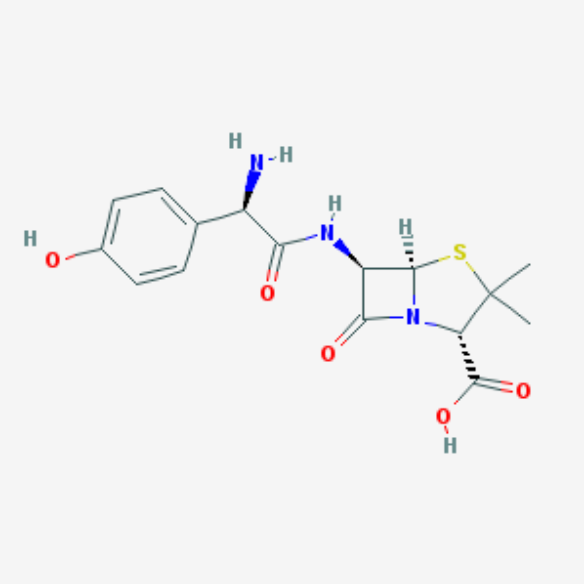
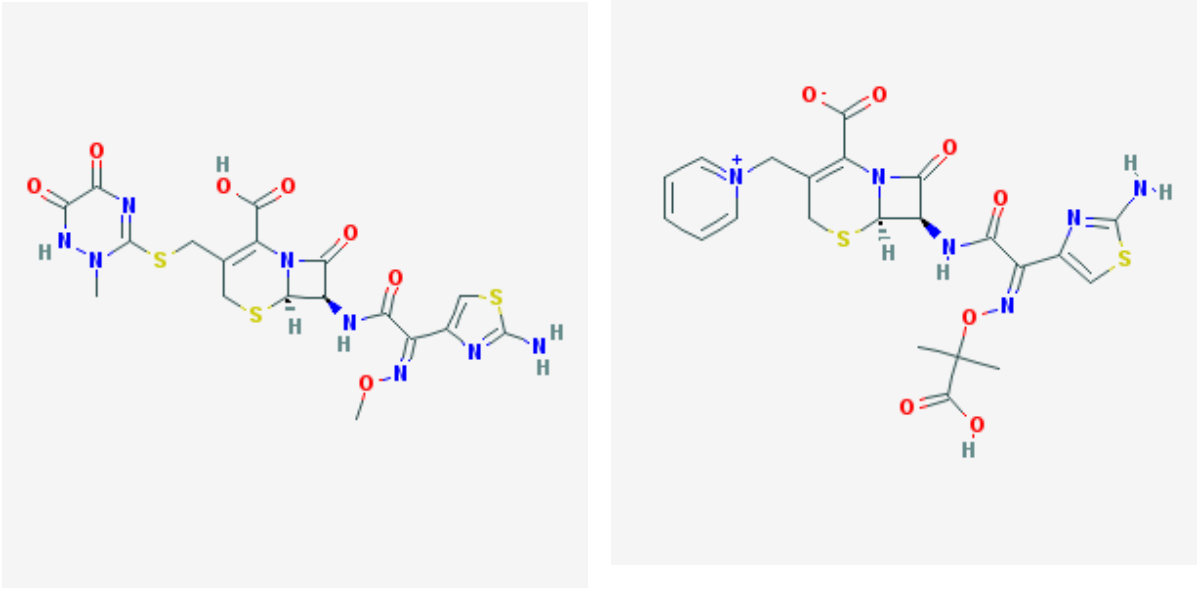


Figure 1: Structure of Amoxycillin

Ceftazidime and Ceftriaxone

Ceftazidime ($C_{22}H_{22}N_6O_7S_2$) and Ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) are semi synthetic, broad spectrum, β-lactam 3rd generation cephalosporin antibiotics with bactericidal activity. They bind to and inactivate Penicillin-Binding Proteins (PBP) located at tha inner membrane of the bacterial cell wall. Inactivation of the PBPs interferes with the cross-linkage of peptidoglycan chain necessary for bacterial cell wall strength and rigidity. This results in the weakening of the bacterial cell wall and causes cell lysis (Pagani *et al.,*2000).



(a) (b)

Figure 2: Structure of (a) Ceftazidime and (b) Ceftriaxone

Vancomycin

Vancomycin ($C_{66}H_{75}Cl_5N_9O_{24}$) is a Glycopeptide antibacterial produce by *Streptomyces orientalis*. Vancomycin is bactericidal against most organisms and bacteriostatic effect on enterococci. It binds tightly to the D-alanyl-D-alanine terminal sequence on the pentapeptide portion of peptidoglycan, thereby interfering with bacterial cell wall synthesis. This leads to activation of bacterial autolysins that destroy the cell wall by lysis. Vancomycin may also alter the permeability of bacterial cytoplasmic membranes and may selectively inhibit RNA synthesis (NIH, 2004).

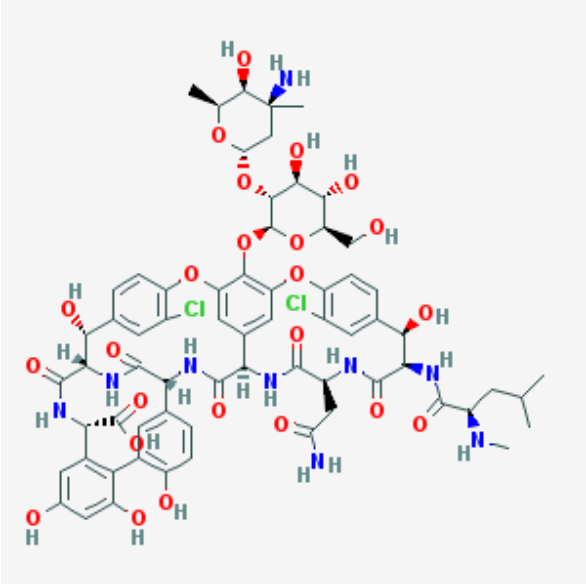


Figure 3: Structure of Vancomycin

Co-trimoxazole

Co-trimoxazole ($C_{24}H_{29}N_7O_6S$) is a synthetic combination of two antibacterial agents- a Sulphonamide called Sulphamethoxazole, and trimethoprim. This synergistic combination inhibits two sequential steps in the bacterial metabolism of folic acid. Trimethoprim is a pyrimidine inhibitor of dihydrofolate reductase; sulfamethoxazole is a sulfamide inhibitor of bacterial dihydrofolate synthase (NIH, 2004).

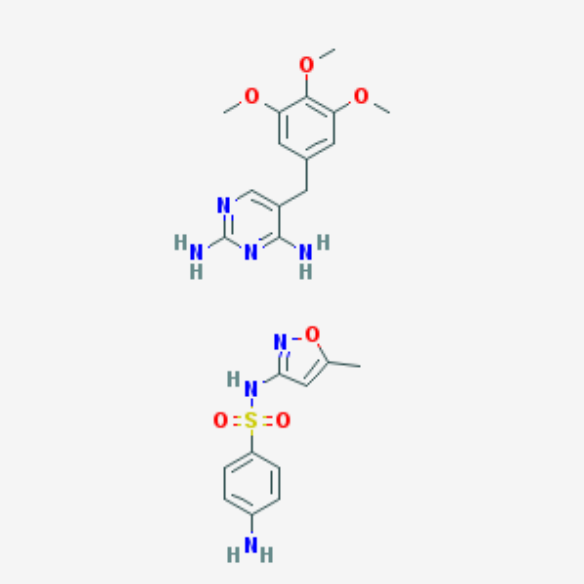


Figure 4: Structure of Sulphamethoxazole/trimethoprim

Gentamicin

Gentamicin ($C_{21}H_{43}N_5O_7$) is a broad-spectrum aminoglycoside antibiotic produced by *Micromonospora purpurea* or *M. echinospora*. It irreversibly binds to the bacterial 30S ribosomal subunit. Specifically, this antibiotic is lodged between 16S rRNA and S12 protein within the 30S subunit. This leads to interference with translational initiation complex, misreading of mRNA, thereby hampering protein synthesis and resulting in bactericidal effect (NIH, 2004).

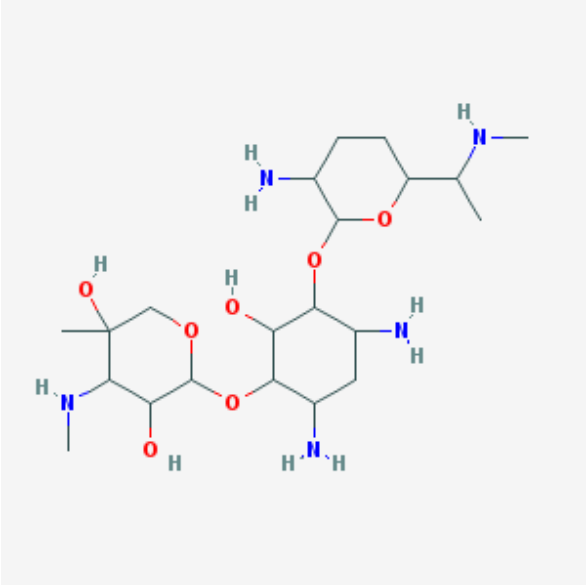


Figure 5: Structure of Gentamicin

.

Erythromycin

Erythromycin ($C_{37}H_{67}NO_{13}$) is a macrolide antibiotic produced by *Streptomyces erythreus*. It is a relatively broad-spectrum antibiotic and it inhibits protein synthesis by binding to 50S ribosomal subunits. This binding process inhibits peptidyl transferase activity and interferes with translocation of amino acids during translation and assembly of proteins (NIH, 2004).

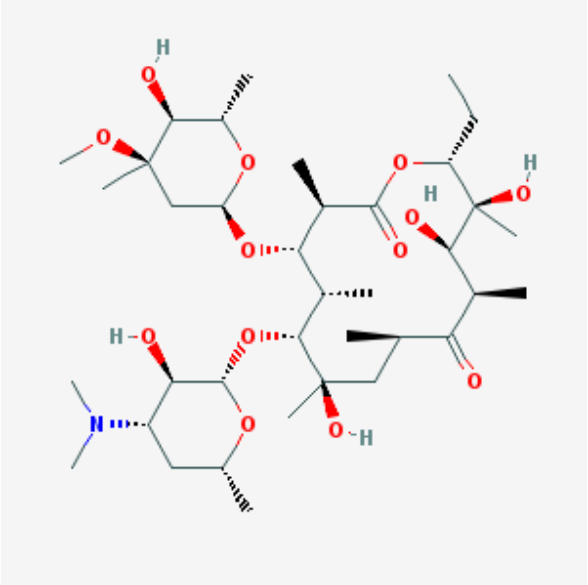


Figure 6: Structure of Erythromycin

Chloramphenicol

Chloamphenicol ($C_{11}H_{12}Cl_2N_2O_5$) is the first broad spectrum antibiotic to be discovered. It is produced by *Streptomyces venequelae* but now produced synthetically. Chloramphenicol diffuses through the bacterial cell wall and reversibly binds to the bacterial 50S ribosomal subunit. The binding interferes with peptidyl transferase activity, thereby prevents transfer of amino acids to the growing peptide chains and blocks peptide bond formation, as result bacterial protein synthesis is blocked and is mainly bacteriostatic (NIH, 2004).

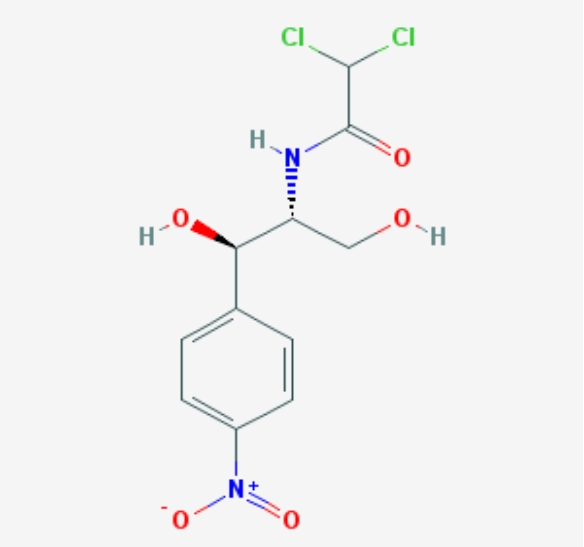


Figure 7: Structure of Chloramphenicol

Ciprofloxacin

Ciprofloxacin ($C_{17}H_{18}FN_3O_3$) is a synthetic broad spectrum Carboxyfluoroquinoline drug that contain 4-quinolone ring. Quinolones inhibit DNA gyrase and topoisomerase IV, thereby blocking DNA replication and transcription. Ciprofloxacin has narrow spectrum of activity (NIH, 2004).

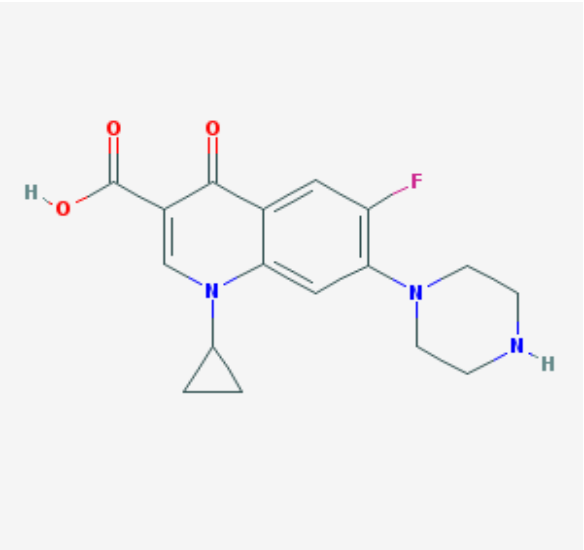


Figure 8: Structure of Ciprofloxacin

2.3.2 Possible Causes of antibiotics resistance

Microbes, such as bacteria, viruses, fungi, and parasites, are living organisms that evolve over time. Their primary function is to reproduce, thrive, and spread quickly and efficiently. Therefore, microbes adapt to their environments and change in ways that ensure their survival. If something stops their ability to grow, such as an antimicrobial, genetic changes can occur that enable the microbe to survive. There are several ways this happens (Levy, 1998).

Natural (Biological) Causes

In the presence of an antimicrobial, microbes are either killed or, if they carry resistance genes, survive. These survivors will replicate, and their progeny will quickly become the dominant type throughout the microbial population (Levy, 1998).

Mutation

Most microbes reproduce by dividing every few hours, allowing them to evolve rapidly and adapt quickly to new environmental conditions. During replication, mutations arise and some of these mutations may help an individual microbe survive exposure to an antimicrobial (Levy, 1998).

Gene Transfer

Microbes also may get genes from each other, including genes that make the microbe drug resistant (Levy, 1998).

Inappropriate Use

Selection of resistant microorganisms is exacerbated by inappropriate use of antimicrobials. Sometimes healthcare providers will prescribe antimicrobials inappropriately, wishing to placate an insistent patient who has a viral infection or an as-yet undiagnosed condition (Levy, 1998).

Inadequate Diagnostics

More often, healthcare providers must use incomplete or imperfect information to diagnose an infection and thus prescribe an antimicrobial just-in-case or prescribe a broad-spectrum antimicrobial when a specific antibiotic might be better. These situations contribute to selective pressure and accelerate antimicrobial resistance (Levy, 1998).

Hospital Use

Critically ill patients are more susceptible to infections and, thus, often require the aid of antimicrobials. However, the heavier use of antimicrobials in these patients can worsen the problem by selecting for antimicrobial-resistant microorganisms. The extensive use of antimicrobials and close contact among sick patients creates a fertile environment for the spread of antimicrobial-resistant germs (Levy, 1998).

