

**FOOD HANDLERS PERCEPTION ON FOOD SAFETY, OCCURRENCE
AND CHARACTERIZATION OF ENTEROTOXIGENIC
STAPHYLOCOCCUS AUREUS ISOLATED FROM FOODS OF ANIMAL
ORIGIN IN NAIROBI, KENYA**

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of Doctor of Philosophy (Microbiology) in the School of Pure and Applied Sciences
of Kenyatta University**

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DECLARATION

This thesis is my original work and has not been presented for degree or other awards in any other University.

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DEDICATION

This PhD Thesis is dedicated to The Almighty God who made it possible for me to pursue my studies, secondly to my wife (Dorothy), my Children (Ian, Tony and Lynn) for their love, patience, understanding, moral support and encouragement during my study.

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ABBREVIATIONS AND ACRONYMS

A260	Absorbance at 260nm
A280	Absorbance at 280nm
agr	accessory gene regulator
ANOVA	Analysis of variance
APC	Antigen presenting cells
ATTC	American type culture collection
API	Analytic profile index <i>Staphylococcus</i> system
aw	Water activity
BLAST	Basic local alignment search tool
bp	base pair
bsa	antibiotic biosynthesis
CBDN	Central Business District of Nairobi
C3	Complement 3 convertase
CDC	Centre for Disease Control
CFU	Colony Forming Unit
CPS	Coagulase-positive <i>Staphylococci</i>
CNS	Coagulase-negative <i>Staphylococci</i>
Da	Dalton
DNA	Deoxyribonucleic acid
dNTPs	deoxynucleoside triphosphate
<i>E.coli</i>	<i>Escherichia coli</i>
EFSA	European Food Safety Authority
EIA	Enzyme immunoassay
ELFA	Enzyme-linked fluorescent assay
Eta and Etb	Exfoliative toxin gene a and b
ETA and ETB	Exfoliative toxin A and B
egc	enterotoxin gene cluster
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food Agriculture Organisation
FDA	Food and Drug Administration
femA	Factor essential for expression of methicillin resistance
GM-CSF	Granulocyte macrophage colony-stimulating factor
G-CSF	Granulocyte colony-stimulating factor
H ₂ O ₂	Hydrogen peroxide
IgG	Immunoglobulin G
IL-1	Interleukin-1
ILRI	International Livestock Research Institute
IQR	Inter quartile range
KAP	Knowledge altitude and practice
Kb	Kilobase
KCl	Potassium chloride
KDa	Kilodalton
KU	Kenyatta University
Lpl	Lipoprotein
LUKED	Leukocidin ExD
MALDI	Matrix assisted laser desorption/ionization
Mec	Methicillin gene
MeA	Methicillin enterotoxin gene A

MCP-1	Monocyte chemoattractant protein 1
MgCl	Magnesium chloride
MHC	Major Histocompatibility Complex
mRNA	messenger RNA
MS	Mass spectrometry
MRSA	Methicillin Resistance <i>Staphylococcus aureus</i>
Mw	Molecular Weight
mM	Millimole
NaCl	Sodium Chloride
NCCLS	National Committee for Clinical Laboratory Standards
ng	nanogram
NHS	Non host-specific
OD	Optical Density
PBP2	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
pH	Power of Hydrogen
RANTEs	Regulated on activation normal T-cell expressed and secreted protein
RNA	Ribonucleic acid
RNase	Ribonuclease
RT-PCR	Reverse transcription polymerase chain reaction
SaPIs	<i>S.aureus</i> pathogenicity islands
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SCC	Staphylococcal cassette chromosome
SCIN	Staphylococcal complement inhibitors
Sd	Standard deviation
SE	Staphylococcal enterotoxin (SEA, SEB, SEC, SED, SEC, SEE)
<i>se</i>	Staphylococcal enterotoxin gene
SET RPLA	<i>Staphylococcus</i> enterotoxin reverses passive latex agglutination
SFP	Staphylococcal Food Poisoning
SFPO	Staphylococcal Food Poisoning Outbreak
Sags	Superantigens
Spa	<i>Staphylococcus</i> protein A
Taq	<i>Thermus aquaticus</i>
TBE	Tris borate ethylenecliamine tetra acetic acid
TCR-VB	T.cell receptor variable beta
TCR-V α	T.cell receptor variable alpha
TNF- α	Tumor necrosis factor alpha
TNFR1	Tumor necrosis factor receptor 1
TOF	Time of flight
tst	Toxic shock toxin
Tris HCl	Trizma hydrochloride
TSST	Toxic Shock Syndrome Toxin -1
USA	United State of America
UV	Ultraviolet Radiation
VanA	Vancomycin A gene
VRSA	Vancomycin resistance <i>Staphylococcus aureus</i>
WHO	World Health Organisation
μ g	Microgram

ABSTRACT

Diarrheal diseases are the commonest manifestation of food poisoning, which are fatal. Knowledge, attitude and practice of food handlers influence the occurrence of food poisoning. *Staphylococcus aureus* is considered the third most important cause of food-borne diseases in the world after *Salmonella* species and *C. perfringens*. Antimicrobial resistance and enterotoxigenic properties of *S. aureus* in food of animal origin in many parts of Kenya are scanty. The aim of the study was to investigate food handlers' perception on food safety and characterization enterotoxigenic *S. aureus* in foods of animal origin in Nairobi Kenya. A cross-sectional descriptive study was conducted in Nairobi and its environs, confined to meat and milk outlets. In the first stage of the study on food safety, food handlers (n=100) were interviewed and questionnaire administered to assess their knowledge, attitudes and practices (KAP). In the second phase, food samples of animal origin (n=420) were randomly purchased from different outlets. Additional 251 samples of various pork products from a meat processing plant were collected for isolation and characterization of enterotoxigenic properties of *S. aureus*. Colonies of *S. aureus* were selected from Baird-Parker medium plates for identification using the biochemical tests. Reverse Passive Latex Agglutination was used to identify enterotoxigenic strains. Gene distributions were detected by multiplex Polymerase Chain Reaction (mPCR) reaction. Their resistance to eight commonly used antibiotics was determined using Kirby-Bauer disc diffusion test. Results on Knowledge, Attitudes and Practices showed that overall scores were not significantly affected by marital status and religion of the respondents. Gender comparisons revealed that the mean KAP scores for men and women food handlers were similar. A total of 251 (37.4%) of *S. aureus* strains were isolated and identified. The proportions of contamination of animal products from the seller and meat processing plant were comparable. The contamination rate of the meat and meat products sample (40.7%) was significantly higher than milk and milk products (25.0%) ($p=0.001$). Most of the strains 187 (74.5%) produced staphylococcal enterotoxin (SE) with staphylococcal enterotoxin A (SEA) being the most frequent (90, 48.1%), followed by a combination of SEA and SEC (22, 11.8%). Most frequent gene detected by multiplex PCR was *sea* (61.8%). Genes *see*, *sed*, *sec*, and *seb* were observed in 33.3%, 17.5%, 15.9% and 13.9% strains, respectively. Combination genes found to occur in pairs were *Sea/See* (21.2%), *Sea/Sed* (9.8%), *Sed/See* (2.1%), *Sea/Sec* (0.7%) and *Seb/Sec* (0.5%). A combinations of four genes *sea/sec/sed/see* accounted for only 0.01%. Relatively low number (2.1%) of discrepancies between multiplex PCR and Reverse Passive Latex Agglutination (RPLA) assay particularly on SED. The results of both methods were identical concerning SEA, SEB and SEC. All of the strains showed multi drug resistance (MDR) to major classes of antibiotics tested with Penicillin G having the highest resistance level (246, 99.6%) followed by Ampicillin (230, 93.1%). The study concluded that knowledge attitude and practice performance seemed to improve along the level of education and work experience. High level of contamination of foods of animal origin by enterotoxigenic and MDR strains of *S. aureus* was evident. The study indicates a need for good hygiene practices in food processing, in order to minimize staphylococcal food poisoning, which poses a health risk for the consumers.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Food safety can be described as handling, preparation and storage of food in ways that prevent food poisoning. Various routes have been described that should be followed to avoid foodborne outbreaks. Teferi *et al.* (2012) stated that food outlets are sources of food borne diseases and food handlers contribute to food borne illness. Foodborne diseases encompass a wide spectrum of illnesses especially in developing countries. They are a growing public health problem, costly yet preventable worldwide. Consumption of contaminated foods by different pathogenic microorganisms, dangerous chemical or toxins can result foodborne illness (Kibret and Abera, 2012). Foodborne illness results also to economic losses, lower work productivity, and hospitalization, or other related health hazard expenses (Dharod *et al.*, 2009).