Agricultural Use

Scientists also believe that the practice of adding antibiotics to agricultural feed promotes drug resistance. More than half of the antibiotics produced in the United States are used for agricultural purposes (Mellon *et al.,* 2001).

2.3.3 Mechanism of drug resistance

Bacteria often develop resistance in several ways. Unfortunately, a particular type of resistance mechanism is not confined to a single class of drug. Two bacteria may use different resistance mechanism to withstand the chemotherapeutic agent. Pathogens often become resistant simply by preventing drug entrance, pumping the drug out of the cell after it has been absorbed, inactivation of the drug through chemical modification, modification of enzyme or cellular structure so that it is no longer susceptible to the drug and by using an alternative pathway to bypass the sequence inhibited by the agent or increase the production of the target metabolite (Willey *et al*., 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Size

Sample size was calculated using epidemiological statistical software (open epi version2) and 60% prevalence of *S. aureus* was adopted as reference prevalence by Ani *et al*. (2011)

3.2 Sample Collection

A total of 370 meat pie samples were purchased from eight local governments of Kano metropolis. Polythene bag was used to pick and wrap the meat pie sample and brought to the laboratory for analyses.

3.3 Sample Analysis

Inoculation of sample

Samples were prepared in two parts that is pastry (dough component covering the filling of the meat pie) and minced meat (fillings of the meat pie) according to the methods of FAO (1979). Twenty five grams of the pastry and minced meat were weighed separately and homogenized in 225ml of peptone water. Dilutions of 10^{-1}, 10^{-2} and 10^{-3} were made and 0.1ml of each dilution was inoculated on Mannitol

Egg Yolk Polymyxin Agar (MYPA) and incubated at 30°C for 24 hours for the isolation of *Bacillus cereus* and on Mannitol Salt Agar (MSA)for the isolation of *Staphylococeus aureus* and incubated at 37°C for 24 hours.

3.4 Identification of Isolates.

3.4.1 Identification of *Staphylococcus aureus*

Isolates that ferment mannitol (yellow colonies on MSA) were subjected to Gram's staining, catalase and coagulase test as described by Cheesbrough (2004).

Gram's staining

A smear was made by emulsifying few colonies in a drop of distilled water on a clean microscope glass slide and air dried. The dried smear was gently heat fixed and placed on a staining rack. The smear was flooded with crystal violet for 1 minute. Crystal violet was rinsed off with clean water and the slide was flooded with lugol's iodine for 1 minute. Lugol's iodine was rinsed off and the smear was decolorized with ethanol for 3 seconds and rinsed off with clean water. The decolorized smear was flooded with neutral red for 2 minutes, after which the slide was rinsed, blotted and air dried. The slide was observed using oil immersion objective lens (×100) (Cheesbrough, 2004). Cells that appeared purple were termed Gram positive while cells that appeared red were termed Gram negative.

Catalase test

With the aid of a swab stick, several colonies of the test organism were picked and placed in 2ml of 3% hydrogen peroxide solution (Cheesbrough, 2004). Immediate bubbling indicated a positive result while no bubbling indicated a negative result.

Coagulase test

The test organism was cultured in 0.8ml of tryptone soy broth at 37°C for 24 hours. Human plasma (0.2ml) was poured in the overnight culture and incubated at 37^0C for 1 hour. Unclear results were incubated for further 2 hours. If results were still not clear after 3 hours of incubation, the test organisms were incubated at room temperature overnight (Cheesbrough, 2004). Coagulase positive bacteria coagulated the plasma and clumping was observed. **Identification of *Bacillus cereus***

Mannitol negative (pink colonies on MYPA), lecithinase positive (colonies that produce white zone of presipitation on MYPA), gram positive bacilli were subjected to catalase test, motility test, test for hemolysis, growth characteristics on nutrient agar and protein crystal test as described by the United States Department of Agriculture (USDA,1998).

A thick suspension of the test organism (isolated gram positive bacill) was prepared by emulsifying the test organism in 0.5ml of peptone water. The suspension was used for motility test, rhizoid growth test, test for heamolysis and protein crystal test.

Motility test

A semisolid medium was prepared as described by Cheesbrough (2004) by weighing 0.75g of nutrient and 1.3g of nutrient broth in 100ml of distilled water and heated to 100^0C. The media was then dispensed in test tubes, autoclaved and allowed to solidify. With the aid of straight wire, the prepared inoculums was inoculated by stabbing down the center and incubated at 37^0C for 24 hours. Motile

organisms produced diffuse growth out into the medium away from the stab. Non-motile organisms produced growth only in and along stab. *B.cereus* was motile.

Rhizoid Growth Test

A loopful of the prepared inoculums was inoculated at near center of a nutrient agar plate. The inoculum was allowed to be absorbed and incubated at 30^0C for 48 – 72 hours. Development of colonies with root like structures (rhizoid) was observed which is a distinctive characteristics of *B. mycoid* but not *B. cereus* and other *B. cereus* group (Tallent *et al*, 2012).

Test for hemolytic activity

A loopful of the prepared inoculum was streaked on blood agar plate and incubated at 37°C for 24 hours. Culture was observed for lysis of red blood cells. Colonies with α-heamolysis produced 1-3mm greenish zone of incomplete heamolysis while β-heamolytic colonies produced a zone of clearing or complete heamolysis. *B. cereus* was strongly heamolytic (β – heamolytic) (Tallent *et al*., 2012).

Test for Protein Crystals

Nutrient agar slant was inoculated with the prepared inoculum and incubated at 30^0C for 24 hours, and then at room temperature for 3 days. A smear was made on a clean glass slide and allowed to air dry. The dried smear was then slightly heat fixed and flooded with methanol. It was allowed to stand for 30 seconds. The methanol was poured off, and the slide was allowed to air dry. The slide was then flooded with carbolfuchsin and heated with bunsen burner from below until it steams. The slide was cooled for 2 minutes and the step was repeated once. The slide was allowed to stand for 30 seconds before it was

rinsed with clean water and air dried. Oil immersion objective lens (×100) was used to examine the presence of free spores and darkly stained tetragonal toxin crystals, which was a typical characteristic of *B. thuringiensis* not *B. cereus* and other *B. cereus* group (Tallent *et al*., 2012).

3.5 Antibiotic susceptibility test

Kirby-Bauier modified antibiotic sensitivity technique was employed as described by Chessbrought (2004) using commercially prepared antibiotic discs (Oxoid Gentamicin (10µg) Sulphamethoxazole/trimethoprim (25µg), Chloramphenicol (10µg), Vancomycin (5µg), Amoxycillin, Ciprofloxacin (5 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg) and Erythromycin (15 µg)). The test was done for both *B. cereus* and *S. aureus*.

Confirmed isolates of *B. cereus* and *S. aureus* were adjusted to 0.5 McFarland standards. With the aid of sterile swab sticks the adjusted inoculum was inoculated on Mueller Hinton agar. The antibiotic discs were placed and pressed on the inoculated plates using sterile forceps. The plates were left for about 30 minute on the bench for proper diffusion, then inverted and incubated aerobically at 37^0C for 24hours. The inhibition zone diameters were measured in millimeter using meter rule and compared with the CLSI, 2006. All the zones of inhibition that fall under the intermediate range were assumed to be resistant.

3.6 Toxin Detection

3.6.1 *Bacillus cereus* diarrhoeal enterotoxin extraction and detection

Confirmed of *B. cereus* isolates were inoculated in brain heart infusion broth and incubated at 37°C for 18 hours. Following incubation, the cultures were cooled to 4^0C by refrigeration and then centrifuged at 3000rpm for 30 minutes. The filtrates were retained for the assay. The microtitre plate was placed in such a way that each row consist of 8 wells (2 of such rows are required for each sample. With the aid of

a micropipette, 25µl of the diluents (phosphate buffered saline containing bovine albumin and sodium hexamethaphosphate) was added to each well of the 2 rows, except the first well of the 2 rows. To each first and second well of both rows, 25µl of the test sample (the filtrate) was added. With the aid of micropipette and starting from the second well, 25µl of the mixture was picked and doubling dilution was performed along each row stopping at the 7th well (so that the 8thwell consists of diluents only). To each well in the first row, 25µl of sensitized latex was added. To each well in the second row, latex control was added. The plate was agitated by hand, covered with a lid and then incubated on a black paper, vibration-free surface at room temperature. Agglutination (formation of lattice structure) was observed in the wells of the 1st row after 24 hours of incubation. Results in row of wells containing latex particles and the last well of each row were negative (Oxoid, 2013).

3.6.2 Staphylococcal enterotoxin extraction and detection

Confirmed colonies of *S. aureus* were inoculated in tryptone soya broth and incubated at 37°C for 18 hours. The cultures were cooled to 4°C by refrigeration and then centrifuged at 3000rpm for 30 minutes. The filterates were retained for the assay. The microtitre plate was placed in such a way that each row contains 8 wells (5 of such rows are required for each sample). With the aid of a micropipette, 25µl of the diluents was dispensed in each well of the 5 rows. To the first well of each row, 25µl of the test sample (the filtrate) was added. With the aid of pipette and staring from the first well of each row, 25µl of the mixture was picked and doubling dilution was made along each of the 5 rows, stopping at 7th well (so that the 8th wells consist of diluents only). To each well of the first row, 25µl of latex sensitized with enterotoxin A was added. To each well of the second row, 25µl of latex sensitized with enterotoxin B was added. To each well of the third row, 25µl of latex sensitized with enterotoxin C was added. To each well of the fourth row, 25µl of latex sensitized with enterotoxin D was added. To the wells of the fifth row, 25µl of latex control was added. The plate was agitated by hand to mix the content of the well and

then covered with a lid to avoid drying out. The plate was incubated at room temperature on a black paper on vibration–free surface. Agglutination (formation of lattice structure) was examined after 24 hours of incubation in the wells of 1^{st}, 2^{nd}, 3^{rd} and 4^{th} rows. All wells of the 5^{th} row and last wells of each row were negative (Oxoid, 2013).

3.7 Statistical analysis

Statistical analysis was done by one way ANOVA using Statistical Package for Social Sciences (SPSS) version 2.0 to compare the means for Staphylococcal enterotoxins and difference were considered significant at value of $P<0.05$. The test was not applicable for *B.cereus* enterotoxin because only one type of enterotoxin was detected.

CHAPTER FOUR

4.0 RESULTS

Out of the 370 meat pie samples analyzed, 143 (38.6%) were positive for *Bacillus* species. The *Bacillus* species isolated were identified as *B. cereus* 86(54.8%), *B. mycoid* 15(9.6%), *B. thuringiensis* 23(14.6%) and *B. megaterium* 33(21.0%) (Appendix II and 4.1). Fifty one 51(59.3%) of the 86 *B. cereus* isolates were detected in the pastry and 35(40.7%) from the minced meat (Table 4.2).

One hundred and sixteen, 116 (31.4%) of the 370 meat pie samples were positive for *S. aureus* (Appendix III). Out of the 147 *S. aureus* isolated, 64(43.5%) were detected in the pastry and 83(56.5%) from the minced meat component of the meat pie (Table 4.3).

*Bacillus cereus a*ntibiotic sensitivity test result is given in Appendix IV. The percentage resistance to amoxycillin, ceftazidime, ceftriaxone,ciprofloxacin, chloramphenicol, erythromycin, gentamicin, sulphamethoxazole/trimethoprim and vancomycin were recorded as 72.1%, 80.2%, 60.5%, 4.7%, 16.3%,19.8%, 0%, 51.2% and 24.4% respectively(Table 4.4).

Similarly *Staphylococcus aureus* antibiotic sensitivity test results are given in Appendix V and the percentage resistance to amoxycillin, ceftazidime, ceftriaxone, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, sulphamethoxazole/trimethoprim and vancomycin were recorded as 58.5%, 35.4%, 64.6%, 10.2%, 8.2%, 6.1%, 2.0%, 12.2% and 6.1% respectively (Table 4.5).

Table 4.1: Frequency of *Bacillus* species isolated from meat pie samples from different locations in Kano.

Local Government	***B. cereus***	***B. mycoid***	***B. thuringiensis***	***B. megaterium***
Dala	9	2	2	1
Fagge	8	2	3	7
Kumbotso	14	3	4	4
Gwale	11	0	2	6
Municipal	11	3	4	5
Nassarawa	13	1	2	1
Tarauni	11	2	3	6
Ungogo	9	2	3	3
Total	**86 (54.8%)**	**15 (9.6%)**	**23 (14.6%)**	**33 (21.0%)**

Table 4.2: Frequency of *B. cereus* isolated from meat pie samples from different locations in Kano

Local govt	**Pastry**	**Minced meat**
Dala	4	5
Fagge	5	3
Kumbotso	8	6
Gwale	8	3
Municipal	4	7
Nassarawa	11	2
Tarauni	7	4
Ungogo	5	4
Total	**51(59.3%)**	**35(40.7%)**

Table 4.3: Frequency of *S. aureus* isolated meat pie samples from different locations in Kano.

Local Government	Pastry	Minced meat
Dala	7	8
Fagge	4	6
Kumbotso	11	14
Gwale	10	8

Municipal	12	12
Nassarawa	7	14
Tarauni	5	8
Ungogo	8	13
Total	**64 (43.5%)**	**80 (56.5%)**

Table 4.4: The percentage resistance and susceptibility pattern of enterotoxigenic *B. cereus* isolated from meat pie samples

Antibiotic	Dala (n=9) (%)		Fagge (n=8) (%)		Gwale (n=11) (%)		Kumbotso (n=14) (%)		Municipal (n=11) (%)		Nassarawa (n=13) (%)		Tarauni (n=11) (%)		Ungogo (n=9) (%)		Total (n=86) (%)	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Amoxycillin	7 (77.8)	2 (22.2)	5 (62.5)	3 (37.5)	8 (72.7)	3 (27.3)	11 (78.6)	3 (21.4)	8 (72.7)	3 (27.3)	9 (69.2)	4 (30.8)	8 (72.7)	3 (27.3)	6 (66.7)	3 (33.3)	62 (72.1)	24 (27.9)
Ceftazidine	7 (77.8)	2 (22.2)	5 (62.5)	3 (37.5)	9 (81.8)	2 (18.2)	12 (85.7)	2 (14.3)	10 (90.9)	1 (9.1)	11 (84.6)	2 (15.4)	9 (81.8)	2 (18.2)	6 (66.7)	3 (33.3)	69 (80.2)	17 (19.8)
Ceftriaxone	5 (55.6)	4 (44.4)	5 (62.5)	3 (37.5)	8 (72.7)	3 (27.3)	8 (57.1)	6 (42.9)	9 (81.8)	2 (18.2)	9 (69.2)	4 (30.8)	5 (45.5)	6 (54.5)	3 (33.3)	6 (66.7)	52 (60.5)	34 (39.5)
Ciprofloxacin	1 (11.1)	8 (88.9)	0 (0)	8 (100)	1 (9.1)	10 (90.9)	1 (7.1)	13 (92.9)	0 (0)	11 (100)	1 (7.7)	12 (92.3)	0 (0)	11 (100)	0 (0)	9 (100)	4 (4.7)	82 (95.3)
Chloramphenicol	1 (11.1)	8 (88.9)	0 (0)	8 (100)	3 (27.3)	8 (72.7)	3 (21.4)	11 (78.6)	3 (27.3)	8 (72.3)	3 (23.1)	10 (76.9)	0 (0)	11 (100)	1 (11.1)	8 (88.9)	14 (16.3)	72 (83.7)
Erythromycin	1 (11.1)	8 (88.9)	2 (25)	6 (75)	3 (27.3)	8 (72.7)	4 (28.6)	10 (71.4)	3 (27.3)	8 (72.3)	2 (15.4)	11 (84.6)	2 (18.2)	9 (81.8)	0 (0)	9 (100)	17 (19.8)	69 (80.2)
Gentamicin	0 (0)	9 (100)	0 (0)	8 (100)	0 (0)	11 (100)	0 (0)	14 (100)	0 (0)	1 (100)	0 (0)	13 (100)	0 (0)	11 (100)	0 (0)	9 (100)	0 (0)	86 (100)
Sulphamethoxa Zole/Trimethoprim	4 (44.4)	5 (55.6)	4 (50)	4 (50)	6 (54.5)	5 (45.5)	7 (50)	7 (50)	6 (54.5)	5 (45.5)	8 (61.5)	5 (38.5)	5 (45.5)	6 (54.5)	5 (55.6)	4 (44.4)	44 (51.2)	42 (48.8)
Vancomycin	2 (22.2)	7	1	7	8 (72.7)	3	4	10	4 (36.4)	7	3	10	2	9	2 (22.2)	7	21 (24.4)	65 (75.6)