Diarrhea is the commonest manifestation of food poisoning and some cases are fatal. Microbial toxin elaborated by disease-causing microbe, cause one form of illness. The other form is due to the body's reactions to the microbe itself following infection (Teplitskie *et al.*, 2009). The impact of foodborne disease internationally is difficult to estimate but approximately 2.1 million children in developing countries including Kenya die as a result of diarrheal-related disease annually (Mead *et al.*, 1999). Food or water is suspected to be the main vehicle for many of these illnesses (WHO, 2002). In China alone, between 2008 and 2010 371 cases of bacterial foodborne diseases outbreaks were reported, involving 20,062 individuals and leading to 41 deaths. Ninety-four (94) outbreaks of Staphylococcal food poisoning, involving 2,223 individuals and leading to 1,186 hospitalizations were also reported in 2003 and 2007 (Wu *et al.*, 2010).

In Kenya, thirty seven food poisoning outbreaks were reported to the Ministry of Health from various parts of the country between 1970 to 1993, and only 13 of 926 people were confirmed to be due to particular etiological agents (Ombui *et al.*, 2001). Foods that were involved included milk and milk products, meat and meat products, maize flour, bread scones and other wheat products, vegetables and lemon pie pudding.

Bacterial foodborne diseases can be recognized in three ways namely, intoxications, infections, and toxic infections. Preformed bacterial toxin like those produced by *S. aureus* and *C. botulinum*, which result from bacterial growth in the food causes foodborne bacterial intoxication. Ingestion of food containing viable bacteria like *Salmonella* or *Listeria*, which grow and establish themselves in the host, results in foodborne infection (Buzby and Roberts, 1997). Some microorganisms reside in the intestinal tracts of normal, healthy animals and humans while some are ubiquitous in nature, occurring on soil and vegetation, in animal wastes and on animal carcasses. For example, human skin surfaces and nasal passages harbor bacteria of the genera *Staphylococcus* (CDC, 2007).

Knowledge, attitude and practice (KAP) on food hygiene are three factors that play major roles in food poisoning occurrence (Sharif and Al-Malki, 2010). To improve knowledge of food hygiene in food handlers in order to reduce disease, training programs is important (Sharif and Al-Malki, 2010). Knowledge of food safety practices to food handlers does not always lead to positive changes in food handling behaviours (Ansari-Lari *et al.*, 2010). Literature shows that several studies of food handler's knowledge, attitudes and practices have been conducted in other parts of the world (Ansari-Lari *et al.*, 2010; Bas *et al.*, 2006; Jevšnik *et al.*, 2007; Sharif and Al-Malki,

2010). However, there is strong statistical evidence that caterers cause most of all bacterial food poisoning. If food handlers become ill they are less likely to exclude themselves from work unlike those in other high-risk occupations, such as health care and day care workers and are therefore of particular concern (Thomas *et al.*, 2006). Various foodborne illness outbreaks have been traced to workers who are ill and poor food handling practices (Ansari-Lari *et al.*, 2010). Education about food safety in such a population is required for proper food handling practices to avoid spreading infectious diseases while at work (Thomas *et al.*, 2006). Educating food handlers to prevent foodborne illness is an important objective for industry and government. There is little if any formal study regarding knowledge, attitudes and practices of food handlers in meat processing plants and meat outlets that protect the consumers from the transmission of disease in Kenya.

In Nairobi, Kenya, the trade in food of animal origin is widespread. Meat and meat products could be of particular importance regarding foodborne illnesses. Two major sources of bacteria causing foodborne disease in meat and meat products could be the living animal carrying pathogenic bacteria while the processing environment harbours them. In addition, the human being is also an important source of pathogenic bacteria, most frequently indirectly by cross-contamination (Bas *et al.*, 2006). Bacteria originating from the animal may, during slaughter, contaminate the carcass, and subsequently are distributed through cut meat or meat products. Limiting the contamination and subsequent inactivation of occurring pathogenic bacteria can determine the quality of meat and meat products (Borch and Arinder, 2002). Infected persons who handle raw agricultural products can introduce foodborne pathogens during processing, storage and

preparation (Thomas *et al.*, 2006). Surface during processing of food should be considered as an important factor for transmission of pathogens.

Although the food particles are removed from the surfaces after a thorough cleaning, the bacteria adhering to these surfaces might not be removed (Kusumaningrum *et al.*, 2003). Food borne diseases continue to bear a heavy toll on the lives of humans both locally and globally but can be prevented and controlled effectively by simple hygiene measures. The incidence and prevalence of such diseases can significantly be reduced if food handler's knowledge, attitude and practices are boosted. *Staphylococcus aureus* is among the major foodborne pathogens, associated with diseases globally. Ingestion of food contaminated with staphylococcal toxin results in food poisoning (Le-Loir *et al.*, 2003). The ability to asymptotically colonize healthy individuals is a fundamental biological property of *S. aureus* (Wertheim *et al.*, 2005; Argudín *et al.*, 2010). Such individuals with *S. aureus* as part of the normal flora are referred to as carriers and are at higher risk of infection. They can also be an important source of the *S. aureus* strains that spread to others by direct contact, usually skin-to-skin contact with a colonized or infected individual. Objects and surfaces containing the organism can also be primary mode of transmission of *S. aureus* (Miller and Diep, 2008).

Pathogenic property of *S. aureus* is determined by the capacity of some strains to synthesis one or more *S. aureus* enterotoxins (Argudín *et al.*, 2010). Staphylococcal enterotoxins are thermostable and resistant to gastrointestinal proteases such as pepsin (Nagarajappa *et al.*, 2012). This explains the ability of the organism to remain active after ingestion (Atanassova, *et al.*, 2001). Biochemically, these enterotoxins are small peptides ranging from 26 to 29 kDa amino acid that are very similar. The amount of

staphylococcal enterotoxins required for establishment of typical symptoms of food poisoning is very low ranging from 20 ng to 1 µg (Normanno *et al.*, 2007). This corresponds to approximately 10⁵ *Staphylococci* colony-forming units (CFU) per gram of food (FDA, 2001).

Staphylococcus aureus may produce a large variety of enterotoxins and A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R and U varieties have been identified. However, about 95% of poisoning outbreaks are caused by classical enterotoxin A, B, C, D and E (Letertre *et al.*, 2003). Food Poisoning caused by Staphylococcal is underestimated (Smyth *et al.*, 2004). Severe gastroenteritis characterised by inflammation of the intestinal tract lining arise after Staphylococcal enterotoxins (SE) ingestion (Berdgoll, 1989). Individual's susceptibility and dose ingested determines the symptoms, which include nausea, abdominal cramps, diarrhea and a characteristic projectile vomiting (Le Loir *et al.*, 2003). Clinical signs of SFP disappear within 24 to 48hrs, but deaths occur rarely, and if it does, it is specifically seen in young or elderly individuals (Martin and Iandolo, 2000). The enterotoxin produced *Staphylococcus aureus* are heat resistant where high temperature only eliminating the bacteria but not the toxins. Several control strategies have been implemented to prevent food poisoning, by *S. aureus* but so far, the number of reported cases has not reduced.

Variety of foods including proteinaceous foods, such as meat and meat products, milk and dairy products, support the growth of *S. aureus* (Bergdoll *et al.*, 1979; FDA, 2003). The bacteria can also be found in utensils used in food preparation air, dust, sewage and water. Raw foods are less frequently implicated as the cause of staphylococcal food poisoning because *Staphylococci* do not compete well with other harmless bacteria

(Balaban and Rasooly, 2000). Cooking eliminates the normal competitive bacteria permitting the growth of contaminating *Staphylococci* in prepared foods such as meat, potato, macaroni salads, custards and bakery products.

Antimicrobials are used for growth promotion and routine disease prevention. They have also been applied sub therapeutically (Gilchrist *et al.*, 2007). Development of multiple antibiotic resistances may result from indiscriminate use of antimicrobials. Antibiotics resistance is when an organism fails to respond to a drug that it is normally susceptible to thereby rendering the drugs treatment ineffective (Devriese *et al.*, 1997). Multiple drug resistance is when the organism is resistant to many drugs. Multi- drug resistant *S. aureus* is becoming an emerging problem in the community (Livermore, 2000; Popovich *et al.*, 2007; Ribeiro *et al.*, 2007). Antibiotic-resistant strains of *S. aureus* have been detected in foods like meat, milk and dairy products (Lee, 2003; Gündoğân *et al.*, 2006; Peles *et al.*, 2007; Normanno *et al.*, 2007; Pesavento *et al.*, 2007; Pereira *et al.*, 2009). In Kenya, multi drug resistant *S. aureus* is becoming common in hospital settings and farms.

Various detection methods of staphylococcal enterotoxins in culture media supernatant such immunodiffusion, agglutination and Enzyme Linked Immunosorbent Assay (ELISA) have been developed (Orwin *et al.*, 2003). Often, these methods do not allow the detection of very low concentrations of toxins, and potential toxin-producing strains may not be discovered. Measured rates of toxin production always refer to particular culture conditions that might not represent conditions present *in vivo* (Schlievert and Case, 2007).

Several oligonucleotide probes for specific detection of staphylococcal enterotoxin genes have been developed. Techniques such as hybridization are time consuming and laborious. Cross-reactions of the probe for SEA with the SEE enterotoxin gene have been reported (Ewald *et al.*, 1990; Jaulhac, *et al.*, 1992; Tsen *et al.*, 1997). The PCR technique offers the possibility of detecting specific gene sequences by DNA amplification and therefore bacterial enrichment is not required before a specific gene can be detected (Saiki *et al.*, 1988). Detection of staphylococcal enterotoxin (SE) genes, such as *sea* and *sec*, *sea* and *seb*, and *sea* to *see* using PCR assays have been reported (Johnson *et al.*, 1991; Grabovetsky *et al.*, 1995 Schmitz *et al.*, 1998). A series of separate reactions is needed to identify a single gene or subset of these genes in all of these studies. Staphylococcal enterotoxin genes that use individual primer sets for each toxin gene by Multiplex PCR reaction are now available (Becker *et al.*, 1998). Detection of individual genes (*sea-sej*) has been developed but the method requires unique primer sets (Monday and Bohach, 1999). The current study evaluated the level of knowledge, attitudes and practices in food safety, the presence of enterotoxin-producing *S. aureus* organisms in food of animal origin in selected areas of Nairobi Kenya and determination of their antibiotic resistance profiles.

1.2 Statement of the problem

Worldwide foodborne diseases constitute a growing public health problem and a significant cause of reduced economic activity. In the United States alone annually, an estimated 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths are reported to be caused by food-borne diseases (Bennett *et al.*, 2013). Throughout the chain of preparation, food handlers play an important role in ensuring food safety. The number of reported food borne cases has not so far reduced in Kenya even if several control

strategies have been implemented. Food handlers' knowledge, attitude and practices (KAP) influence the outcome of food preparation, which reduces the risk of contamination hence food poisoning among food services is a major public health concern. *Staphylococcus aureus* has been reported to be a significant source of food borne infection worldwide due to its ability to produce several enterotoxins. When food contaminated with *S. aureus* is consumed, the enterotoxins are reabsorbed into the blood in the gastrointestinal tract activating the emetic reflex resulting in food poisoning. Due to its ability to produce over 50 genes, *S. aureus* is a significant source of food borne infection worldwide.

Over the past two decades, *S aureus* has evolved from a controllable infectious disease mainly because of increased antibiotic resistance both in hospital and in community. Food of animal origin such milk has been shown to have *S. aureus*, which can induce disease condition as well as antibiotics resistant to the human particularly to the young and old age people. With increasing prevalence of *S. aureus* strains resistant to one or several classes of antimicrobial agents, treatment of these infections becomes increasingly difficult.

1.3 Study justification

Ingestion of contaminated food and drinking water has attributed to most cases of diarrheal diseases according to previous reports (WHO, 2005). Due to limited foodborne disease investigations and surveillance in most countries including Kenya, most outbreaks go undetected. To guarantee that meals served are hygienic, food handlers plays a prime role in food production possessing. During large production of food, deliberate or accidental contamination might endanger the health of consumers, which

may have very expensive repercussions on a country. For improving the knowledge of food, handlers training programs are important but more knowledge on food safety practices might not lead to positive changes in food handling behaviors. This study describes the knowledge, attitudes and practices of food handlers, with regard to food hygiene and safety. It is anticipated that findings from this study will promote food safety knowledge amongst food handlers in order to minimize food poisoning.

Foodborne disease caused by staphylococcal food poisoning (SFP) is prevalent worldwide (Pigott, 2008). Staphylococcal enterotoxin and human food poisoning is not clear at present. To date, even when staphylococcal food poisoning outbreaks are reported, in Kenya no report of *S. aureus* strains are characterized. Identification of Staphylococcal enterotoxin genes is important to improve SFP diagnosis with the emergence of new staphylococcal enterotoxins (SEs). Multi drug resistant *S. aureus* is common in Kenyan hospital settings and farms. Extensive use of antibiotics for both prophylaxis and as growth promoters in agriculture, contributes to the development of antibiotic resistant bacteria, which has emerged as a threat to human health (Fontes *et al.*, 2013).

1.4 Research questions

- i) What is the level of knowledge, attitudes and practices of food safety by food handlers in Nairobi, Kenya?
- ii) What is the level of occurrence of enterotoxigenic strains of *S. aureus* in foods of animal origin in Nairobi, Kenya?
- iii) What is the distribution of staphylococcal enterotoxins genes in the isolated *S. aureus*?

- iv) What are the antibiotic resistance profiles of the isolated *S. aureus* strains isolated from food of animal origin?

1.5 Null Hypotheses

- i) Lack of knowledge, negative attitude and poor food safety practices do not results in poor hygiene and occurrence of foodborne diseases.
- ii) There is no relationship between occurrence of *S. aureus* and enterotoxigenic strains in foods of animal origin.
- iii) Antibiotic resistance is rare in *S. aureus* isolated from foods of animal origin.

1.6 Objectives of the study

1.6.1 General objectives

To investigate food handlers' perception, occurrence and characterization of enterotoxigenic *Staphylococcus aureus* isolated from foods of animal origin in Nairobi, Kenya.

1.6.2 Specific objectives

- i) To investigate food handlers knowledge, attitudes and practices on safety in Nairobi, Kenya.
- ii) To determine the occurrence of enterotoxigenic strains of *S. aureus* in food of animal origin in Nairobi, Kenya
- iii) To determine the distribution of staphylococcal enterotoxin genes from the isolated *S. aureus*.
- iv) To determine the antibiotic resistance profiles of *S. aureus* strains in food of animal origin in Nairobi, Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Foodborne diseases

Globally, especially in developing countries, food-borne diseases are a major public health concern, costly yet preventable. Food-borne diseases originate from ingesting contaminated foodstuffs, containing many different microbes or pathogens, like poisonous mushrooms chemical hazards or other harmful toxins that are present in foods (Kibret and Abera, 2012). WHO defines food-borne disease (FBD) as “disease of infectious or toxic nature caused by, or thought to be caused by, the consumption of food or water” (Le Loir, 2003). In the USA, foodborne microbes cause an estimated 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths annually. About 31 known pathogenic microorganisms cause 9.4 million illnesses, 56,000 hospitalizations, and 1,300 deaths annually (Scallan *et al.*, 2011). The figure may be low, because it fails to account for the foodborne infections that are asymptomatic which can result in serious disease with complications such as renal failure and death.

The contamination of food may occur at any stage in the process of preparation from food production to consumption (FAO/WHO, 2005). Food or water contaminated with pathogens or their toxins can cause foodborne diseases. Bacteria, viruses, fungi and parasites that cause foodborne diseases are often referred to as foodborne pathogens (Zhao *et al.*, 2014). Bacteria most commonly responsible for foodborne disease outbreaks includes *L. monocytogenes*, *E. coli* O157:H7, *S. aureus*, *S. enterica*, *B. cereus*, *Vibrio* spp., *C. jejuni*, *C. perfringens*, and Shiga toxin-producing *Escherichia coli* (STEC) (Scallan *et al.*, 2011; Zhao *et al.*, 2014).

Production, processing, and distribution of food increasingly takes place across vast and complex networks, each part or pathway of which must be working optimally without the introduction of contaminants and/or adulterants that could taint the final product(s).

The increased distance between the sources of production and consumption is a global phenomenon where more than two-thirds of countries are importers of food (Buzby *et al.*, 1996). Increased interactions between humans, domestic animals, and wildlife often caused by habitat destruction, changing land-use patterns, and hunting of animals for food or for the food trade, increased contact between humans, animals, and their associated microbes also increases the potential for pathogen transmission between animal species or between humans and animals.

Contaminations of foods by microbes have reduced shelf life and food quality leading to infections and poisoning outbreaks hence continuous monitoring of food processing is essential (Pike *et al.*, 2010; Cohen, 2000). Although foodborne disease has decreased in developed countries like USA, it is still a problem in the developing countries, Kenya included. Foodborne pathogens such as *S. aureus* have also been reported to be present in food of animal origin (Sofos, 2008; Syne *et al.*, 2013). This puts consumers at high risk and imposes grave economic losses to the producers due to recall of food products implicated in food poisoning.