	(77.8)	(12.5)	(87.5)		(27.3)	(28.6)	(71.4)		(63.7)	(23.1)	(76.9)	(18.2)	(81.8)		(77.8)

Key:

R = Resistant

S= Susceptible

Table 4.5: The percentage resistance and susceptibility pattern of enterotoxigenic *S. aureus* isolated from meat pie samples

Antibiotic	Dala (n=15) (%)		Fagge (n=10) (%)		Gwale (n=18) (%)		Kumbotso (n=25) (%)		Municipal (n=24) (%)		Nassarawa (n=21) (%)		Tarauni (n=13) (%)		Ungogo (n=21) (%)		Total (n=147) (%)	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Amoxycillin	8 (53.3)	7 (46.7)	6 (60)	4 (40)	9 (50)	9 (50)	16 (64)	9 (36)	14 (58.3)	10 (41.7)	16 (76.2)	5 (23.8)	7 (53.8)	6 (46.2)	10 (47.6)	11 (52.4)	86 (58.5)	61 (41.5)
Ceftazidime	4 (26.7)	10 (73.3)	1 (10)	9 (90)	5 (27.7)	13 (72.2)	12 (48)	13 (52)	8 (33.3)	16 (66.7)	9 (42.9)	12 (57.1)	3 (23.1)	10 (76.9)	10 (47.6)	11 (52.4)	52 (35.4)	95 (64.6)
Ceftriaxone	5 (33.3)	11 (66.7)	7 (70)	3 (30)	13 (72.2)	5 (27.7)	17 (68)	8 (32)	16 (66.7)	8 (33.3)	14 (66.7)	7 (33.3)	8 (61.5)	5 (38.5)	15 (71.4)	6 (28.6)	95 (64.6)	52 (35.4)
Ciprofloxacin	1 (6.7)	14 (93.3)	1 (10)	9 (90)	1 (5.6)	17 (94.4)	4 (16)	21 (87.5)	4 (16.7)	20 (83.3)	19 (90.5)	2 (9.5)	0 (0)	13 (100)	3 (14.3)	18 (85.7)	15 (10.2)	132 (89.8)
Chloramphenicol	0 (0)	15 (100)	0 (0)	10 (100)	2 (11.1)	16 (88.9)	2 (8)	23 (92)	2 (8.3)	22 (91.7)	3 (14.3)	18 (85.7)	1 (7.7)	12 (92.3)	2 (9.5)	19 (90.5)	12 (8.2)	135 (91.8)
Sulphomethoxazole/ Trimethoprim	1 (6.7)	14 (93.3)	1 (10)	9 (90)	2 (11.1)	16 (88.9)	5 (20)	20 (80)	2 (8.3)	22 (91.7)	3 (14.3)	18 (8579)	3 (23.1)	10 (76.9)	1 (4.8)	20 (95.2)	18 (12.2)	129 (87.8)
Gentamicin	0 (0)	15 (100)	0 (0)	10 (100)	0 (0)	18 (100)	2 (8)	23 (92)	0 (0)	24 (100)	0 (0)	21 (100)	0 (0)	13 (100)	1 (4.8)	20 (95.2)	3 (2.0)	144 (98)
Erythromycin	1	14	1	9	1	17	2	23	2	22	0	2	0	13	0	21 (100)	9 (6.1)	138 (93.9)

	(6.7)	(93.3)	(10)	(90)	(5.6)	(94.4)	(8)	(92)	(8.3)	(91.7)	(0)	(9.5)	(0)	(100)	(0)			
Vancomycin	0 (0)	15 (100)	0 (0)	10 (100)	0 (0)	18 (100)	2 (8)	23 (92)	3 (12.5)	21 (87.5)	1 (4.8)	20 (95.2)	1 (7.7)	12 (92.3)	2 (9.5)	19 (90.5)	9 (6.1)	138 (93.9)

Key:

R = Resistant

S = Susceptible

The result of diarrhoeal enterotoxin detection from *B. cereus* is given in Appendix VI. Out of the 86 *B. cereus* isolated, 26(30.2%) were found to produce *B. cereus* diarrhoeal enterotoxin, 25(96.2%) were detected in *B. cereus* isolated from the pastry and 1(3.8%) from the minced meat (Table 4.6).

Similarly, the result of enterotoxin detection from *S. aureus* is presented in Appendix VII. Four Staphylococcal enterotoxin (SEA, SEB, SEC and SED) were detected in this study. Out of the 147 *S. aureus* isolates, 55 (37.7%) were found to produce the enterotoxins with SEA having the highest occurrence 32(52.46%) followed by SEC 19(31.15%), SEB 8(13.11%) and the least is SED 2(3.28%) (Table 4.7). Five (9.1%) out of the 55 *S. aureus* isolates produce more than one enterotoxin. SEA in combination with SEC has 3(60%) occurrence, SEA in combination with SEB and SEB in combination with SED has 1(20%) occurrence each.

Table 4.6: Detection of *B. cereus* diarrhoeal enterotoxin from *B. cereus* isolated from meat pie samples

	No. positive		No. of negative	
Local Govt	P (n=51)	M (n=35)	P (n=51)	M (n=35)
Dala	4	0	5	0
Fagge	3	0	2	3
Kumbotso	1	0	7	6
Gwale	5	0	3	3
Municipal	1	0	3	7
Nasarawa	6	0	5	2
Tarauni	1	0	6	4
Ungoggo	4	1	1	3
Total	25	1	32	28

Key:

P= Pastry

M= Minced meat

Table 4.7: Detection of Staphylococcal enterotoxins from *S. aureus* isolated from meat pie samples

	SEA		SEB		SEC		SED	
Local Govt	P (n=64)	M (n=83)	P (n=64)	M (n=83)	P (n=64)	M (n=83)	P (n=64)	M (n=83)
Dala	1	1	0	1	1	1	0	0
Fagge	0	3	0	0	0	0	0	0
Kumbotso	3	2	0	1	1	2	0	0
Gwale	2	2	0	0	0	3	0	0
Municipal	2	1	0	2	0	2	0	0
Nasarawa	2	4	0	2	2	3	0	1
Tarauni	1	4	0	0	0	2	0	0
Ungoggo	2	2	1	1	0	2	0	1

Total	13	19	1	7	4	15	0	2

Key:

SEA=Staphylococcal enterotoxin A

SEB= Staphylococcal enterotoxin B

SEC= Staphylococcal enterotoxin C

SED= Staphylococcal enterotoxin D

P=Pastry

M=Minced meat

CHAPTER FIVE

5.0 DISCUSSION

The *Bacillus* species isolated in this study were *B. cereus, B. megaterium, B. mycoid* and *B. thuriengiensis* as shown in Table 4.1. The presence of *Bacillus* species in street foods can be explained due to the fact that may be the ingredients such as wheat flour and spices used in the preparation contain spores of Bacillus. Presence of *Bacillus* species more specifically *B. cereus* in street foods is of great significance since the organism is known to produce many types of toxins, two of which are most frequently associated with food poisonings. The diarrhoeal enterotoxins that are destroyed when food is heated and the emetic enterotoxin toxin which is not inactivated by heating of food (Jay *et al.,* 2005; Granum, 2007).

Members of *Bacillus* species isolated in this study were similar to the species isolated by Rusul and Yaacob (1995) from rice noodles, wet noodles, spices, and grains, legumes and legumes products while Rath and Patra (2012) isolated slightly different species (*B. coagulans, B. subtilis, B. megaterium* and *B. cereus)* from street foods in India.

The frequency of *B. cereus* isolated from the pastry component was 59.3% which is higher than 40.7% isolated from the minced meat component of the meat pie as shown in Table 4.2. This may be due to contamination of agricultural products (wheat, wheat flour and spices) used in the preparation since *B. cereus* is widespread in nature and frequently isolated from soil and growing plants, the way the products are handled in an open air (Guinebretière *et al.,*2002). The re-packaging materials are also possible source of contamination.

The prevalence of *S. aureus* recorded in this study was 31.4% as shown in Appendix III and 43.5% of the *S. aureus* were detected from the pastry while 56.5% were detected from the minced

meat as shown in Table 4.3. From the results, it is clear that minced meat sample had more *S. aureus* contamination than the pastry. This may be because meat offers a rich nutrient media for microbial growth (Phillips, 2003).

Staphylococcus aureus is a normal flora of up to 30-50% of human population (Le loir *et al.,* 2003). It is able to grow at wide range of temperature (Schmitt *et al.,* 1990) and Sodium Chloride concentration of up to 15% (Le loir *et al.,* 2003). These characteristics enable *S. aureus* to grow in a wide variety of foods. This and there ecological niche can explain their incidence in food stuffs ((Le loir *et al.,* 2003). The presence of *S. aureus in* ready to eat foods depicts a deplorable state on the processing and packaging of these foods (Clarencel *et al.,* 2009).

The prevalence of *S.aureus* recorded in this study is lower than the findings of Ani *et al., (*2011) who reported 60% prevalence of *S. aureus* from meat pie and African salad in Abia (Nigeria) and 56.25% reported by Sina *et al.,* (2011) from street foods in Cotonou (Benin) and higher than the findings of Oh *et al.* (2007) in Korean ready to eat foods which yielded 8.6% *S. aureus.*

The resistance of *B. cereus* to amoxycillin (72.1%), cetriaxone (60.5%) and ceftazidime (80.2%) recorded in this study were the highest of all antibiotics as shown in Table 4.4. Resistance to β-lactam antibiotics is attributed to the production of β-lactamase by *B. cereus.* Resistance to amoxycillin, ceftazidime and ceftriaxone have been reported by Meena *et al.* (2000) in India, Whong and Kwaga (2007) in Nigeria, Chon (2012) in Korea and Rusul and Yaacob (1995) in Malaysia. The results show *B. cereus* resistance to ciprofloxacin (4.7%), gentamicin (0%) and vancomycin (24.4%) (Table 4.4) which is in conformity with the reports of Whong and Kwaga (2007) in Nigeria who reported 0%, 1% and 20% *B. cereus* resistance to ciprofloxacin, gentamicin and vancomycin respectively. Lee *et al.* (2004) also reported the susceptibility of *B. cereus* to ciprofloxacin, chloramphenicol, gentamicin and vancomycin. Erythromycin (19.8%) resistance of

B. cereus recorded in this study as shown in Table 4.4 is in conformity with the findings of Rusul and Yaacob (1995) who reported 22.6% *B. cereus* resistance to erythromycin. Sulphamethoxazole/trimethoprim (51.2%) resistance of *B. cereus* is also in conformity with 45% reported by Neslihan and Ebru (2014) in Turkey.

Ceftriaxone, amoxycillin and ceftazidime has the highest *S. aureus* resistance (64.6%, 58.5% and 35.4% respectively) as shown in Table 4.5. *S. aureus* resistance to cephalosporins (ceftriaxone and ceftazidime) is very common. Attien *et al.* (2007) reported 79% and 68% *S. aureus* resistance to ceftriaxone and amoxycillin. Daka *et al.* (2012) reported 23.1% *S. aureus* resistance to ceftazidime in south Ethiopia. Resistance of *S. aureus* to these antibiotics is due to the production of the enzyme β-lactamase which destroys the β-lactam ring of the antibiotic.

Ciprofloxacin, erythromycin, chloramphenicol, gentamicin, vancomycin and sulphamethoxazole/trimethoprim shows low levels of *S. aureus* resistance (9.5%, 6.1%, 8.2%, 2.0%, 6.1% and 12.2% respectively) (Table 4.5). These is similar to 5.8%, 0.8% and 5.8% for ciprofloxacin, erythromycin and chloramphenicol respectively reported by Aichi and Madubuike (2007) from street foods in Nigeria, 1.5% for gentamicin reported by Yucel *et al.* (2011) from foods in Turkey, 9.99% and 0% for sulphamethoxazole/trimethoprim and vancomycin respectively reported by Sina *et al.* (2011) from street food in Ghana.

The rate at which resistance occurs among microbial populations is often driven by the overuse and abuse of antimicrobial agents in many clinical settings (Khan *et al.,* 2005). Differences in susceptibility to antibiotics by microorganisms have become a major factor in drug choice and success of treatment. Great concerns have been raised regarding emerging antimicrobial resistance among bacteria that may result in unpredictable antimicrobial susceptibility and failure of therapy (Khameneh and Afshar, 2009). Some trends in the antimicrobial resistance

patterns and rates are universal, while others appear to be unique for specific regions (Irfan *et al.,* 2008).

The occurrence rate of diarrhoeal enterotoxigenic *B. cereus* recorded in this study was 30.2% as shown in Appendix VI. The spores of *B. cereus* are highly resistant to adverse environmental factors and the vegetative form starts to grow when cooked foods are exposed to warm temperature for prolonged period. The heat treatment induces germination of the spore and the vegetative cells then produce toxins. *B. cereus* produces many types of toxins, two of which are most frequently associated with food poisoning.

The presence of diarrhoeal enterotoxigenic *B. cereus* is of great significance since the toxin is known to cause diarrhea and abdominal cramps within the incubation period of 8-16 hours and symptoms persist for 12-24 hours. This result is in agreement to the findings of Reyes *et al.* (2006) who reported 29.8% prevalence of diarrhoeal enterotoxigenic *B. cereus* from pudding milk, milk with rice and milk-cereal-rice in Chilean School Feeding Program and lower than the findings of Rusul and Yaacob (1995) who reported 84% prevalence of diarrhoeal enterotoxigenic *B. cereus* from cooked foods (rice noodles, wet noodles, spices, and grains, legumes and legumes products) in Malaysia.

Staphylococcal enterotoxins detected in this study as shown in Appendix VII were SEA, SEB, SEC and SED. Out of the 147 *S. aureus* isolated, 54 (36.7%) were found to produce one or more of the toxins. *Staphylococcus aureus* is one of the leading causes of food-borne illnesses. *S. aureus* food-borne disease is a typical intoxication due to enterotoxins ingestion preformed in food by the enterotoxinogenic strain. These toxins are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. Ingestion of staphylococcal enterotoxins may cause symptoms such as diarrhea, vomiting,

nausea, loss of appetite, severe abdominal cramps and mild fever within 1-6 hours of incubation period and may persist for 24-48 hour. The illness is usually self-limiting and only occasionally it is severe enough to warrant hospitalization. The result of the present study is higher than the findings of Rosec *et al.* (1997) who reported 15.9% prevalence of Staphylococcal enterotoxins detected from *S. aureus* isolated from raw milk cheese in France and lower than 47% reported by Sina *et al*. (2011) from street foods in Cotonou and 43% reported by Rosec *et al.* (1997) from food stuffs in France.