2.2 Knowledge, Attitudes and Practices (KAP) on food safety

2.2.1 Food handlers knowledge

Food safety is a scientific discipline describing handling, preparation and storage of food in ways that prevent foodborne illness. To avoid potentially severe health hazards a number of routines are followed. Food handlers and food service establishments are

sources that contribute to food borne illness (Teferi *et al.*, 2012). Kalua, (2001) defined knowledge as the ability to recall or recognize something such as a fact concept, principle or custom. Knowledge can further be acquired through formal or informal settings either by the help of someone or alone. For everyone to make informed decisions about one's health and participate actively in promoting health of the community, knowledge is necessary (Kalua, 2001). For more than three hundred years, knowledge of the self has been considered at the core of human behaviour and seems to agree with Alfred Lord Tennyson who said, "Self reverence, self knowledge, self control, and these three alone lead to sovereign powers". Messinger urges, "He that would govern others must first master himself".

Knowledge we possess about ourselves, are central to improving our management skills. For anyone to improve or develop new capacity, one needs to know the level of capability he possesses (Whetten and Cameron, (1995). Skills and knowledge must be identified to be meaningful and applicable to use and for the business. Food service staff who had attended educational courses on food safety and hygiene were more knowledgeable about pathogens responsible for foodborne diseases (Tokuc *et al.*, 2009). Similar results have been reported in studies among food service staff in hospitals in other countries (Buchhheri *et al.*, 2007; Bas *et al.*, 2006). In Irish populace, knowledge of food risks assessed concluded on certain issues including the fact that, the characteristics of individuals influence their level of understanding. They showed that educational level and age were linked as factors that affect the understanding of food risk issues (Bas, 2006).

In the categories of high risk foods, foodborne diseases, food storage temperatures, and sources of food contamination, food handlers demonstrated excellent knowledge with a mean percentage score of $84.83\% \pm 11.71\%$ (Labib *et al.*, (2013). Nevertheless, the respondent lacked knowledge of proper method of thawing frozen food. Ninety percent thought that thawing is to keep frozen meat and broiler overnight at room temperature. According to the study, level of knowledge is influenced by age, motivation and training. Another study demonstrated that participants had good knowledge on personal hygiene and definition of foodborne diseases (Nee and Norrakiah, 2011). Food handlers have shown to display reasonable level of knowledge in personal hygiene and cross contamination (Santos *et al.*, 2008). Knowledge on food hygiene in South Africa in small and micro enterprises by food handlers had an average 46% correct answers (Marais *et al.*, 2007), while the mean food safety knowledge score in Ankara, Turkey was $43.4\% \pm 16.3\%$ (Cakiroglu and Ucar, 2008).

2.2.2 Food handlers attitudes

Attitude can be defined as a persistent tendency to feel and behave in a particular way towards some object (Luthans, 1989). Three components of attitudes are emotional, informational and behavioural. Emotional component includes the person's feelings about an object. Informational component consists of the beliefs and information the individual has about the object. Behavioural component consists of a person's tendencies to behave in a particular way towards an object. Unless something is done to change them, attitudes tend to persist. Attitudes can also fall anywhere along a continuum from very favourable to unfavourable.

Personal feelings and beliefs can direct attitudes towards some object (Luthans, 1989). Food handlers are reluctant to participate in research and fears for their possible forceful removal until assurance is given that no action will be taken against them (Kitagwa, 2005). Excellent attitudes and poor practices toward food hygiene measures by the respondents was revealed by Maryam *et al.* (2010), and this was in agreement with studies conducted elsewhere (Jevšnik *et al.*, 2007; Labib *et al.*, 2013).

2.2.3 Food handlers practices

Sanitary handling practices and good personal hygiene at work are an essential part of any prevention program for food safety. Human handling errors have been implicated in outbreaks of food poisoning although majority of food handlers have the skills and knowledge to handle food safely (Ehiri and Morris, 1996). Foodborne illnesses would never diminish due to inappropriate handling of foods by food handlers in the food service industry (Greig *et al.*, 2007). Food preparation outcome is influenced by Food handlers' knowledge, attitude and practices (KAP) (Ansari-Lari *et al.*, 2010; Baş *et al.*, 2006). In Nairobi Kenya, the risk of contamination that leads to food poisoning can be reduced by good knowledge attitude and practice.

2.3 Bacteria associated with human foodborne diseases

In 60% of cases that require hospitalization, bacteria are the causative agents of foodborne illness (Mead *et al.*, 1999). Foodborne illness is difficult to estimate internationally. In developing countries, about 2.1 million children die due to diarrheal-related illnesses annually. Food or water is suspected to be the vehicle for many of these illnesses (WHO, 2002). Food supplying consumers with nutrients is equally capable of supporting the growth of contaminating microorganisms.

Foodborne gastroenteritis worldwide is frequently caused by Staphylococcal food poisoning (SFP) (Le Loir *et al.*, 2003; Wieneke *et al.*, 1993). Between 2008 and 2010, an outbreak of bacterial foodborne diseases, involving 20,062 individuals resulting in 41 death cases in China, *S. aureus* was the fifth most frequently observed pathogen after *V. parahaemolyticus*, *B. cereus*, *B. proteus*, and *Salmonella* (Mao *et al.*, 2010).

Between 2006 and 2009, in Shenzhen, eleven outbreaks of SFP were reported representing the second most frequent cause of bacterial food poisoning after *V. parahaemolyticus*. The actual number of SFP cases is expected to be much higher than is reported since most SFP cases are mild (Mead *et al.*, 1999). A survey conducted in Kenya where 5000 samples were collected in meat chain, microbial contaminations varied between meat species and were dynamic along their supply chain. *Campylobacter* spp. (mainly *C. jejuni*) was predominant in poultry and in chicken meat, almost half of the sample were contaminated (Kariuki *et al.*, 2013).

2.4 Staphylococcal Food Poisoning

2.4.1 *Staphylococcus aureus*

S. aureus is a non-motile Gram-positive facultative anaerobe. Cells are spherical, single and often form grape-like clusters. They form spherical clusters in two planes and have no flagella. The bacteria are about 0.5 – 1.0 µm in diameter. The organism produces catalase and coagulase. *Staphylococci* form clusters while *Streptococci* forms chains, a character used to distinguish the two bacteria. Colonies of *S. aureus* on solid medium appear as golden or cream white depending on the culture media. The genus *Staphylococcus* is taxonomically in the bacterial family Staphylococcaceae that include three lesser known genera, *Gamella*, *Macrococcus* and *Salinicoccus*. *S. aureus* are able

to grow in a wide range of physical conditions for example optimum temperatures of 30°C to 37°C, and of pH 7.0 to 7.5. The organism can survive in sodium chloride concentrations of up to 15%.

A wide variety of foods that require manipulation during processing, including fermented food products can support the growth of these bacteria (Cruickshank *et al.*, 1973). Distinguishing *Streptococci* (catalase-negative) from *Staphylococci*, the catalase test is important. Catalase-positive organism produce O₂ and bubble once the test is performed by adding 3% hydrogen peroxide on an agar plate. Blood itself contains catalase so the test should not be done on blood agar (Schneewind *et al.*, 1995).

2.4.2 Factors influencing the growth of *Staphylococcus aureus*

Many multiplicity parameters, which are described as intrinsic and extrinsic factors have effects for the growth of microorganisms. These bacteria are able to grow over a much wider water activity (a_w) range than other food associated pathogens. The organism can grow at a minimum water activity of 0.83–0.86 equivalent to about 20% NaCl (Smith *et al.*, 2001). The growth conditions of *S. aureus* water activity are different from those conditions of toxin productions but this depends on the type of toxin (Ewald *et al.*, 1990).

Staphylococcal enterotoxin A and D (SEA and SED) production occur under nearly all water activity (a_w) range as long as all other conditions are optimal (Smith *et al.*, 2001). Production of Staphylococcal enterotoxin B (SEB) is very sensitive to reduced water activity and hardly any of the toxin is produced at 0.93 a_w . The effects of water activity on Staphylococcal enterotoxin C (SEC) production are the same as SEB production.

Staphylococcal enterotoxin E (SEE) production in media containing 10% NaCl has been demonstrated (Ewald *et al.*, 1990).

Factors affecting growth and SE production are the humectants used to lower the water activity, the pH, the atmospheric composition and the incubation temperature. Size of inoculum, the type of growth medium, the Sodium Chloride concentration, the temperature and the atmosphere are important parameters that influences the response of *S. aureus* to pH (Genigeorgis,1989). At a pH of 5.1 majority of *S. aureus* strains tested aerobically produced detectable amounts of SE but in anaerobic conditions, most strains did not produce detectable SE below pH 5.7 (Bergdoll, 1989; Smith *et al.*, 2001). The bacteria grow between 7 and 48 °C, with optimal temperature of 37 °C. Bergdoll, (1989) revealed that *S. aureus* was completely inactivated in milk after application of the different temperature and time conditions. Staphylococci probably become more heat resistant as the water activity is lowered to a range between 0.70 and 0.80 (Troller, 1986; Hudson, 2011).

2.4.3 *Staphylococcus aureus* behaviour in food environments

More than 50 species and subspecies of *Staphylococci* have been described according to their potential to produce coagulase. Designation between coagulase positive staphylococci (CPS) and noncoagulase-producing strains, called coagulase-negative *Staphylococci* (CNS) has been classified (Becker *et al.*, 2001). Some of CNS plays a role in the fermentation of meat and milk-based products. The CNS enterotoxigenic potential has been a subject of controversy. In most investigations cases, enterotoxin production or enterotoxin-like have failed to detect gene in CNS (Rosec *et al.*, 1997). Production of enterotoxins, in some studies showed that CNS strains could lead to food poisoning

(Vernozy-Rozand *et al.*, 1996; Zell *et al.*, 2008). Even *et al.* (2010) demonstrated that, among 129 CNS strains isolated from fermented foodstuffs, only one carried SE genes.

The main causative agent described in staphylococcal food poisoning outbreaks is *S. aureus*. *S. intermedius* has been described as a potential enterotoxigenic CPS (Becker *et al.*, 2001). Enterotoxigenic potential particularly for SEC of this species has been isolated from dogs. Such strains producing toxins raises possible health hazard, especially when carried by animals such as dogs, which come in close contact with humans (Hirooka *et al.*, 1988). In the United States *S. intermedius* was involved in one outbreak caused by blended margarine and butter (Khambaty *et al.*, 1994; Bennett, 1996). Other enterotoxigenic potentials are *S. delphini* and *S. pseudintermedius*, which is the main species, isolated from dogs.

2.4.4 Reservoirs of *Staphylococcus aureus*

The bacteria *S. aureus* can be found as a normal flora of skin and mucous membranes of mammals and birds. This bacterium when disseminates in the environment survives for long periods in the host (Schmitt *et al.*, 1990). Close adaptation of this bacterium to its host has been demonstrated and identified based on four biochemical tests namely staphylokinase, β -haemolysin production, coagulation of bovine plasma and growth on crystal violet agar following the simplified biotyping scheme described by Devriese, (1997). Based on this, human, poultry, cattle and sheep/goat were described. Many strains could not be assigned to these host-specific biotypes and belong to non host-specific (NHS) biotypes, particularly those associated with several hosts (Schmitt *et al.*, 1990).

Protein A production, and phage typing are additional biochemical test that allowed researchers to differentiate the poultry biotype from the new biotype. Protein-A test is no longer commercially available, and as phage typing cannot be routinely used, therefore these two biotypes test cannot be easily distinguished (Rosec *et al.*, 1997). Biotyping has been useful in tracing or estimating the origin of *S. aureus* in various food products despite these drawbacks (Devriese *et al.*, 1997), and for epidemiological investigations and food poisoning outbreaks in the food industry (Kerouanton *et al.*, 2007).

2.4.5 Contamination by *S. aureus*

When food or its ingredients are contaminated with enterotoxigenic strains of *Staphylococcus* spp., it will result to Staphylococcal food poisoning (SFP). To induce SFP five conditions for Staphylococci growth and enterotoxin production is required. First, there must be a source containing enterotoxin producing *Staphylococci* such as raw materials, healthy or infected carrier. Second is the transfer of *Staphylococci* from source to food, like unclean food preparation tools because of poor hygiene practices. Third is that food should compose of favourable physicochemical characteristics for *S. aureus* growth and toxinogenesis (Asao *et al.*, 2003). Temperature should be favourable and sufficient time for bacterial growth and toxin production. Lastly, food containing sufficient amounts of toxin to provoke symptoms should be ingested. Most SFPOs arise because of poor hygiene practices during processing, cooking or distributing the food product (Pereira *et al.*, 2009). Inadequate cooling of foods after contamination can induce *Staphylococcus* growth that can stimulate toxin production, resulting in food poisoning (Bergdoll *et al.*, 1989).

Transfer of *Staphylococci* found in mammals and birds to food has two main sources that include human carriage during food processing and dairy animals in cases of mastitis. The human strains are mainly involved in SFPOs but animals are known to be potential source of primary contamination. *Staphylococcus aureus* can be carried over from the udder into the milk in cases of staphylococcal mastitis of ruminants such as cows, goats or ewes. A study on 178 *S. aureus* strains associated with 31 SFPOs isolates, animal strains were demonstrated for the first time to be responsible for two outbreaks (Kerouanton *et al.*, (2007).

2.4.6 Foods associated with Staphylococcal food poisoning

Frequently involved in staphylococcal food poisoning are foods that require considerable handling during preparation, and kept at slightly elevated temperatures after preparation (Le Loir *et al.*, 2003). These foods differ widely from one country to another. In the United Kingdom, 53% of the staphylococcal food poisonings between 1969 and 1990 were due to meat products (Wieneke *et al.*, 1993). Among the staphylococcal food poisoning cases reported between 1975 and 1982, in the United States, the highest (36%) were due to red meat while in 17.1% of the cases, the food involved was unknown (Genigeorgis, 1989). This may be attributed to differences in the consumption and food habits in each of the countries.

2.4.7 Virulence and pathogenicity of *S. aureus*

The antigenic property of *S. aureus* which is the teichoic acid (Polymers of glycerol or ribitol phosphate) is attached to peptidoglycan molecules on the cell wall of the bacteria (Bukowski *et al.*, 2010). When released during bacterial cell lysis into the bloodstream may cause fever, decrease in blood pressure due to blood vessel dilation, and possibly

toxic shock (Forbes *et al.*, 2007). In some strains of *S. aureus*, there is presence of a capsule composed of protein-A (SpA) that makes the bacteria to vary from others in morphology. This can delay or prevent host polymononuclear phagocytosis by binding to immunoglobulin G (IgG) Fc (Bukowski *et al.*, 2010).

Induction of tumor necrosis factor α (TNF- α) and TNF-converting enzyme-dependent soluble TNFR1 from *Staphylococcus* protein-A (SpA) which is a potent activator has anti-inflammatory consequences, particularly in the lung. Presence of coagulase and fibrogenin on their cell wall surface, causes aggregation of this bacteria which bind together to form clusters in *cocci* seen under the microscope (Bukowski *et al.*, 2010).

Depending on a particular strain, virulence of *S. aureus* is attributed to several kinds of toxins. These include exotoxin such as exfoliatins, toxic shock syndrome toxin-1 (TSST-1), and enterotoxins. Large amounts of interleukin-1 (IL-1) by human monocytes, interleukin-2 (IL-2), and tumour necrosis factor are released after stimulation. Expression of IL-2 receptors and the proliferation of human Tcells binding to MHC class II molecules is also induced (Scholl *et al.*, 1990). Some strains may also produce bicomponent toxins such as Panton-Valentine leukocidin, beta-toxin, alpha-toxin and delta-toxin.

Clumping factor, hyaluronidase, Staphyloxanthin pigment, protein-A, coagulase, leukocidin, and biofilm production can also affect the virulence (Forbes *et al.*, 2007). Coagulase either frees or bound clots plasma and coats the bacteria cell preventing phagocytosis. The *coa* gene is a virulence factors for *S. aureus*, and expression of this gene enhances bacterial growth which promote infection in the host defense mechanisms, such as phagocytosis (Karahan *et al.*, 2009). Hyaluronic acid is broken

down by Hylurodidase, also known as spreading factor and helps the bacteria to spread. DNase (deoxyribonuclease) produced by *S. aureus* which breaks down the DNA, lipase to digest lipids, staphylokinase to dissolve fibrin aid in spread of the bacteria. Beta lactamase induce antibiotic resistance (Schneewind *et al.*, 1995).

2.4.8 Staphylococcal foodborne disease

Worldwide Staphylococcal food disease is considered as one of the most common Foodborne Diseases, which are of major concern to the public (Le Loir *et al.*, 2003; Hennekinne *et al.*, 2012). In United States of America, this organism is one of the most common causes of reported Food borne diseases (Balaban and Rasooly 2000; Murray, 2005). Vaughan and Sternberg in Michigan, USA, investigated the first documented event of SFD due to the consumption of contaminated cheese in 1884 (Hennekinne *et al.*, 2012).