The predominant toxin type is SEA with 32 (52.46%) prevalence followed by SEC 19 (31.15%), SEB (8 (13.11%) and the least was SED 2 (3.28%) (Table 4.7). This is in agreement with Sina *et al.* (2011) who reported the predominance of SEA enterotoxin from street foods. Balaban and Rasooly (2000) also reported the predominance of SEA genes. In contrast, Rosec *et al.* (1997) reported SEC as the predominant toxin in raw milk cheese and food stuffs. Statistical analysis showed that P=0.682 which is >0.05. This showed that there was no significant difference between the enterotoxins production by *S. aureus* within and across the local governments.

5.1 Conclusion

Bacillus cereus and *Staphylococcus aureus* are well established as causative agents of food poisoning. From the results obtained, it can be conclusively stated that the prevalence of *Bacillus cereus* (38.6%) and *Staphylococcus aureus* (31.4%) were high and some of these isolates produces enterotoxins (30.2% diarrhoeal enterotoxin produced by *Bacillus cereus* and 37.7% Staphylococcal enterotoxin produced by *Staphylococcus aureus*). These highlight the potential risk for consumers in the absence of strict hygienic and preventive measures to avoid the presence of *Bacillus cereus, Staphylococcus aureus* and their toxins in foods, emphasizing the

need for improved hygiene practice during processing, packaging, distribution and consumption of the food.

5.2 Recommendations

I. The results suggest the need to educate street food salesmen and the community on food hygiene to avoid food intoxication and subsequent diseases.

II. Dust and flies should be denied access to the meat pie as they may be good agent of bacterial transmission.

III. Continuous surveillance using susceptibility test should be carried out as antibiotic susceptibility profile varies from time to time.

REFERENCES

Achi, O.K and Madubuike, C.N. (2007). Prevalence and Antimicrobial Resistance of *S.aureus* Isolated from Retail Ready to eat Foods in Nigeria. *Research Journal of Microbiology* 2: 516-523

Agata, N., Ohta, M., Arakawa, Y. and Mosi, M. (1995). The bcet genes of *Bacillus cereus* encodes an enterotoxin protein. *Journal of Microbiol*ogy 141(4): 983-980.

Andersson, M. A., Hakulinen, P., Honkalampi-Hämäläinen, U., Hoornstra, D., Lhuquenot, J.C., Mäki-Paakkanen, J., Savolainen, M., Severin, I., Stammati, A. L., Turco, L.,Weber, A.,von Wright, A., Zucco, F. and Salkinoja-Salonen, M.(2007). Toxicological profile of cereulide, the *Bacillus cereus* emetic toxin, in functional assays with human, animal and bacterial cells. *Official Journal of the International Society on Toxinology* 49: 351-367.

Ani, O. C., Uhuo, A. C. and Nzenwa, N. J. (2011). The prevalence of *S. aureus* in food samples at selected areas of Aba, Abia state, Nigeria. *Journal of Biological Science.* 4.

Argudin, M. A., Mendoza, M. C. and Radicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins*.Toxins* 2(7): 1751-1773.

Attien, P., Sina, H., Moussaoui, W., Dadie, T., Chabi, S. K., Djeni, T., Bankole, H. S., Kotchoni, S. O., Edoh, V., Prevost, G., Dje, M. and Baba-Moussa, L. (2003). Prevalence and antibiotic resistance of Staphylococcal strains isolated from meat products sold in Abidjan street (Ivory Coast). *African Journal of Microbiology Reseach* 7(26): 3285-3293

Awny, N. M., Abou Zeid, A. A. M. and Mohamed Ahmed Abdo, M. A. (2010).Prevalence of toxigenic bacteria in some egyptian food. *Proceeding of fifth scientific environmental conference*. Zagazig University: 107 – 124.

Bailey, R. (2013). *Bacteria and food poisoning*. U.S. Center for disease control and prevention.1-4.

Balaban, N. and Rasooly, A. (2000). Staphylococcal enterotoxins. *International Journal of Food Microbiology* 61: 1-10.

Behling, R. G., Eifert, M. C., Gurtler, J. B., Kornacki, J. L., Line, E., Radcliff, R., Ryser, E. T., Stanwick, B. and Yan, Z. (2010). Selected pathogens of concern to industrial food processors: infectious, toxigenic,toxico-infectious, selected emerging pathogenic bacteria. *Principles of Microbiology Trouble Shooting in the Industrial Food Processing Environment, Food Microbiology and Food Safety* 1-48.

Bergdoll M. S., Borja C .R., Robbins R. N., Weiss K. F.(1989). Identification of enterotoxin E. *Infectious Immunology* 4: 593–595.

Becker, K., A. W. Friedrich, G. Lubritz, M. Weilert, G. Peters, and C. Von Eiff. (2003). Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *Journal of Clinical Microbiology* 41:1434-1439.

Becker, K., A. W., Friedrich, G., Peters, A. and von Eiff, C. (2004). Systematic survey on the prevalence of genes coding for staphylococcal enterotoxins SElM, SElO, and SElN. Mol. *Nutritional Food Reseach* 48:488-495.

Belkum, A., Melles, D. C., Snijders S. V., van Leeuwen, W. B. . Wertheim, H. F. L., Nouwen, L. J., Verbrugh, H. A. and Etienne , J. (2006). Clonal Distribution and Differential Occurrence of the Enterotoxin Gene Cluster, *egc*, in Carriage- versus Bacteremia-Associated Isolates of *Staphylococcus aureus. Journal of Clinical Microbiology* 44(4): 1555–1557.

Betley, M. J., Borst, D. W. and Regassa, L. B. (1992). Staphylococcal enterotoxins, toxic shock syndrome toxin and Streptococcal pyogenic exotoxins:a comparative study of their molecularBiologicallogy. *Chemical Immunology* 1-35.

Bukar, A., Yushau, M., Adikwu, E. M. (2009). Incidence and identification of potential pathogens on hands of some personnel in some small scale food industries in Kano Metropolis. Nigeria.*Biologicall. Environmental Science Journal of Tropical Countries* 6 :4.

Bukar, A., Uba, A. and Oyeyi, T. I. (2010). Occurance of some enteropathogenic bacteria in some ready to eat foods in Kano metropolis, Nigeria. *African Journal of Food Science* 4 (2): 32-36.

Buzby, J. C. and Robert, T. (1997). Economic cost and trade impacts of microbial food borne illness. *World Health Statistics* 50: 57-66.

Cheesbrough, M. (2004). *District laboratory practice in tropical countries*, 2nd edition. Cambridge University press, U.K. 178-187.

Chen, T. R., Chiou, C. S and Tsen, H. T. (2004). Use of Novel PCR Primers Specific to the Genes of Staphylococcal Enterotoxin G, H, I for the survey of staphylococcus aureus strains Isolated from Food Poisoning Cases and Food Samples in Taiwan. *International Journal of Food Microbiology* 95(2): 189-197.

Chon, J. W., Kim, J. H., Lee, S. J., Hyeon, J. Y. and Seo, K. H. (2012). Toxin profile, antibiotic resistance, phenotypic and molecular characterization of *B. cereus* in Sunsik. *Journal of Food Microbiology* 32(1): 217-222.

Clarence, S.Y., Obinna, C.N. and Shalom, N.C. (2009). Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin city metropolis, Nigeria. *African Journal of Microbiology Research* 3(6): 390-395.

Clinical Laboratory Standards Institute (2006). *Performance standard for antimicrobial disk susceptibility test; Approved standard 9th edition. CLSI document M2-A9*. Clinical Laboratory standards Institute, Wayne, PA.

Daka, D**., G/silassie, S.** and **Yihdego, D. (**2012). Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia .*Analytical Clinical Microbiology and Antimicrobials* **11**:26.

Deizelles, S. Dilasser, F., Duquenne, M. and Deperrois, V. (2009). Differential temporal expression of the Staphylococcal enterotoxin gene during cell growth. *Journal of Food Microbiology* 26: 896-904.

Dinges, M.M., Orwin, P.M. and Schlievert, P.M. (2000). Exotoxins of *Staphylococcus aureus*.*Clinical Microbiology Reviews* 13: 16–34.

Duff, S. B., Scott, E.A., Mastilios, M.S., Todd, E.C., Krilov, L.R.G., Eddes, A.M., Acknerman, S.J. (2003). Cost effectiveness of a target disinfection program in household kitchens to prevent food borne illnesses in the United States, Canada and the United Kingdom. *Journal of Food Protection* (11): 2104 – 2156.

Durand, G., Bes, M., Meugnier, H., Enright, M.C, Forey, F., Liassine, N., Wenger, A., Kikuchi, K., Lina, G., Vandenesch, F. and Etienne, J.(2006) Detection of new methicillin-resistant *Staphylococcus aureus* clones containing the toxic shock syndrome toxin 1 gene responsible for hospital- and community-acquired infections in France. *Journal of Clinical Microbiology* 44(3):847-53.

Fagerlund A., Ween A., Lund T., Hardy S.P. and Granum P.E. (2004). Genetic and functional analysis of the *cytK* family of genes in *Bacillus cereus*. *Microbiology* 150: 2689-2690.

Finberg, R.W., Moellering, R.C., Tally, F.P. (November 2004). The importance of bactericidal drugs: future directions in infectious disease. *Clinical Infectious Diseases* 39 (9): 1314–20.

Fitzgerald, J. R., Sturdevant, D.E., Mackie, S.M., Gill, S.R. and Musser, J.M. (2001). Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic*. Proceeding of the National Academy of Sciences of the United States of America* 98(15): 8821-8826.

Food and agriculture organization of United states (1979). *Manual of food quality control for microbiological analysis*. Graw Hill Publishers USA. 840-841.

Food and Agricultural Organisation. (1989). *Street foods*. A summary of FAO studies and other activities relating to street foods. Rome; FAO. 122-145

Forsythe, S. J. (2000). Basic aspects. *In: The microbiology of safe food* 2nd edition. Blackwell Science, USA. 10-52.

Foster, T. (1996). Chapter 12 Staphylococcus, *Medical Microbiology* 4th Edition. University of Texas Medical Branch at Galveston.

Geny, B. and Popoff, M. R. (2006). Bacterial protein toxins and lipid pore formation or toxin entry into cells Biology*. Cell.* 98: 667-678.

Granum, P. E. (1994). *Bacillus cereus* and its toxins. *Journal of Bacteriology Symposium Supplement* 76:61S-66S.

Griffiths, M. W. and Schraft, H. (2002). *Bacillus cereus food poisoning. In: Foodborne diseases*, edited by Clicer D. O. and Riemann H. P. Academic press, California USA. 261-270

Guinebretière, M-H., Broussolle, V. and Nguyen-The, N. (2002). Enterotoxigenic profiles of food-poisoning and food-borne *Bacillus cereus* strains. *Journal of Clinical Microbiology* 40: 3053-3056

Hazariwala, A., Sanders, Q., Hudson, C. R., Hofacre ,C., Thayer, S .G., Mauer, J. (2002). Distribution of Staphylococci enterotoxin genes among *Staphylococcus aureus* isolates from poultry and humans with invasive Staphylococcal disease. *Avian Diseases* 46(1): 132-136.

Helgason, E., Okstad, O.A., Caugant, D. A., Johansen, H. A., Fouet, A., Mock, M., Hegna, I. and Kolsto A. B. (2000). *Bacillus anthracis, Bacillus cereus* and *Bacillus thuringiensis* - one species on the basis genetic evidence. *Applied and Environmental Microbiology* 66: 2627-2630.

Holt, J.G., Frieg, N. R., Sneath, P.H.A., Staley, S.T. and Williams, S.T. (1994). Genus Bacillus. In Bergey´s manual: *Determinative bacteriology* 9th edition. Edited by Hensyl W.R. Baltimore, USA Williams & Wilkins. 559-564

Holtfreter, S., K. Bauer, D. Thomas, C. Feig, V. Lorenz, K. Roschack, E. Friebe, K. Selleng, S. Lovenich, T. Greve, A. Greinacher, B. Panzig, S. Engelmann, G. Lina and B. M. Broker. (2004). *egc*-encoded superantigens from *Staphylococcus aureus* are neutralized by human sera much less efficiently than are classical staphylococcal enterotoxins or toxic shock syndrome toxin. *Infectious Immunology* 72: 4061-4071.

Ikeda, T., Tamate, N., Yamaguchi, K. and Makino, S. (2005). Mass Outbreak of Food Poisoning Disease Caused by Small Amount of Staphylococcal Enterotoxin A and H. *Applied and Environmental Microbiology* 71: 2793-2795. `

Irfan, S., Idrees, F., Mehraj,. V, Habib, F., Adil, S. and Hasan, R. (2008). Emergence of Carbapenem resistant Gram negative and vancomycin resistant Gram positive organisms in bacteremic isolates of febrile neutropenic patients: a descriptive study. *Infectious Diseases* 8 (80): 2334-80.

Jaaskelainen, E. (2008). Assessment and control of *Bacillus Cereus* emetic toxin in food. *A dissertation submitted to Department of Applied Chemistry and Microbiology University of Helsinki* 1-59

Jay, J.M., Loessner, M.J. and Golden, A.D (2005). *Bacillus cereus* gastroenteritics. *Modern food microbiology*, 7th edition. Springer science + Business media, Inc., New York. USA. 583-590.

Jensen, G.B., Hansen, B.M., Eilenberg, J and Mahillon, J. (2003). The hidden lifestyles of *Bacillus cereus* and relatives. *Minireview in Environmental Microbiology* 5: 631-640

Khameneh, Z. R. and Afshar, A. T. (2009) Antimicrobial susceptibility pattern of urinary tract pathogens. *Saudi Journal of Kidney Diseases Transplant* 20(2): 251-253.

Khan, S., Gupta, D. K. and Khan, D. N. (2005). Comparative study of three antimicrobial drugs protocol (Ceftriaxone, Gentamicin/Amikacin and Metronidazole) versus two antimicrobial drugs protocol (Ceftriaxone and Metronidazole) in cases of intra-abdominal sepsis. *Kathmandu University Medical Journal (KUMJ)* 3(1): 55-63.

Larkin, E.A., Carman, R.J., Krakauer, T. and Stiles, B.G. (2009.). *Staphylococcus aureus*: the toxic presence of a pathogen extraordinaire. *Current Medical Chemistry* 16: 4003–4019.

Le loir, Y., Baron F. and Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. *Journal of Genetic and Molecular Research* 2: 63-76

Lechner, S. Mayr, R.; Francis, K. P. Pruss, B. M. Kaplan, T. Wiessner Gunkel, E. Stewart, G. S., and Scherer, S. (1998). *Bacillus weihenstephanensis* sp. nov. is a new psychrotolerant species of the *Bacillus cereus* group. *International Journal of Systematic Bacteriology* 48(4): 1373-1382.

Lee, J., Wallace, S. and Wong-Beringer, A. (2004). Persistent *B. cereus* Bacteremia in an immunocompetent host. *Infectious Diseases in Clinical Practice* 12 (5): 294-296.

Ler, S. G., Lee, F. K. and Hopalakrishnakone, P. (2006). Trends in Detection of warfare Agents. Detection methods for Ricin, Staphyloccal enterotoxin B and T – 2 toxin. *Journal of Chromatography* 1133 (1-2): 1-12.

Levy, S. B. (1998).Certain bacterial infections now defy all antibiotics. The resistance problem may be reversible, but only if society begins to consider how the drugs affect "good" bacteria as well as "bad". *The Challenge of Antibiotic Resistance*. Scientific American Archive. 46-53

Lund, T. and Granum, P. E. (1996). Characterisation of a non-haemolytic enterotoxin complex from *Bacillus cereus* isolated after a foodborne outbreak. *Federation of European Microbiological Societies* 141(2/3): 151-156.

Lund, T.; DeBuyser, M. L. and Granum, P. E. (2000). A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. *Molecular Microbiology.* 38(2): 254-261.

Mead, P.S., Slutskker, L., Dietz, V., mccaig, L.f, Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe, R. (1999). Food related illness and Death in the United States. *Emerging Infectious Diseases* 5: 607-625.

Meena, B. S., Kapoor, K. N. and Agarwal, R. K. (2000). Occurance of multi-drug resistant *Bacillus cereus* in foods. *Journal of Food Science and Technology (Mysore)* 37(3): 289-291.

Mellon, M., Benbrook, C. and Benbrook, K. L. (2001).Estimates of Antimicrobial Abuse in Livestock. *Hogging It.* Union of concerned scientists. 1-14

Moravek M., Buerk C., Broussolle V., Guinebretiere M-H., Granum P.E., Nguyen-The C. and Märtlbauer E. 2006. Determination of the toxin potential of *Bacillus cereus* isolates by quantitative enterotoxin analyses. *Federation of European Microbiological Societies* 257: 293-298.

Mortimer, P. R. and McCann, G. (1974). Food-poisoning episodes associated with *Bacillus cereus* in fried rice. *London, England* 1(7865): 1043-1045.

Nakamura, L. K. (1998). *Bacillus pseudomycoides* sp. nov. *International Journal of Systematic Bacteriology* 1031-1035.

National Institute of Health (2004). National center forBiologicaltectechnology information. *U.S National Lirary of Medicine.*

Neslihan, G., Ebru, A. (2014). Occurance and antibiotic resistance of *E. coli, S. aureus* and *B. cereus* in raw milk and diry products in Turkey. *International journal of Dairy Technology* 67(4): 562-569.

Nester, E. W., Anderson, D. G., Roberts,C.E.P. and Nester. (2004). *Microbiology: A human perspective* 4th edition. Mcgraw Hills. 610-628.

Niskanen, T., Johansson, T., Siitonen, A. and Kuusi, M. (2007). Ruokamyrkytykset Suomesa vuonna. *Eviran Julkaisuja* 15-28

Oh, S., Lee, N., Cho, Y.S., Shin, D., Choi, S. Y. and Koo, M. (2007). Occurance of toxigenic *S. aureus* in ready to eat foods in Korea. *Journal of Food Protection* 70 (5): 1153-1158.

Ogston, A. (1883). Micrococcus poisoning. *Journal of Analytical Physiology* 17: 24-58.

Oxoid limited (2013). Toxin detection kits: *Oxoid Microbiology Products*. Themo fisher scientific inc.

Pagani, L., Migliavacca, R., Pallecchi, L., Matti, C., Giacobone, E., Amicosante, G., Romero, E. and Rossolini, G. M. (2000). Emerging extended spectrum beta lactamases in protes mirabilis. *Journal of Clinical Microbiology* 40: 1549-1552.

Panneerseelan, L. (2008). Detection of *S. aureus* Enterotoxins and Enterotoxin producing strains. *A dissertation submitted to the faculty of the Graduate College of Oklahoma State University* 1-127

Phillips, M. (2003). *Analysis of Microbial Hazards related to time/temperature control of foods for safety; Comprehensive Review in Food Science and Food safety* 2: 33-35.

Pinchuk, I. V., Bes wick, E. J. and Reyes, V. E. (2010). Staphylococcal Enterotoxin. *National Center for Biological technology Information, U.S National Library of Medicine.*

Pirttijärvi, T. (2000). Contaminant aerobic sporeforming bacteria in the manufacturing processes of food packaging board and food. *DissertationesBiologicalcentri Viikki Universitatis Helsingiensis*, Finland.

Popoff, M. R. (2011). Multi faceted Interactions of Bacterial Toxins with the Gastrointestinal Mucosa. *Future Microbiology* 6 (7): 763 – 797.

Public Health Agency of Canada (2001). Office of Laboratory Security. *Material Safety Data Sheet: Staphylococcus aureus.*

Rath, C. C. and Patra, S. (2012). Bacteriological Quality Assessment of Selected Street Foods and Antibacterial Action of Essential Oils Against Food Borne Pathogens. *Journal of Food Safety* 14: 5-10

Rapid test methods (2013). Detection of Bacterial Toxins in Food. *Rapid Microbiology focued on Microbiology.* thermoscience. 1-7.

Reyes, J. E., Bartias, J. M., Gutierrez, M. R. and Rodriguez, M. (2007). Prevalence of *B. cereus* in dried milk products used by Chilean Feeding Program. *Journal of Food Microbiology* 3(37): 1-6.

Roberts, T. A., Baird-Parker, A. C. and Tompkin, R. B. (1996). *Bacillus cereus*. *In: Microorganisms in Foods.* Microbiological Specification of Food Pathogens Blackie Academic & Professional Great Britain. 20-35

Rosec, J. P., Guiraud, J. P., Dalet, C. and Richard, N. (1997). Enterotoxin production by Staphylococci isolated from foods in France. *International Journal of food Microbiology* 35(3): 213-221.

Rusul, G. and Yaacob, N. H. (1995). Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. *International Journal of Food Microbiology* 25(2):131-139

Schmid, D., Fretz, R., Winter, P., Mann, M., Höger, G., Stöger, A., Ruppitsch, W., Ladstätter, J., Mayer, N., de Martin, A., Allerberger, F. (2009). Outbreak of staphylococcal food intoxication after consumption of pasteurized milk products, June 2007, Austria. *Wien. Klin. Wochenschr* 121:125–131.

Sina, H., Baba-Moussa, F., Kayode, A. P., Noumava, P. A., Sezan, A., Hounhouigan, J. D., Kotchoni, S. O., Prevost, G. and Baba-Moussa, L. (2011). Characterization of *S. aureus* isolated from street foods: Toxin profile and prevalence of antibiotic resistance. *Journal of Applied Biological Science* 46:3133-3143.

Stenfors, A., Fagerlund, A. and Granum, P. E. (2008). From soil togut *Bacillus cereus* and its food poisoning toxins. *Federation of European Microbiological Societies* 32(4): 579-606.

Stoppler, M. C. (2011). What is Staphylococcal food poisoning. *Med. Net. Com.*119-120.

Talaro, K. and Talaro, A. (1996). *Foundations in Microbiology* 2nd Edition Mc- Graw Hill Publishers USA; 840-841.

Tallent, S. M., Rhodehamel, E. J., Harmon, S. M. and Bannett, R. W. (2012). BAM: *Bacillus cereus. Bacteriological Analytical Manual Chapter 14.* U.S. Department of Health and Human Services.

Todar, K. (2008). The genus Bacillus. *Todar's Online text book of Bacteriology*. Madison, Wisconsin.1-6.

Torok, T. J., Tauze, R. V., Wise, R. P., Livengood, J. R., Sokolow, R. and Manvans, S. (1997). A large community outbreak of Salmonellosis caused by International contamination of a restaurant salads /bars. *Journal of American Medical Association* 278(8): 389-395.

Tsang, D. (2002). *Microbiological guidelines for ready to eat food Road and Environmental Hygiene department Hong Kong.* 115-116.

United State Department of Food Agriculture (1998). *Bacteriology-identification*. Identification of Bacillus species.Public health agency New york: 3. 1-27.

Vammam, A. H. and Evans, M. G. (1991). Food poisoning: *Medical and Microbiological overview; Bacillus. In foodborne pathogens, an illustrated text*, London, UK. 267-288

Weingartner, L. (2004). The concept of food and Nutrition Security. *Food and Nutrition security assessment instruments and intervention strategies.*1-14.

Whong, C. M. and Kwaga, J. K. (2007). Antibiogram of *Bacillus cereus* isolates from some Nigerian foods. *Nigerian Food Journal* 25(1): 178-183.

Wijnand, L. M., Dufrenne, J. B. and vanLeusden, F. M. (2003). Characterization of *Bacillus cereus*. *Quantitative research of Bacillus cereus within the scope of hazard characterisation and exposure assessment.* Health Protection and Veterinary Public Health, Nertherland.

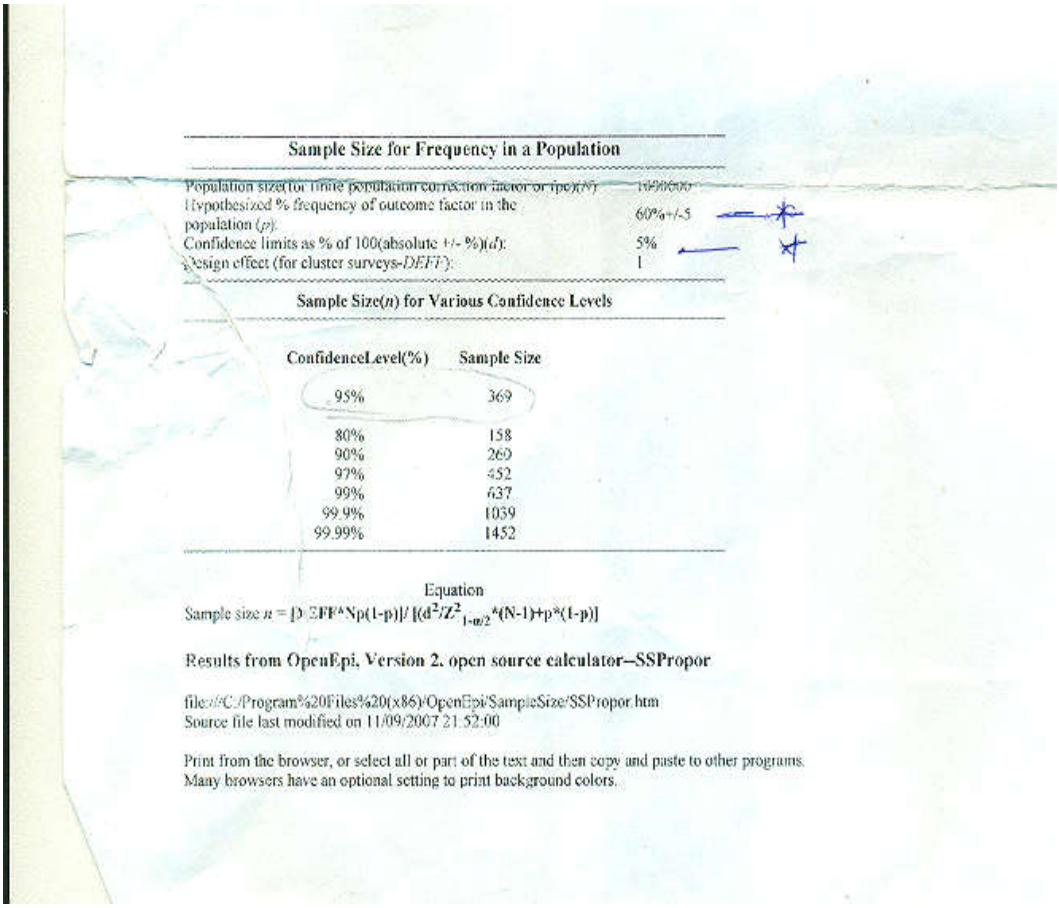
Wijnand, L. M., Dufrenne, J. B., van Leusden, F. M. and Abee, T. (2007). Germination of *Bacillus cereus* spores is induced by germinants from differentiated Caco-2 cells, a human cell line mimicking the epithelial cells of the small intestine. *Applied and Environmental Microbiology* 5052-5054

Willey, J. M., Sherwood, L. M. and Woolverton, C. J. (2008). *Prescott, Harley, and Klein's Microbiology, 7th edition*. McGraw Hill New York. 980-981.

Yucel, N., Citak, S. and Bayhun, S. (2011). Antimicrobial resistance profile of *S. aureus* isolated from clinical samples and foods of animal origin. *Foodborne Pathogenic Diseases* 8(3): 427-431.

Zhang, S. Iandolo, J., and Stewart, G. C. (1998). The Enterotoxin D Plasmid of *Staphylococcus aureus* Encodes a Second Enterotoxin Determinant (Sej). *Federation of European Microbiological Societies* 168: 227 – 233.

APPENDIX I



Sample Size for Frequency in a Population

Population size(for finite population correction factor or fpc)(*N*):	16000000
Hypothesized % frequency of outcome factor in the population (*p*):	60%+/-5
Confidence limits as % of 100(absolute +/- %)(*d*):	5%
Design effect (for cluster surveys-*DEFF*):	1

Sample Size(*n*) for Various Confidence Levels

ConfidenceLevel(%)	Sample Size
95%	369
80%	158
90%	260
97%	452
99%	637
99.9%	1039
99.99%	1452

Equation

Sample size $n = [DEFF*Np(1-p)] / [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

Results from OpenEpi, Version 2, open source calculator--SSPropor

file://C:/Program%20Files%20(x86)/OpenEpi/SampleSize/SSPropor.htm
Source file last modified on 11/09/2007 21:52:00

Print from the browser, or select all or part of the text and then copy and paste to other programs.
Many browsers have an optional setting to print background colors.

APPENDIX II

Biochemical tests for the identification of Bacillus species isolated from meat pie samples

Sample name	Catalase	Man	Lec	Heam	Mot	Lac	Prot	Rhi
D6B	+	-	+	+	++	-	-	-
D7P	+	-	+	+	++	-	-	-
D9B	+	-	+	+	-	-	-	+
D10P	+	-	+	+	++	-	-	-
D11B	+	-	+	+	+++	-	-	-
D19P	+	-	+	+	++	-	-	-
D22P	+	-	+	+	++	-	-	-
D23B	+	-	+	+	-	-	-	+
D29P	+	-	+	+	++	-	+	-
D29P	+	+	-	-	+	+	-	-
D32P	+	-	+	+	++	-	+	-
D37B	+	-	+	+	+++	-	-	-
D39B	+	-	+	+	++	-	-	-
F1B	+	+	-	-	+	+	-	-
F8B	+	-	+	+	++	-	-	-
F9P	+	-	+	+	++	-	-	-
F9P	+	+	-	-	+	+	-	-
F11B	+	-	+	+	++	-	-	-
F11B	+	+	-	-	+	+	-	-
F13P	+	-	+	+	+	-	+	-
F15P	+	-	+	+	-	-	-	+
F17P	+	-	+	+	++	-	-	-
F17B	+	+	-	-	+	+	-	-

F20P	+	-	+	+	++	-	-	-
F24P	+	-	+	+	+++	-	-	-
F26P	+	-	+	+	-	-	-	+
F28P	+	-	+	+	+	-	+	-
F30B	+	+	-	-	+	+	-	-
F39P	+	-	+	+	+	-	+	-
F39P	+	-	+	+	++	-	-	-
F39P	+	+	-	-	+	+	-	-
F40B	+	-	+	+	++	-	-	-
F40B	+	+	-	-	+	+	-	-
K1P	+	-	+	+	+	-	+	-

Sample name	Catalase	Man	Lec	Heam	Mot	Lac	Prot	Rhi
K5P	+	+	-	-	+	+	-	-
K6P	+	-	+	+	++	-	-	-
K7B	+	-	+	+	-	-	-	+
K8B	+	-	+	+	-	-	-	+
K9B	+	-	+	+	+++	-	-	-
K10P	+	-	+	+	+	-	+	-
K10P	+	+	-	-	+	+	-	-
K14P	+	-	+	+	++	-	-	-
K15B	+	-	+	+	++	-	-	-