Staphylococcus aureus foodborne poisoning has a rapid onset usually 3–5 hours following ingestion of contaminated food with this bacterium. During growth at permissive temperatures, these bacteria produce one or more enterotoxins (Le Loir *et al.*, 2003). The incubation period of SFD depends on the amount of toxin ingested and immunological state of an individual. As low as 0.5 ng/mL dose of Staphylococcal enterotoxins can cause food poisoning. Staphylococcal food poisoning has an abrupt onset to cause symptoms which includes nausea, vomiting, hypersalivation, abdominal cramping, with or without diarrhea. Dehydration and hypotension may occur if significant fluid is lost (Argudin *et al.*, 2010). Within 24–48 hours of onset, although self-limiting SFD can be severe, especially in infants, elderly, and immune compromised patients (Argudin *et al.*, 2010; Hennekinne *et al.*, 2012).

Reported foodborne outbreaks in 2008 by European Food Safety Authority from 27 European Union Member States, showed that *S. aureus* was the fourth most common causative agent (EFSA, 2010). An extensive staphylococcal food poisoning outbreak occurred in Kansai district in Japan affecting as many as 13,420 people (Asao *et al.*, 2003). Dairy products from a factory in Hokkaido, which experienced a transient shortage of power supply during the manufacturing process, were incriminated. In 2009 Ministry of Health, Labour and Welfare recorded 7.6% incidents caused by *S. aureus*, affecting 690 persons (Asao *et al.*, 2003).

2.5 Staphylococcal enterotoxins

Staphylococcus aureus produces a wide range of toxins. Staphylococcal enterotoxins (SEs) are a family of nine major serological types of heat stable enterotoxins that belong to the large family of pyrogenic toxin superantigens (Argudin *et al.*, 2010). *Staphylococcus aureus* synthesizes potent gastrointestinal exotoxins throughout the logarithmic phase of growth or during the transition from the exponential to the stationary phase (Derzelle *et al.*, 2009). Enterotoxins are active in high nanogram to low microgram quantities and are resistant to heat treatment, low pH and proteolytic enzymes hence retaining their activity in the digestive tract after ingestion (Argudin *et al.*, 2010; Larkin *et al.*, 2009). Staphylococcal enterotoxins share nucleotide and amino acid sequence identity (32 to 82% and 21 to 82%, respectively). Within the enterotoxin family, based upon amino acid fall one group (SEA, SEE, and SED) have 52% to 83% amino acid identity. Another group (SEB and the SECs) have 62 to 64% amino acid identity (Ren *et al.*, 1994). The percentage relatedness of amino acid differ from one staphylococcal enterotoxin to another (Orwin *et al.*, 2003).

2.5.1 Properties of Staphylococcal enterotoxins

Environmental conditions such as freezing, drying, and heat treatment and low pH destroy *S. aureus* enterotoxin-producing strain but enterotoxins are resistant to such conditions (Bergdoll, 1989). Thermal resistance is dependent on the relative purity of the SE preparation. After heating at 100 °C for 130 min, crude SEA in buffer was reduced from 21- $\mu\text{g mL}^{-1}$ to $< 1 \mu\text{g mL}^{-1}$, however, in buffer heating at 80 °C for 3 min or 100 °C for 1 min completely inactivates purified SEA (0.2 mg mL⁻¹). Crude SEB is considerably more heat resistant than purified SEA (Minor and Martha, 1972).

In food processing, heat treatments commonly used are not effective for complete destruction of SE (Bergdoll, 1989). Loss of the serological reactivity of the SE is determined by thermal inactivation. Biological activity such as clumping and coagulation may be lost before the serological activity. Therefore eating foods that have been heated after SE was produced can result in some food poisoning. The nature of the food, pH, presence of NaCl, and by the type of toxin influences thermal stability of SE. At pH 6.0 or higher than at pH 4.5–5.5 SEA, is relatively more stable to heat while SED is relatively more stable at pH 4.5–5.5 than at pH 6.0 or higher. Reactivation may occur under certain circumstances like cooking, storage or incubation if SE is not completely inactivated by heat (Bergdoll, 1989).

2.5.2 Staphylococcal enterotoxin nomenclature

In 1959 to 1960, Casman and Bergdoll first characterized SEA and SEB but since then, 22 different SEs have been described. In the chronological order of their discovery, they are designated SEA to SEIV2 except for SEF which was renamed later as TSST1 (Ono *et al.*, 2008; Thomas *et al.*, 2007; Casman, 1960; Bergdoll *et al.*, 1959). Oral

administrations in a primate model producing emetic activity made the proteins named accordingly (Lina *et al.*, 2004). Because either no emetic properties were detected or they were not tested in primate models, some of these were renamed SE-like toxins (SEI) (Thomas *et al.*, 2007; Lina *et al.*, 2004). SEs belongs to the broad family of phylogenic toxin superantigens.

Unlike conventional antigens, superantigens (SAGs) do not need processing by antigen-presenting cells (APC) before being presented to T cells. By Cross-linking major histocompatibility complex (MHC) class II molecules on APC these superantigens can directly stimulate T lymphocytes with the variable portion of the T-cell antigen receptor β chain (TCR $V\beta$) or the T-cell antigen receptor α chain for SE (TCR $V\alpha$), inducing polyclonal cell proliferation (Pu *et al.*, 2011). Super antigen binding sites lie outside the peptide-binding groove and therefore do not depend on T-cell antigenic specificity but rather on the $V\beta$ and/or $V\alpha$ region of the TCR.

Massive release of chemokines and pro inflammatory cytokines result from this, which may lead to potentially lethal toxic shock syndrome (Balaban and Rasooly, 2000). The mechanisms leading to the emetic activity are less documented although the superantigenic activity of SEs has been well characterized. Results are still limited and controversial for emesis despite the considerable efforts to identify specific amino acids and domains within SEs. For example, SEI displays weak emetic activity whereas SEIL and SEIQ are nonemetic (Ono *et al.*, 2008). The disulphide loop found at the top of the N-terminal domain of other SEs is characteristically lacking in these toxins. Although it may stabilize a crucial conformation important for this activity the loop, itself does not appear to be an absolute requirement for emesis (Hovde *et al.*, 1994).

2.5.3 Mechanisms of action of staphylococcal enterotoxin

Staphylococcal enterotoxin has not unequivocally linked specific cells and receptors in the digestive system to oral intoxication in contrast to many other bacterial enterotoxins. Staphylococcal enterotoxins have been suggested to stimulate the vagus nerve in the abdominal viscera that transmits the signal to the vomiting center in the brain (Bergdoll and Wong, 2006). A small molecular weight compound from chilli peppers that depletes peptidergic sensory nerve fibers also diminishes SE effects in mammals, which supports this idea (Hu *et al.*, 2007). Release of inflammatory mediators such as neuroenteric peptide substance P, leukotrienes, histamine causes vomiting, and the emetic response when staphylococcal enterotoxins penetrate the gut lining, activates local and systemic immune responses (Hu *et al.*, 2007).

Local immune system activation could be responsible for the gastrointestinal damage associated with SE ingestion. Several regions of the gastrointestinal tract shows inflammatory changes but the most severe lesions appear in the stomach and the upper part of the small intestine (Argudin *et al.*, 2010). Inhibition of water and electrolyte reabsorption in the small intestine is due to diarrhea associated with SEs intoxication (Larkin *et al.*, 2009). In an attempt to link the two distinct activities of SEs, that is superantigenicity and enterotoxicity, enterotoxin activity could facilitate transcytosis, enabling the toxin to enter the bloodstream and circulate through the body, allowing the interaction with antigen presenting cell and T-cells that leads to superantigen activity (Kappler *et al.*, 1997). The spread of *S.aureus* from an infection site and the circulation of SEs following ingestion could have a more effects than when the toxin remains localized (Larkin *et al.*, 2009).

2.5.4 Environmental factors on SE production by *S. aureus*

Many different protein rich foods such as meat and dairy products are often associated with SFP. Products such as cream, butter, cheeses, cream-filled pastries, sausages, ham, cooked meals, canned meat, salads, and sandwich fillings favour the growth of *S. aureus* with production of SE (Le Loir *et al.*, 2003). Cheese made from milk contaminated after pasteurization and before inoculation with lactic starter culture has been involved in outbreaks. The starter culture was reported not to have grown properly, resulting in a fermentation accident that allowed production of SE by the *S. aureus* strain (Duquenne *et al.*, 2010; Bergdoll, 1989).

Production of SE does not always accompany growth of *S. aureus*. In some few cases, toxin has been observed in non-replicating cell cultures in ham products (Bang *et al.*, 2008). Formation of SEA and prolonged *sea* gene expression were observed over the course of a week, instead of a short term growth of the bacteria (Wallin-Carlquist *et al.* (2010). Márta *et al.* (2011) observed same general pattern of unexpectedly *sed* gene expression followed by the prophage encoded SEA in ham products. Both of the genes (*sed* and *sea*) are regulated differently. Panton-Valentine leukocidin gene in *S. aureus* has also been demonstrated (Sumbly and Waldor, 2003). In USA, Chocolate milk was contaminated with *S. aureus* that was stored for 4 to 5h before pasteurization. The bacteria were reported killed during the process of pasteurization but SEs was not affected (Bennett, 1996).

2.5.5 Distribution of enterotoxigenic strains of *S. aureus*

The majority of reported Staphylococcal food poisoning outbreaks are associated with classical enterotoxins, SEA-SEE. However, enterotoxin A was the most incriminated

(Argudin, 2010). Investigation by Moustafa *et al.* (2014) revealed that 38% of the workers examined were positive for *S. aureus* in which 14% of the isolates being enterotoxigenic. Enterotoxin type A was found to be the most prevalent of isolates (68%) followed by enterotoxin C (46%), SEB (36%) and SED (18%) respectively. Results also indicated that some isolates produced more than one type of toxin. Among 61 strains isolated from raw milk cheeses, 15.9% were reported as enterotoxin producer (Rosec *et al.*, 1997). Larsen *et al.* (2000), reported only one of 414 *S. aureus* isolates from cows with mastitis carried an SE gene. A similar study done elsewhere on *S. aureus*, isolates from bovine mastitis was also indicated as SE producer (Cardoso *et al.*, 1999).

Strains isolated from milk of cows with mastitis have shown that high levels (72.8%) of the strains being enterotoxigenic (Akineden *et al.*, 2001). In US, Staphylococcal enterotoxin A (SEA) was recovered from 77.8% of all SFD outbreaks followed by SED (37.5%) and SEB (10%) respectively (Argudin, 2010). In various part of the world, SEA is the most commonly reported enterotoxin among SFD outbreaks. However, SEC and SEE are also implicated in Staphylococcal Food poisoning. Staphylococcal enterotoxin C (SEC) was involved in the SFD outbreak during 2001–2003 (Chiang *et al.*, 2008). In 2009, Six SFD outbreaks were caused by SEE present in cheese made from unpasteurized milk. Although rare, SEE has also been implicated in the SFD outbreaks in USA and UK (Argudin, 2010).

2.5.6 Detection of foodborne bacterial pathogen

Bacterial pathogens present in food are detected by culturing the microbes on an agar followed by standard biochemical identifications (Mandal *et al.*, 2011). The methods are simple and inexpensive but because of the ability of some microorganisms growing in

different culture media such as pre-enrichment media, selective enrichment media, it can be laborious and time consuming. Two to three days for identification and more than a week for confirmation of the species of the pathogens are required when using conventional methods. The methods have low sensitivity, and false negative results may occur due to viable but non-culturable (VBNC) pathogens. Failure to detect the specific foodborne pathogens would increase transmission risk resulting in food poisoning outbreaks (Zhao *et al.*, 2014). To overcome the limitations of conventional methods for the detection and identification of foodborne pathogens different rapid methods with high sensitivity and specificity have been developed.

To detect the presence of pathogens in raw and processed foods immediately, researchers are still developing novel methods with improvement in terms of rapidity, sensitivity, specificity and suitability for in situ analysis and distinction of the viable cells (Zhao *et al.*, 2014). Low numbers of pathogens in the food can also be detected using rapid methods, they are more time efficient, labor saving and able to reduce human errors. However, each of the rapid method has advantages and limitations (Mandal *et al.*, 2011).

2.5.7 Immunological methods for detection of SE

The use of anti-enterotoxin polyclonal or monoclonal antibodies has most commonly been used detecting SEs in food. Different kits are now available based on two different principles; enzyme immunoassay (EIA) comprising ELISA and enzyme-linked fluorescent assay (ELFA), and RPLA (Schlievert and Case, 2007). Lack of specificity and sensitivity of the immunological methods is a difficult task to detect contaminants in food matrices (Wieneke, 1993). Development and use of these techniques for detecting SEs is impaired by many drawbacks. First, highly purified toxins are needed to raise

specific antibodies to develop an EIA and purified toxins are difficult and expensive to obtain. Only antibodies against SEA to SEE, SEG, SEH and SEIQ are available (Schlievert and Case, 2007).

Some outbreaks remained uncharacterized without a known etiological agent because ELISA test will not detect the other SEs. False positives may occur depending on food components. Another drawback is the low specificity of some commercial kits (Wieneke, 1993). Proteins, such as protein A, may extent to interfere with binding to the Fc fragment. Fragments in immunoglobulin G (Fab) from several animal species, such as mouse or rabbit, but not rat or goat may also interfere. Endogenous enzymes, such as alkaline phosphatase or lactoperoxidase also cause some interference. It is crucial to concentrate the extract before performing detection assays regardless of the detection method used and owing to the low amount of SEs present in food (Macaluso *et al.*, 2000; Meyrand *et al.*, 1999). Conclusive diagnosis of SFPs has mainly been based on demonstrating the presence of SEs in food after enumerating CPS strains, using commercial EIA kits designed to detect SEA to SEE or using a confirmatory in house ELISA method to differentiate and quantify these types of SEs (Lapeyre *et al.*, 1996; Bennett, 2005).

2.5.8 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is one of the most commonly used molecular based method for the detection of foodborne bacterial pathogens. PCR was invented more than 30 years ago and it allows the specific target DNA sequence detection to be used to detect single bacterial pathogen present in food (Velusamy *et al.*, 2010). A specific target DNA is amplified in sequence in a three steps process cycle (Mandal *et al.*, 2011). Target

Single stranded DNA is first denatured at high temperature. Specific primers, which are the forward and reverse primer will anneal to the DNA strands in the second step. Primers complementary to the single stranded DNA will then be extended with the presence of deoxyribonucleotides and a thermostable DNA polymerase polymerization process. Products of PCR amplification are visualized on electrophoresis gel as bands by staining with ethidium bromide (Zhao *et al.*, 2014).

Genes encoding enterotoxins have been detected using PCR in strains of *S. aureus* isolated from contaminated foods (Ostyn *et al.*, 2010). Major limitations for this method are that, staphylococcal strains must be isolated from food, and results do not inform whether there in absence or presence of SEs, in food. PCR cannot be the sole method for confirming *S. aureus* as causative agent in an outbreak. However, this method being specific, highly sensitive and rapid can provide highly valuable information in characterization *S. aureus* strains involved in SFPOs (Velusamy *et al.*, 2010).

2.5.9 Multiplex PCR (mPCR)

The basic principle of mPCR is similar to conventional PCR but offers a more rapid detection method as compared to simple PCR through the simultaneous amplification of multiple gene targets. In conventional PCR assay one set of specific, primers are used but in mPCR several sets of specific primers are used. For the development of mPCR, primer design is very important, as the primer sets should have similar annealing temperature for a successful mPCR assay product (Zhao *et al.*, 2014). Primers concentration is also important factor in mPCR. The concentration of primers may need to be adjusted to ensure the production of reliable PCR products because of primer dimers (Zhao *et al.*, 2014).

Other important factors to consider for a successful mPCR assay include the PCR buffer concentrations, the balance between magnesium chloride and deoxynucleotide concentrations, the quantities of DNA template, cycling temperatures and TaqDNA polymerase (Cheah *et al.*, 2008). Two to three staphylococcal enterotoxin genes have been detected previously using mPCR. Currently five or more Staphylococcal enterotoxin can be simultaneously detected using mPCR. Multiplex PCR has been used for the simultaneous detection of genes for *S. aureus* isolates that revealed *sea* genes as dominant (Gucukoglu *et al.*, 2011). Results carried out elsewhere also indicated that 15.6% of the *S. aureus* isolates possessed the SEA gene, 9.3% had the SEB gene, and 6.2% possessed both genes (Fooladi *et al.*, 2010). *S. aureus* strains were isolated from cheese samples showed a prevalence of 7.3% being enterotoxigenic (Lamprell *et al.*, 2004). Katsuda *et al.* (2005) observed that 183 (67.8%) of 270 *S. aureus* isolates had genes coding for one or more enterotoxins. Morandi *et al.* (2007) reported that 67% of the *S. aureus* strains isolated from milk and dairy products were positive for the presence of toxin genes.