K16P	+	-	+	+	+	-	+	-
K16P	+	+	-	-	+	+	-	-
K16B	+	-	+	+	++	-	-	-
K17P	+	-	+	+	++	-	-	-
K20P	+	-	+	+	++	-	-	-
K24P	+	-	+	+	+	-	+	-
K25B	+	+	-	-	+	+	-	-
K26B	+	-	+	+	+++	-	-	-
K29P	+	-	+	+	++	-	-	-
K30P	+	-	+	+	++	-	-	-
K36B	+	-	+	+	-	-	-	+
K37B	+	-	+	+	++	-	-	-
K42P	+	-	+	+	++	-	-	-
K42B	+	-	+	+	++	-	-	-
K43P	+	-	+	+	++	-	-	-
G1B	+	-	+	+	++	-	-	-
G7P	+	-	+	+	++	-	-	-
G7B	+	-	+	+	++	-	-	-
G10P	+	-	+	+	++	-	-	-
G11P	+	-	+	+	+++	-	-	-
G11B	+	+	-	-	+	+	-	-
G15B	+	+	-	-	+	+	-	-
G18P	+	-	+	+	++	-	-	-
G20P	+	-	+	+	++	-	-	-
G22P	+	-	+	+	+	-	+	-

G27P	+	-	+	+	++	-	-	-
G27P	+	+	-	-	+	+	-	-
G29P	+	-	+	+	+	-	+	-
G32P	+	+	-	-	+	+	-	-

Sample name	Catalase	Man	Lec	Heam	Mot	Lac	Prot	Rhi
G33P	+	-	+	+	++	-	-	-
G34P	+	+	-	-	+	+	-	-
G35P	+	-	+	+	++	-	-	-
G38B	+	+	-	-	+	+	-	-
G45B	+	-	+	+	++	-	-	-
U4P	+	-	+	+	+++	-	-	-
U12P	+	-	+	+	++	-	-	-
U14B	+	-	+	+	-	-	-	+
U15P	+	-	+	+	-	-	-	+
U16P	+	-	+	+	+++	-	-	-
U23B	+	-	+	+	++	-	-	-
U26B	+	-	+	+	+	-	+	-
U28B	+	+	-	-	+	+	-	-
U30P	+	-	+	+	+	-	+	-
U31P	+	-	+	+	+	-	+	-
U31B	+	-	+	+	++	-	-	-
U36P	+	-	+	+	+	-	-	-
U36B	+	-	+	+	++	-	-	-

U37P	+	+	-	-	+	+	-	-
U38B	+	-	+	+	++	-	-	-
U42P	+	-	+	+	++	-	-	-
U44B	+	+	-	-	+	+	-	-
M4B	+	-	+	+	+++	-	-	-
M9P	+	-	+	+	+	-	+	-
M10P	+	+	-	-	+	+	-	-
M12P	+	-	+	+	+	-	+	-
M13B	+	-	+	+	++	-	-	-
M18P	+	-	+	+	+	-	+	-
M19B	+	-	+	+	++	-	-	-
M19B	+	-	+	+	-	-	-	+
M21P	+	-	+	+	++	-	-	-
M25P	+	+	-	-	+	+	-	-
M25P	+	-	+	+	+	-	+	-
M26P	+	-	+	+	++	-	-	-
M28P	+	+	-	-	+	+	-	-
M29B	+	-	+	+	++	-	-	-
M29B	+	+	-	-	+	+	-	-
M30B	+	-	+	+	-	-	-	+
M34P	+	-	+	+	-	-	-	+

Sample name	Catalase	Man	Lec	Heam	Mot	Lac	Prot	Rhi
M37B	+	-	+	+	+++	-	-	-
M38P	+	-	+	+	++	-	-	-

M38B	+	-	+	+	++	-	-	-
M43B	+	-	+	+	++	-	-	-
M44P	+	-	+	+	++	-	-	-
M44P	+	+	-	-	+	+	-	-
N1P	+	-	+	+	-	-	-	+
N5B	+	-	+	+	++	-	-	-
N12P	+	-	+	+	++	-	-	-
N17P	+	-	+	+	++	-	-	-
N20P	+	-	+	+	++	-	-	-
N21P	+	-	+	+	++	-	-	-
N21B	+	+	-	-	+	+	-	-
N22P	+	-	+	+	++	-	-	-
N25P	+	-	+	+	+	-	+	-
N25B	+	-	+	+	++	-	-	-
N29P	+	-	+	+	++	-	-	-
N33P	+	-	+	+	+++	-	-	-
N34B	+	-	+	+	+	-	+	-
N37P	+	-	+	+	++	-	-	-
N38P	+	-	+	+	++	-	-	-
N41P	+	-	+	+	++	-	-	-
N42P	+	-	+	+	++	-	-	-
T4P	+	-	+	+	++	-	-	-
T6B	+	-	+	+	-	-	-	+
T7P	+	-	+	+	++	-	-	-
T8B	+	+	-	-	+	+	-	-

T15B	+	-	+	+	+++	-	-	-
T16P	+	-	+	+	+	-	+	-
T16P	+	+	-	-	+	+	-	-
T16B	+	+	-	-	+	+	-	-
T18P	+	-	+	+	++	-	-	-
T18B	+	-	+	+	++	-	-	-
T18B	+	+	-	-	+	+	-	-
T24P	+	-	+	+	++	-	-	-
T27P	+	-	+	+	+++	-	-	-
T29B	+	-	+	+	+	-	+	-
T35P	+	+	-	-	+	+	-	-
T35P	+	-	+	+	++	-	-	-

Sample name	Catalase	Man	Lec	Heam	Mot	Lac	Prot	Rhi
T36P	+	-	+	+	-	-	-	+
T37B	+	+	-	-	+	+	-	-
T39P	+	-	+	+	+	-	+	-
T40P	+	-	+	+	++	-	-	-
T43B	+	-	+	+	++	-	-	-
T44B	+	-	+	+	+++	-	-	-

Key:

Man= Mannitol fermentation

Lec= Lecithinase reaction

Heam= Heamolytic reaction

Mot= Motility test

Lac= Lactose fermentation

Prot= Protein crystal test

Rhi= Rhizoid growth test

APPENDIX III

Biologicalchemical tests for the identification of *S. aureus* isolated from meat pie samples

Sample name	Catalase test	Coagulase test
U4B	+	+
U8B	+	-
U9P	+	-
U10P	+	+
U10B	+	+
U12P	+	-
U13P	+	-
U14P	+	-
U14B	+	+
U15P	+	-
U16B	+	+
U18P	+	-
U18B	+	-
U19P	+	-
U19B	+	+
U23P	+	+
U24P	+	+
U25P	+	-
U25B	+	+
U25B	+	-
U26P	+	-
U26B	+	-
U27P	+	+

U27B	+	-
U28P	+	-
U28B	+	-
U29P	+	-
U30B	+	+
U32P	+	-
U32P	+	-
U33P	+	-
U33B	+	+
U34P	+	-
U34B	+	-
U35P	+	+
U35B	+	+
U36P	+	+

Sample name	Catalase test	Coagulase test
U36B	+	+
U38B	+	+
U39P	+	-
U39B	+	+
U40B	+	-
U41B	+	-
U42P	+	+
U42B	+	+
U45B	+	-

M1P	+	-
M1B	+	-
M2B	+	-
M4B	+	+
M7P	+	-
M9P	+	-
M9B	+	-
M10P	+	-
M12P	+	+
M12B	+	+
M13P	+	+
M14B	+	+
M15B	+	+
M16B	+	-
M17B	+	+
M18B	+	+
M19B	+	+
M20B	+	+
M21P	+	+
M21B	+	-
M22P	+	-
M22B	+	+
M23P	+	+
M24P	+	+
M24B	+	-

M25P	+	+
M26P	+	-
M26B	+	+
M27P	+	-
M27B	+	-
M28P	+	-
M28P	+	-

Sample name	Catalase test	Coagulase test
M28B	+	-
M29P	+	-
M29B	+	-
M30P	+	-
M30B	+	-
M31P	+	-
M31B	+	-
M32P	+	-
M33B	+	-
M34P	+	+
M35P	+	+
M35B	+	-
M36P	+	-
M36B	+	+
M36B	+	-
M37P	+	+

M37B	+	-
M38B	+	-
M39P	+	+
M39B	+	+
M40P	+	-
M40B	+	-
M41P	+	+
M43P	+	+
M45P	+	-
M45B	+	-
G1B	+	+
G5P	+	-
G5B	+	-
G6P	+	-
G6B	+	+
G9P	+	+
G10P	+	+
G10B	+	-
G11P	+	+
G13P	+	-
G17P	+	-
G18P	+	-
G19P	+	-
G21P	+	+
G21B	+	+

Sample name	Catalase test	Coagulase test
G22P	+	+
G22B	+	+
G23B	+	+
G24B	+	-
G27B	+	-
G28P	+	-
G28P	+	-
G28B	+	-
G29B	+	+
G30P	+	-
G30B	+	-
G31P	+	+
G31B	+	+
G32P	+	+
G32B	+	-
G33P	+	-
G33B	+	-
G34P	+	-
G35B	+	+
G36B	+	-
G37P	+	+
G38P	+	+
G39P	+	-

G39B	+	-
G40P	+	-
G42P	+	-
G43P	+	-
G43B	+	-
G45B	+	+
K1P	+	+
K1B	+	+
K2P	+	+
K2B	+	+
K3B	+	+
K4B	+	-
K7B	+	-
K8P	+	-
K9P	+	-
K9B	+	+
K11P	+	-
K11P	+	-

Sample name	Catalase test	Coagulase test
K11B	+	-
K12P	+	-
K12B	+	+
K13P	+	-
K13B	+	-

K15P	+	+
K15B	+	-
K17P	+	-
K17B	+	-
K18P	+	-
K18B	+	+
K19P	+	-
K19B	+	-
K22P	+	+
K22B	+	+
K24P	+	+
K24B	+	+
K26P	+	+
K26B	+	-
K27P	+	-
K27B	+	+
K28B	+	-
K30P	+	+
K30B	+	+
K31P	+	+
K31B	+	+
K35B	+	-
K36P	+	-
K37P	+	+
K37B	+	-

K39P	+	-
K39P	+	-
K39B	+	-
K40P	+	-
K40B	+	+
K41P	+	-
K41B	+	-
K43P	+	+
K43B	+	+
K44P	+	+
K44B	+	+

Sample name	Catalase test	Coagulase test
N1B	+	+
N5B	+	+
N7B	+	+
N8B	+	+
N12B	+	+
N18P	+	+
N18B	+	+
N19P	+	-
N19B	+	-
N20P	+	-
N21P	+	-
N22P	+	+

N22B	+	+
N23P	+	+
N26P	+	+
N26B	+	+
N28P	+	-
N29P	+	-
N30P	+	-
N32P	+	-
N32B	+	-
N33P	+	-
N34B	+	-
N35P	+	-
N36B	+	-
N39P	+	+
N39B	+	+
N40P	+	-
N40B	+	+
N41P	+	+
N41B	+	+
N42P	+	+
N42B	+	+
N43B	+	+
N45B	+	-
T4P	+	-
T6B	+	-

T10P	+	-
T11B	+	+
T15P	+	+
T15B	+	-

Sample name	Catalase test	Coagulase test
T16B	+	-
T17P	+	-
T17B	+	-
T18P	+	-
T18B	+	-
T19P	+	-
T19P	+	-
T19B	+	-
T20B	+	+
T21B	+	+
T22B	+	-
T22B	+	-
T24P	+	+
T26P	+	-
T26B	+	-
T27B	+	-
T28P	+	-
T28B	+	-
T29B	+	-

T31B	+	+
T34P	+	-
T36P	+	-
T36B	+	-
T37P	+	-
T39P	+	+
T39B	+	+
T40P	+	+
T40B	+	-
T41B	+	+
T42B	+	+
T43B	+	-
T44B	+	+
T45P	+	+
F3P	+	-
F4B	+	+
F8P	+	+
F8B	+	-
F9B	+	-
F10P	+	-
F10B	+	-
F11P	+	-

Sample name	Catalase test	Coagulase test
F11B	+	-

F12P	+	-
F12P	+	-
F12B	+	-
F13B	+	-
F14P	+	-
F14B	+	+
F15B	+	-
F17P	+	+
F17B	+	-
F19P	+	-
F19B	+	-
F21B	+	-
F22B	+	-
F24P	+	+
F24B	+	-
F26B	+	-
F27P	+	-
F27B	+	+
F28B	+	+
F29P	+	-
F29B	+	-
F30P	+	-
F30B	+	-
F31P	+	-
F31B	+	-

F32B	+	-
F33P	+	+
F33B	+	-
F37P	+	-
F37B	+	-
F38P	+	-
F38B	+	-
F39P	+	-
F39B	+	-
F40P	+	-
F40B	+	-
F42B	+	+
F43B	+	-
F45P	+	-
F45B	+	+

Sample name	Catalase test	Coagulase test
D2B	+	+
D3B	+	-
D5P	+	+
D5B	+	+
D6P	+	+
D6B	+	+
D7P	+	-
D8P	+	-
D9B	+	-
D11B	+	+

D13P	+	-
D13B	+	-
D14P	+	-
D15P	+	-
D16P	+	-
D16B	+	-
D17P	+	+
D20B	+	-
D21P	+	-
D23B	+	-
D24P	+	-
D24B	+	-
D25P	+	-
D25B	+	-
D26B	+	+
D28P	+	+
D28B	+	+
D29B	+	-
D31P	+	-
D31B	+	-
D32P	+	-
D33B	+	-
D35P	+	-
D36P	+	-
D38P	+	+
D38B	+	+

D39P	+	+
D39B	+	+
D44P	+	-
D45P	+	+
D45P	+	-
D45B	+	-

APPENDIX IV

Antibiotic sensitivity test results of *B. cereus* isolated from meat pie samples

	Inhibition zone diameter in mm								
Sample name	AMX	CRO	CAZ	CHL	CIP	ERY	CN	VAN	SXT
D7P	0	7	10	25	20	20	28	21	29
D6B	7	12	12	25	18	37	29	20	31
D10P	10	14	14	28	24	36	27	10	15
D11B	12	20	17	20	26	25	30	15	10
D19P	21	25	20	19	30	31	24	17	22
D22P	7	0	10	30	29	30	24	17	40
D23B	11	27	16	22	31	37	32	21	12
D37B	13	19	14	11	29	25	26	7	29
D39B	24	29	20	25	28	27	30	18	14
F8B	20	20	22	19	24	25	24	19	19
F9P	7	16	16	28	28	20	29	18	10
F11B	16	19	11	24	27	30	31	9	12
F17P	27	24	24	27	29	29	30	17	27
F20P	0	12	14	18	29	20	28	19	8
F24P	19	24	19	28	27	28	27	15	20

F39P	10	10	7	20	20	24	26	20	24
F40B	16	17	10	25	22	28	22	19	8
K6P	9	0	10	24	19	13	30	21	20
K9B	14	14	11	12	24	26	24	19	11
K14P	7	10	9	26	34	29	19	17	11
K15B	25	20	14	25	31	19	31	21	28
K16B	17	21	16	12	25	23	28	9	15
K17P	20	26	17	29	28	30	26	19	25
K20P	9	9	11	10	31	22	22	22	19
K26B	0	7	9	31	28	20	24	10	10
K29P	12	19	17	29	28	28	23	18	31
K30P	10	21	12	28	27	29	29	21	7
K37B	27	24	20	30	25	27	18	19	21
K42P	14	20	24	27	27	25	28	7	21
K42B	7	9	0	27	29	23	27	19	12
K43P	10	14	15	20	21	28	26	21	9
G1B	12	21	17	24	25	27	22	11	15
G7P	0	9	12	17	31	28	30	14	25
G7B	10	16	14	29	16	14	24	11	9
G10P	24	26	21	22	27	20	19	10	12
G11P	11	14	9	29	28	24	28	14	22

	Inhibition zone diameter in mm								
Sample name	AMX	CRO	CAZ	CHL	CIP	ERY	CN	VAN	SXT
G18P	7	10	9	20	29	23	26	9	30

G20P	8	12	14	22	29	14	22	10	10
G27P	20	27	20	12	21	29	24	22	24
G33P	19	19	16	8	26	30	23	10	18
G35P	12	15	10	18	28	31	29	10	12
G45B	0	7	10	20	21	23	18	10	10
M4B	12	20	12	30	30	27	16	17	10
M13B	10	20	14	17	30	28	25	11	25
M19B	10	20	10	29	21	30	24	15	9
M21P	24	26	20	21	27	20	30	22	12
M26P	11	8	8	29	24	29	23	20	22
M29B	0	10	8	22	23	27	21	21	30
M37B	8	20	16	22	22	11	25	8	7
M38P	20	21	17	12	24	26	29	14	24
M38B	19	25	11	8	22	24	18	11	28
M43B	17	24	17	18	26	27	28	18	12
M44P	12	25	9	20	31	9	27	10	10
N5B	12	14	16	31	21	30	26	21	26
N12P	10	10	9	17	25	29	22	20	0
N17P	10	16	16	10	30	20	30	9	17
N20P	24	27	21	27	27	24	24	17	10
N21P	10	8	0	13	26	27	19	10	21
N22P	7	14	7	22	25	20	21	19	23
N25B	8	12	10	25	29	26	28	9	7
N29P	20	27	17	20	17	25	27	15	12
N33P	19	18	17	20	30	29	24	17	7

N37P	17	25	17	18	33	31	24	19	10
N38P	12	19	11	22	21	33	32	22	12
N41P	27	27	21	31	29	27	29	21	14
N42P	13	14	15	19	25	29	30	19	17
T4P	12	21	14	23	27	31	18	20	20
T7P	24	26	14	29	26	24	21	16	25
T15B	10	24	16	26	26	10	28	18	9
T18P	24	29	19	19	30	26	27	10	12
T18B	11	20	13	21	33	28	39	12	22
T24P	0	8	10	30	21	22	25	15	30
T27P	8	12	15	25	29	29	25	21	10
T35P	10	15	16	21	25	30	26	8	24

	Inhibition zone diameter in mm								
Sample name	AMX	CRO	CAZ	CHL	CIP	ERY	CN	VAN	SXT
T40P	20	22	20	31	27	26	29	22	18
T43B	10	16	10	27	24	26	18	14	12
T44B	12	18	12	20	27	27	28	18	10
U4P	28	27	28	19	30	31	27	20	32
U12P	28	28	22	29	21	28	26	16	21
U23B	14	14	17	30	30	26	22	9	12
U16P	0	7	7	24	30	30	30	20	9
U31B	7	20	16	28	31	28	24	19	10
U36P	13	24	24	22	24	24	19	21	16
U36B	10	18	10	16	29	23	24	21	26

U38B	19	24	17	26	22	24	26	9	11
U42P	7	22	10	25	27	29	30	15	10

APPENDIX V

Antibiotic sensitivity test result of *S. aureus* isolated from meat pie samples

	Inhibition zone diameter in mm								
Sample name	AMX	CRO	CAZ	CHL	CIP	ERY	GEN	VAN	SXT
D2B	13	10	16	27	39	37	23	20	29
D5P	21	24	22	22	31	19	31	17	31
D5B	22	26	22	21	32	36	21	14	11
D6P	10	11	24	27	25	23	20	16	20
D6B	7	13	12	29	24	28	24	21	28
D11B	12	20	14	24	34	31	31	16	22
D17P	23	27	20	18	39	32	30	19	33
D26B	14	20	20	26	18	37	24	20	34
D28P	21	19	19	22	36	30	26	17	28
D28B	7	15	18	28	30	29	25	19	33
D38P	11	25	9	23	31	37	31	21	40
D38B	20	21	20	27	33	32	28	15	21
D39P	24	28	23	24	30	36	26	20	18
D39B	22	23	20	20	30	24	32	14	27

D45P	15	26	21	31	34	37	21	13	29
F4B	9	10	19	24	26	30	21	21	15
F8P	21	19	20	18	28	31	28	16	29
F14B	11	20	21	22	30	26	27	14	20
F17P	10	9	18	27	17	37	30	19	28
F24P	18	18	20	23	35	29	24	20	19
F27B	7	0	0	22	39	26	24	13	33
F28B	24	20	28	20	34	12	32	24	21
F33P	14	10	20	18	26	30	26	26	18
F42B	19	16	19	29	25	31	26	28	40
F45B	12	22	21	25	21	37	30	20	20
G1B	25	23	22	29	31	30	21	13	26
G6B	16	11	19	24	32	35	20	21	27
G9P	9	8	8	19	30	31	28	15	30
G10P	10	10	18	21	26	30	27	19	20
G11P	22	18	21	23	16	24	26	13	20
G21P	9	10	8	13	28	28	28	12	19
G21B	22	18	20	17	27	32	30	15	17
G22P	17	12	12	26	29	34	24	17	30
G22B	21	14	20	29	30	32	19	13	15

	Inhibition zone diameter in mm								
Sample name	AMX	CRO	CAZ	CHL	CIP	ERY	GEN	VAN	SXT
G23B	24	26	21	22	33	14	23	20	24
G29B	7	15	14	25	32	31	31	19	31

G31P	16	12	19	27	31	25	20	14	10
G31B	26	27	25	29	30	36	24	16	28
G32P	20	19	20	24	26	26	28	21	37
G35B	23	16	24	23	21	32	26	16	27
G37P	9	12	0	21	34	30	31	17	26
G38P	17	20	18	19	31	29	30	20	29
G45B	24	28	24	20	25	24	24	14	18
K1P	19	21	19	27	29	35	30	19	32
K1B	10	14	18	29	27	27	27	17	29
K2P	19	17	18	18	24	29	28	20	26
K2B	11	10	14	22	21	29	21	14	34
K3B	9	11	9	27	22	35	24	15	12
K9B	12	11	14	23	29	35	18	17	22
K12B	21	24	21	28	20	32	12	16	31
K15P	12	19	20	15	30	28	28	21	14
K18B	17	20	17	22	32	32	29	16	28
K22P	13	14	15	20	24	14	30	14	19
K22B	16	17	26	25	26	30	27	19	28
K24P	15	27	25	27	30	34	31	20	21
K24B	7	10	12	32	34	28	25	10	40
K26P	9	14	10	17	16	31	26	13	32
K27B	28	24	20	27	32	30	14	17	24
K30P	11	15	16	18	40	24	31	15	11
K30B	21	22	21	29	20	31	24	12	15
K31P	12	18	19	29	33	9	23	13	20

K31B	10	16	16	22	17	30	30	9	27
K37P	0	9	10	22	21	29	28	19	26
K40B	18	20	21	20	26	27	26	15	29
K43P	12	10	11	22	26	35	32	21	24
K43B	20	19	20	27	30	24	24	13	10
K44P	21	221	23	25	24	23	30	19	30
K44B	8	16	16	28	29	36	28	15	28
M4B	21	18	18	26	30	31	21	17	29
M12P	19	21	16	12	25	24	24	21	12

	Inhibition zone diameter in mm								
Sample name	AMX	CRO	CAZ	CHL	CIP	ERY	GEN	VAN	SXT
M12B	8	12	0	19	26	30	28	12	28
M13P	0	9	21	20	31	32	19	19	19
M14B	21	18	18	24	30	28	30	13	26
M15B	20	19	17	21	18	27	25	15	12
M17B	11	16	19	29	24	14	20	16	30
M18B	18	22	20	20	30	34	27	18	20
M19B	24	27	22	21	20	31	31	20	19
M20B	22	21	12	21	25	30	28	14	32
M21P	9	10	8	24	23	20	32	10	29
M22B	12	9	12	12	17	25	30	19	21
M23P	20	11	21	24	17	35	28	14	19
M24P	14	11	19	21	22	34	17	10	26
M25P	19	24	21	21	30	30	27	9	30

M26B	11	14	13	24	32	27	28	13	30
M34P	0	10	18	25	28	32	23	12	17
M35P	7	15	17	27	35	29	21	16	24
M36B	10	14	19	19	39	27	31	15	31
M37P	25	23	22	18	26	31	28	17	24
M39P	21	17	22	25	28	35	24	21	21
M39B	20	20	19	22	31	28	28	18	30
M41P	27	24	21	26	33	30	26	19	26
M43P	7	10	21	24	33	30	26	12	32
N1B	18	15	21	20	30	31	34	17	27
N5B	12	17	21	24	27	28	30	20	26
N7B	8	12	12	21	28	29	26	18	30
N8B	14	10	0	19	32	30	24	15	31
N12B	20	18	18	14	34	32	30	12	10
N18P	9	11	7	25	24	35	28	12	29
N18B	20	15	19	28	28	13	24	19	27
N22P	19	23	26	27	31	28	21	13	15
N22B	11	10	16	25	36	14	27	8	28
N23P	27	24	18	18	36	27	20	16	28
N26P	19	20	16	20	39	24	18	17	17
N26B	15	22	10	15	18	32	20	20	30
N39P	12	11	23	21	29	30	25	21	32
N39B	7	9	11	26	28	31	26	13	22

	Inhibition zone diameter in mm								
Sample	AMX	CRO	CAZ	CHL	CIP	ERY	GEN	VAN	SXT

name									
N40P	8	9	11	25	30	27	21	19	24
N40B	17	20	19	18	31	32	30	13	14
N41P	16	19	22	19	29	33	22	15	29
N41B	14	10	24	28	27	29	28	12	19
N42P	10	12	14	21	21	28	25	17	27
N42B	22	22	24	20	24	30	29	18	26
N43B	28	21	20	28	23	32	27	18	31
T11B	11	15	19	23	28	30	23	14	27
T15P	10	9	10	25	28	35	21	20	29
T20B	29	26	24	29	30	32	28	17	31
T21B	27	27	25	20	32	24	27	15	21
T24P	21	14	19	25	34	37	30	21	34
T31B	18	20	21	26	29	23	24	23	10
T39P	20	19	22	22	30	20	19	20	33
T39B	9	0	14	16	21	32	26	24	28
T40P	24	24	26	32	31	28	24	26	27
T41B	11	10	19	21	30	31	30	22	14
T42B	9	9	20	24	21	25	21	10	13
T44B	26	28	20	19	25	36	23	24	21
T45P	0	7	10	24	27	32	28	17	20
U4B	12	0	10	27	26	36	21	20	28
U10P	21	27	24	24	29	26	28	17	31
U10B	24	8	12	28	40	24	30	15	29
U14B	12	7	12	29	26	32	24	21	22

U16B	26	20	11	21	28	26	32	19	11
U19B	34	11	23	27	35	30			
U23P	14	10	25	23	25	28	13	10	24
U24P	7	12	10	25	30	30	26	15	28
U26B	30	27	19	7	20	24	28	20	19
U27P	10	19	26	22	35	28	30	14	20
U30B	11	10	17	25	24	30	24	21	24
U33B	29	16	12	29	32	30	28	17	34
U35P	18	16	10	15	25	30	21	16	26
U35B	0	12	21	19	17	31	23	11	29
U36P	14	12	20	22	31	35	25	18	25
U36B	26	21	20	21	26	28	30	19	28

	Inhibition zone diameter in mm								
Sample name	AMX	CRO	CAZ	CHL	CIP	ERY	GEN	VAN	SXT
U38B	24	19	`7	20	30	27	26	21	30
U39B	22	20	0	23	29	34	31	13	23
U40P	20	14	22	21	24	29	32	12	31
U42P	12	0	18	28	19	28	30	22	26
U42B	28	28	27	26	21	24	24	18	40

APPENDIX VI

Result for *B.cereus* diarrhoeal enterotoxin detection from *B.cereus* isolated from meat pie

Sample name	*B.cereus* diarrhoeal enterotoxin
D7P	+
D6B	-
D10P	+
D11B	-
D19P	+
D22P	-
D23B	-
D37B	+
D39B	-
F8B	-
F9P	-
F11B	-
F17P	+
F20P	+
F24P	+
F39P	-

F40B	-
K6P	+
K9B	-
K14P	-
K15B	-
K16B	-
K17P	-
K20P	-
K26B	-
K29P	-
K30P	-
K37B	-
K42P	-
K42B	-
K43P	-
G1B	-
G7P	-
G7B	-
G10P	+
G11P	+
G18P	+
G20P	+
G27P	-
G33P	-

Sample name	*B.cereus* diarrhoeal enterotoxin

G45B	-
M4B	-
M13B	-
M19B	-
M21P	+
M26P	-
M29B	-
M37B	-
M38P	-
M38B	-
M43B	-
M44P	-
N5B	-
N12P	-
N17P	+
N20P	+
N21P	-
N22P	-
N25B	-
N29P	+
N33P	+
N37P	+
N38P	+
N41P	-
N42P	-

T4P	+
T7P	-
T15B	-
T18P	-
T18B	-
T24P	-
T27P	-
T35P	-
T40P	-
T43B	-
T44B	-
U4P	+
U12P	+
U23B	-
U16P	+
	+
U31B	

Sample name	*B.cereus* diarrhoeal enterotoxin
U36P	+
U36B	-
U38B	-
U42P	-

APPENDIX VII

Result for the detection of Staphylococcal enterotoxin from *S. aureus* isolated from meat pie samples

Sample name	SEA	SEB	SEC	SED
U4B	-	-	-	-
U10P	+	-	-	-
U10B	+	-	-	-
U14B	-	-	-	-
U16B	-	-	+	-
U19B	-	-	-	-
U23P	+	-	-	-
U24P	-	-	-	-
U26B	-	-	-	-

U27P	-	+	-	-
U30B	+	-	-	-
U33B	-	-	-	-
U35P	-	-	-	-
U35B	-	-	-	-
U36P	-	-	-	-
U36B	-	-	+	-
U38B	-	-	-	-
U39B	-	-	-	-
U40P	-	-	-	-
U42P	-	-	-	-
U42B	-	+	-	+
M4B	-	-	-	-
M12P	-	-	-	-
M12B	-	-	-	-
M13P	-	-	-	-
M14B	-	-	-	-
M15B	-	-	-	-
M17B	-	-	-	-
M18B	-	-	-	-
M19B	+	-	-	-
M20B	-	-	+	-
M21P	-	-	-	-
M22B	-	+	-	-
M23P	-	-	-	-

				-
M24P	-	-	-	

Sample name	SEA	SEB	SEC	SED
M25P	-	-	-	-
M26B	-	-	-	-
M34P	-	-	-	-
M35P	-	-	-	-
M36B	-	-	-	-
M37P	-	-	-	-
M39P	-	-	-	-
M39B	-	-	+	-
M41P	+	-	-	-
M43P	+	-	-	-
G1B	-	-	+	
G6B	-	-	-	-
G9P	-	-	-	-
G10P	-	-	-	-
G11P	-	-	-	-
G21P	-	-	-	-
G21B	-	-	-	-
G22P	+	-	-	-
G22B	-	-	+	
G23B	-	-	-	-
G29B	+	-	-	-
G31P	-	-	-	-

G31B	-	-	-	-
G32P	-	-	-	-
G35B	+	-	-	-
G37P	-	-	-	-
G38P	+	-	-	-
G45B		-	+	-
K1P	+	-	-	-
K1B	+	+	-	-
K2P	-	-	-	-
K2B	-	-	-	-
K3B	-	-	-	-
K9B	-	-	-	-
K12B	-	-	-	-
K15P	-	-	-	-
K18B	-	-	-	-

Sample name	SEA	SEB	SEC	SED
K22P	-	-	-	-
K22B	-	-	-	-
K24P	-	-	+	-
K24B	-	-	+	-
K26P	-	-	-	-
K27B	-	-	-	-
K30P	-	-	-	-
K30B	-	-	+	

K31P	-	-	-	-
K31B	-	-	-	-
K37P	+	-	-	-
K40B	-	-	-	-
K43P	-	-	-	-
K43B	-	-	-	-
K44P	+	-	-	-
K44B	+	-	-	-
N1B	-	-	-	+
N5B	-	-	+	-
N7B	+	-	+	-
N8B	+	-	-	-
N12B	-	+		
N18P	-	-	+	-
N18B	+	-	+	-
N22P	+	-	-	-
N22B	-	-	-	-
N23P	-	-	-	-
N26P	-	-	+	-
N26B	-	-	-	-
N39P	-	-	-	-
N39B	-	-	-	-
N40P	+	-	-	-
N40B	+	-	-	-
N41P	-	-	-	-

N41B	-	-	-	-
N42P	-	-	-	-
N42B	-	+	-	-
N43B	-	-	-	-
T11B	+	-	-	+

Sample name	SEA	SEB	SEC	SED
T15P	-	-	-	-
T20B	+	-	-	-
T21B	-	-	-	-
T24P	-	-	-	-
T31B	+	-	+	-
T39P	-	-	-	-
T39B	+	-	-	-
T40P	-	-	-	-
T41B	-	-	-	-
T42B	-	-	-	-
T44B	-	-	-	-
T45P	+	-	-	-
F4B	+	-	-	-
F8P	-	-	-	-
F14B	-	-	-	-
F17P	-	-	-	-
F24P	-	-	-	-
F27B	-	-	-	-
F28B	+	-	-	-

F33P		-	-	-
F42B	+	-	-	-
F45B	-	-	-	-
D2B	-	-	-	-
D5P	-	-	-	-
D5B	-	-	-	-
D6P	+	-	-	-
D6B	-	+		-
D11B	-	-	+	-
D17P	-	-	+	-
D26B	-	-	-	-
D28P	-	-	-	-
D28B	-	-	-	-
D38P	-	-	-	-
D38B	+	-	-	-
D39P	-	-	-	-
D39B	-	-	-	-
D45P	-	-	-	-

APPENDIX VIII

STATISTICAL ANALYSIS

Oneway

Descriptives

RES

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
DAL	4	1.2500	.95743	.47871	-.2735	2.7735	.00	2.00
FAG	4	.7500	1.50000	.75000	-1.6368	3.1368	.00	3.00
GWAL	4	1.7500	2.06155	1.03078	-1.5304	5.0304	.00	4.00
KUMB	4	1.7500	1.25831	.62915	-.2522	3.7522	.00	3.00
MUNIC	4	3.2500	2.75379	1.37689	-1.1319	7.6319	.00	6.00
NASS	4	2.5000	1.91485	.95743	-.5470	5.5470	1.00	5.00
TARAU	4	1.7500	2.36291	1.18145	-2.0099	5.5099	.00	5.00
UNGO	4	2.2500	1.25831	.62915	.2478	4.2522	1.00	4.00
Total	32	1.9063	1.78451	.31546	1.2629	2.5496	.00	6.00

Test of Homogeneity of Variances

RES

Levene Statistic	df1	df2	Sig.
1.833	7	24	.127

ANOVA

RES

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.469	7	2.353	.686	.682
Within Groups	82.250	24	3.427		
Total	98.719	31			

Robust Tests of Equality of Means

RES

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	.686	7	17.200	.682

a. Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: RES

	(I) FAC	(J) FAC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	DAL	FAG	.50000	1.30902	1.000	-3.8354	4.8354
		GWAL	-.50000	1.30902	1.000	-4.8354	3.8354
		KUMB	-.50000	1.30902	1.000	-4.8354	3.8354

	MUNIC	-2.00000	1.30902	.785	-6.3354	2.3354
	NASS	-1.25000	1.30902	.977	-5.5854	3.0854
	TARAU	-.50000	1.30902	1.000	-4.8354	3.8354
	UNGO	-1.00000	1.30902	.994	-5.3354	3.3354
	DAL	-.50000	1.30902	1.000	-4.8354	3.8354
	GWAL	-1.00000	1.30902	.994	-5.3354	3.3354
	KUMB	-1.00000	1.30902	.994	-5.3354	3.3354
FAG	MUNIC	-2.50000	1.30902	.558	-6.8354	1.8354
	NASS	-1.75000	1.30902	.876	-6.0854	2.5854
	TARAU	-1.00000	1.30902	.994	-5.3354	3.3354
	UNGO	-1.50000	1.30902	.939	-5.8354	2.8354
	DAL	.50000	1.30902	1.000	-3.8354	4.8354
	FAG	1.00000	1.30902	.994	-3.3354	5.3354
	KUMB	.00000	1.30902	1.000	-4.3354	4.3354
GWAL	MUNIC	-1.50000	1.30902	.939	-5.8354	2.8354
	NASS	-.75000	1.30902	.999	-5.0854	3.5854
	TARAU	.00000	1.30902	1.000	-4.3354	4.3354
	UNGO	-.50000	1.30902	1.000	-4.8354	3.8354
	DAL	.50000	1.30902	1.000	-3.8354	4.8354
	FAG	1.00000	1.30902	.994	-3.3354	5.3354
KUMB	GWAL	.00000	1.30902	1.000	-4.3354	4.3354
	MUNIC	-1.50000	1.30902	.939	-5.8354	2.8354
	NASS	-.75000	1.30902	.999	-5.0854	3.5854

	TARAU	.00000	1.30902	1.000	-4.3354	4.3354
	UNGO	-.50000	1.30902	1.000	-4.8354	3.8354
	DAL	2.00000	1.30902	.785	-2.3354	6.3354
	FAG	2.50000	1.30902	.558	-1.8354	6.8354
	GWAL	1.50000	1.30902	.939	-2.8354	5.8354
MUNIC	KUMB	1.50000	1.30902	.939	-2.8354	5.8354
	NASS	.75000	1.30902	.999	-3.5854	5.0854
	TARAU	1.50000	1.30902	.939	-2.8354	5.8354
	UNGO	1.00000	1.30902	.994	-3.3354	5.3354
	DAL	1.25000	1.30902	.977	-3.0854	5.5854
	FAG	1.75000	1.30902	.876	-2.5854	6.0854
	GWAL	.75000	1.30902	.999	-3.5854	5.0854
NASS	KUMB	.75000	1.30902	.999	-3.5854	5.0854
	MUNIC	-.75000	1.30902	.999	-5.0854	3.5854
	TARAU	.75000	1.30902	.999	-3.5854	5.0854
	UNGO	.25000	1.30902	1.000	-4.0854	4.5854
	DAL	.50000	1.30902	1.000	-3.8354	4.8354
	FAG	1.00000	1.30902	.994	-3.3354	5.3354
	GWAL	.00000	1.30902	1.000	-4.3354	4.3354
TARAU	KUMB	.00000	1.30902	1.000	-4.3354	4.3354
	MUNIC	-1.50000	1.30902	.939	-5.8354	2.8354
	NASS	-.75000	1.30902	.999	-5.0854	3.5854
	UNGO	-.50000	1.30902	1.000	-4.8354	3.8354

		DAL	1.00000	1.30902	.994	-3.3354	5.3354
		FAG	1.50000	1.30902	.939	-2.8354	5.8354
		GWAL	.50000	1.30902	1.000	-3.8354	4.8354
	UNGO	KUMB	.50000	1.30902	1.000	-3.8354	4.8354
		MUNIC	-1.00000	1.30902	.994	-5.3354	3.3354
		NASS	-.25000	1.30902	1.000	-4.5854	4.0854
		TARAU	.50000	1.30902	1.000	-3.8354	4.8354
		FAG	.50000	.88976	.998	-3.6072	4.6072
		GWAL	-.50000	1.13652	1.000	-6.2248	5.2248
		KUMB	-.50000	.79057	.996	-4.0115	3.0115
	DAL	MUNIC	-2.00000	1.45774	.834	-9.9018	5.9018
		NASS	-1.25000	1.07044	.910	-6.5319	4.0319
		TARAU	-.50000	1.27475	1.000	-7.1599	6.1599
		UNGO	-1.00000	.79057	.883	-4.5115	2.5115
		DAL	-.50000	.88976	.998	-4.6072	3.6072
Games-Howell		GWAL	-1.00000	1.27475	.987	-6.7106	4.7106
		KUMB	-1.00000	.97895	.953	-5.2844	3.2844
	FAG	MUNIC	-2.50000	1.56791	.742	-10.0525	5.0525
		NASS	-1.75000	1.21621	.814	-7.1252	3.6252
		TARAU	-1.00000	1.39940	.992	-7.4687	5.4687
		UNGO	-1.50000	.97895	.772	-5.7844	2.7844
		DAL	.50000	1.13652	1.000	-5.2248	6.2248
	GWAL	FAG	1.00000	1.27475	.987	-4.7106	6.7106

	KUMB	.00000	1.20761	1.000	-5.6391	5.6391
	MUNIC	-1.50000	1.71998	.978	-9.1625	6.1625
	NASS	-.75000	1.40683	.999	-6.8522	5.3522
	TARAU	.00000	1.56791	1.000	-6.8328	6.8328
	UNGO	-.50000	1.20761	1.000	-6.1391	5.1391
	DAL	.50000	.79057	.996	-3.0115	4.0115
	FAG	1.00000	.97895	.953	-3.2844	5.2844
	GWAL	.00000	1.20761	1.000	-5.6391	5.6391
KUMB	MUNIC	-1.50000	1.51383	.954	-9.1596	6.1596
	NASS	-.75000	1.14564	.995	-6.0005	4.5005
	TARAU	.00000	1.33853	1.000	-6.4886	6.4886
	UNGO	-.50000	.88976	.998	-4.3518	3.3518
	DAL	2.00000	1.45774	.834	-5.9018	9.9018
	FAG	2.50000	1.56791	.742	-5.0525	10.0525
	GWAL	1.50000	1.71998	.978	-6.1625	9.1625
MUNIC	KUMB	1.50000	1.51383	.954	-6.1596	9.1596
	NASS	.75000	1.67705	1.000	-6.8354	8.3354
	TARAU	1.50000	1.81430	.984	-6.4199	9.4199
	UNGO	1.00000	1.51383	.994	-6.6596	8.6596
	DAL	1.25000	1.07044	.910	-4.0319	6.5319
	FAG	1.75000	1.21621	.814	-3.6252	7.1252
NASS	GWAL	.75000	1.40683	.999	-5.3522	6.8522
	KUMB	.75000	1.14564	.995	-4.5005	6.0005

	MUNIC	-.75000	1.67705	1.000	-8.3354	6.8354
	TARAU	.75000	1.52069	.999	-5.9360	7.4360
	UNGO	.25000	1.14564	1.000	-5.0005	5.5005
	DAL	.50000	1.27475	1.000	-6.1599	7.1599
	FAG	1.00000	1.39940	.992	-5.4687	7.4687
	GWAL	.00000	1.56791	1.000	-6.8328	6.8328
TARAU	KUMB	.00000	1.33853	1.000	-6.4886	6.4886
	MUNIC	-1.50000	1.81430	.984	-9.4199	6.4199
	NASS	-.75000	1.52069	.999	-7.4360	5.9360
	UNGO	-.50000	1.33853	1.000	-6.9886	5.9886
	DAL	1.00000	.79057	.883	-2.5115	4.5115
	FAG	1.50000	.97895	.772	-2.7844	5.7844
	GWAL	.50000	1.20761	1.000	-5.1391	6.1391
UNGO	KUMB	.50000	.88976	.998	-3.3518	4.3518
	MUNIC	-1.00000	1.51383	.994	-8.6596	6.6596
	NASS	-.25000	1.14564	1.000	-5.5005	5.0005
	TARAU	.50000	1.33853	1.000	-5.9886	6.9886

APPENDIX IX

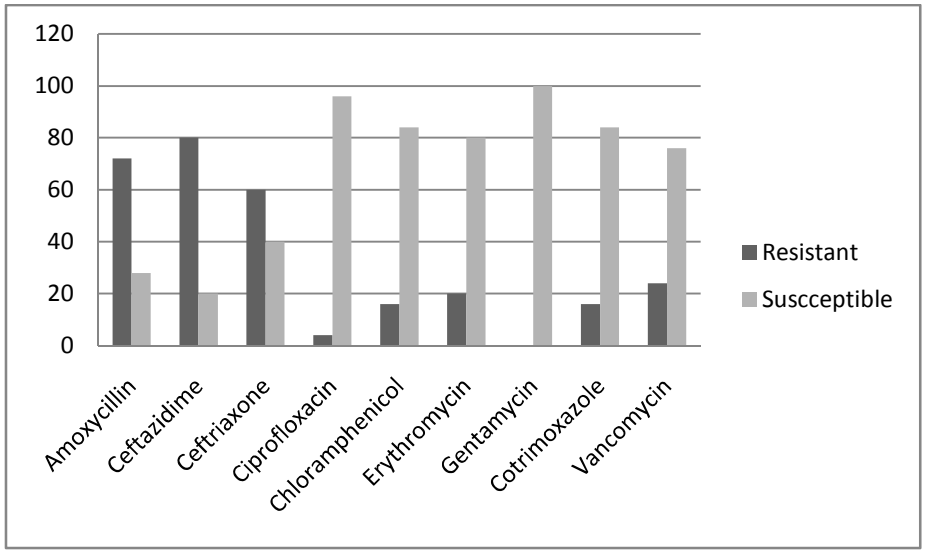


Figure 4.1: Graphical representation of antibiotic susceptibility and resistance of *Bacillus cereus*\

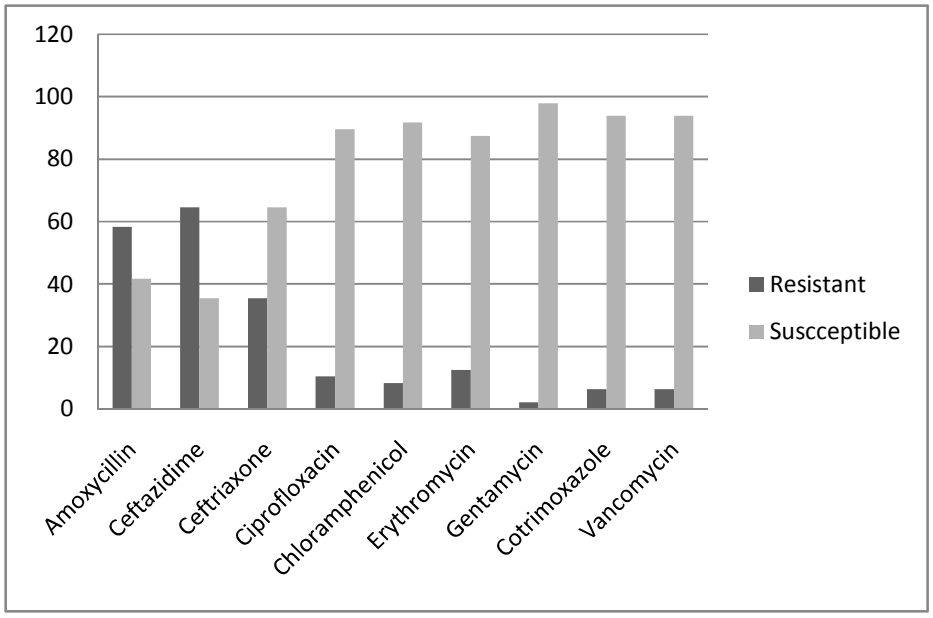


Figure 4.2: Graphical representation of antibiotic susceptibility and resistance of *Staphylococcus aureus*