2.6 Antibiotic resistance of *S. aureus*

In many countries, the increased frequency of methicillin-resistant *S. aureus* (MRSA) associated with nosocomial infections have been documented (Henry and Frank 2009; Kock *et al.*, 2010). This has led to a tendency of multidrug resistant (MDR), leading to reduced effectiveness of antibiotics and growing healthcare costs, which is a major public health concern (Kock *et al.*, 2010). In 1943, antibiotic resistance in *S. aureus* was uncommon when Alexander Fleming of Imperial College London who observed the antibacterial activity of the Penicillium fungus when growing a culture of *S. aureus*. By 1950, 40% of hospital *S. aureus* isolates were penicillin-resistant and by 1960 had raised

to 80 % (US Meat and Poultry, 2011). The extensive use of antibiotics in animals for prophylaxis and as growth promoters in agriculture, have contributed to the development of antibiotic resistant bacteria. Antibiotic resistant *S.aureus* (ARSA) in foodstuffs linked with healthcare associated reservoirs has been reported (Smith *et al.*, 2002). Distribution of *S. aureus* on food surfaces could be transmitted through the food supply chain and this can provide the opportunity for host jumps among species, which could cause human illness (Singer *et al.*, 2003).

In many countries, Penicillin resistance is extremely common, and first-line therapy is most commonly a penicillinase-resistant β -lactam antibiotic such as oxacillin or flucloxacillin (Cosgrove *et al.*, 2009). For treatment of serious infections, such as endocarditis, combination therapy with gentamicin may be used but because of high risk of damage to the kidneys, its use is controversial (Bayer *et al.*, 1998). Treatment duration depends on the site of infection and on severity (Chamber, 2001).

2.6.1 Mechanisms of antibiotic resistance

Wide varieties of mechanisms of resistance to antibiotics by microbial pathogens have developed. Genes encoding antibiotic resistance have strong indicator of a resistance phenotype, and bacteria possessing these genes have a survival advantage (Normanno *et al.*, 2007). Genes encoding proteins mediates antibiotic resistance in bacteria as a result of exclusion, export, and/or modification of an antibiotic. Resistance may also occur because of a mutation in genes encoding proteins or processes that are targeted by antibiotics (Negri *et al.*, 2000). An enzyme that cleaves the β -lactam ring of the Penicillin molecule mediates Staphylococcal resistance to penicillin rendering the antibiotic ineffective. Antibiotics such as Nafcillin, Methicillin, Coxacillin,

Dichoxacillin, oxacillin, and flucloxacillin, which are penicillinase-resistant β -lactam, are able to resist degradation by staphylococcal penicillinase (Cosgrove *et al.*, 2009).

Methicillin resistance is mediated through the *mec* operon, part of the staphylococcal cassette chromosome *mec* (SCC*mec*). Resistance is conferred by the *mecA* gene, which codes for an alternative penicillin binding protein (PBP2a or PBP2') that has a lower affinity for binding β -lactams (Penicillins, Cephalosporin, and Carbapenems). This allows for resistance to all β -lactam antibiotics, and obviates their clinical use during MRSA infections (Normanno *et al.*, 2007). As such, the glycopeptide vancomycin is often deployed against MRSA. Resistance gene can widely be disseminated through plasmids, integrons, transposons, or other mobile genetic elements (Wright and Sutherland 2007).

Resistance of *S. aureus* to many used antibiotics is common with only 2% of all *S. aureus* isolates in the United Kingdom, are sensitive to Penicillin (Laupland *et al.*, 2006). The β -lactamase-resistant Penicillins for example Oxacillin, Cloxacillin, Methicillin, and Flucloxacillin were developed to treat Penicillin-resistant *S. aureus*, and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used. In 1959, methicillin was introduced but only two years later, the first case of MRSA was reported in England (Johnson *et al.*, 2001). In hospital settings, MRSA generally remained an uncommon finding until the 1990s, when there was an explosion in MRSA prevalence in hospitals, where it is now endemic (Blot *et al.*, 2002).

2.6.2 Methicillin-resistant *S. aureus* (MRSA) contamination and transmission

Methicillin resistant *S. aureus* abbreviated MRSA is one of a number of greatly feared strains of *S. aureus*, which have become resistant to most β -lactam antibiotics (Cosgrove *et al.*, 2009). Transfer of antimicrobial resistance in food can occur by means of residues of antibiotics in food. Ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms can also transmit resistance (Pesavento *et al.*, 2007). Animals may be colonized with *S. aureus*, but only recently, MRSA strains were isolated from several food production animals, including pigs, cattle, chicken and other animals (Normanno *et al.*, 2007).

Contact with pigs was recognized as a risk factor for MRSA carriage (de Neeling *et al.*, 2007). Pigs in particular, and also pig farmers and their families, were found colonized with MRSA and an association between the emergence of MRSA strains in pigs and the use of antibiotics in pig farming has been suggested (Pesavento *et al.*, 2007). Different foods, including bovine milk, meat products, raw chicken meat and cheese have been detected with MRSA strains (Normanno *et al.*, 2007). From 444 raw chicken meat products sampled from supermarkets in Japan 0.5% were MRSA strains (Kitai *et al.*, 2005). Strains of MRSA from samples of raw pork and beef have also been isolated in Italy (Normanno *et al.*, 2007). Jones *et al.* (2002) reported for the first time an outbreak of gastrointestinal illness caused by community-acquired MRSA in which various *S. aureus* strains were isolated from food remnants, affected people and food handlers. This isolate was resistant to Penicillin and oxacillin but sensitive to all other antibiotics tested. Foods of animal origin, such as meat and milk products have been suggested to be potential sources of resistant strains of *S. aureus*, which is a health risk to the consumers (Normanno *et al.*, 2007).

2.7 Gaps in research

To prevent food poisoning, several control strategies have been implemented but so far the number of reported cases has not reduced. According to World Health Organisation, (2002) it has been observed that 10% to 20% of food borne diseases are due to contamination by the food handlers. Hygienic measures in handling foods have been disregarded worldwide resulting in foodborne disease outbreaks. In developing countries, Kenya included information is limited on hygienic practices and appropriate ways of handling the food by food handlers that could potentially enhance the transmission of the disease to susceptible consumers. In some cases, pathogenic bacteria can come into contact with food, survive and multiply in sufficient numbers to cause illness consumers (Kasturwar and Shafee, 2011). Personal hygiene and environmental sanitation are key factors in the transmission of foodborne diseases.

Many food poisoning outbreaks investigated have successfully traced food handlers as a source of contamination but only a few that have specifically assessed knowledge, attitude and practice on meat sellers and processor. Numerous studies conducted suggested that meat and dairy products might be a potential vehicle for the transmission of enterotoxigenic strains of *S.aureus* from the farm into the community, but additional research is needed to investigate the relationship between occurrences of classical enterotoxin with corresponding gene expression. Antibiotic resistance of *S. aureus* in food has emerged as a concern of paramount importance to human health as underscored by many researchers (Daka *et al.*, 2012; Ho *et al.*, 2012; Fontes *et al.*, 2013). Continues monitoring and occurrence of multi drug resistance *S. aureus* in different kinds of foods of animal origin is however required as this is creating a major health concern.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study area was within the Central Business District of Nairobi (CBDN) and its environment, which comprised Githurai, Kahawa Wendani and City Market (Figure 3.1). A nearby pork meat processing plant known to maintain high standards of hygiene was also included in the study for comparison purposes. The study area was selected for project research due to its position as the commercial hub of the food of animal origin in Nairobi. The area is among the largest outlets of food of animal origin in Kenya and are prone to contamination with foodborne disease causing bacteria.

3.2 Study population

The study was confined to food handlers and samples of foods of animal origin outlets specifically milk shops, supermarkets and butcheries. Within this area large population of residences are served with ready to eat foods of animal origin.

3.3 Study design

The survey was a cross-sectional descriptive study. The study involved a field survey and a laboratory assessment of microbiological status of food samples. In the field survey, pre tested questionnaires were used to interview food handlers in the sampled food of animal origin to evaluate knowledge, attitude and practice of food hygiene. The laboratory analysis involved collection of milk and meat products and subsequently determination of the enterotoxigenic and resistance strains of *Staphylococcus aureus* from the samples. All bacteriological analyses were performed at Kenyatta University (KU) microbiology laboratory.



Figure 3.1 Map of Nairobi and its environs

3.4 Determination of sample size for the respondents

The minimum required sample size determination for the study was computed using Equation 1.

$$n_o = \frac{z^2 P(1-P)}{e^2} \dots\dots\dots \text{Equation 1}$$

Where:

n_o The minimum required sample size

z is the normal standard deviate for 95% confidence interval (1.962)

p is the estimated proportion of an attribute that is present in the population (The level of knowledge is unknown thus a conservative proportion of 50% will be used/gives maximum sample size)

e is the desired level of precision (10%)

Therefore; $n_o = \frac{1.962^2 \times (0.5)(1-0.5)}{0.1^2} = 96$

$$0.1^2 \qquad \qquad \qquad n_o = 96$$

Thus, the minimum required sample size for the current study is 96 participants.

3.5 Perception of food handlers on food safety

Three main topics regarding knowledge, attitude and practices of food safety were considered.

3.5.1 Pretesting of questionnaires

Questionnaires were piloted at one outlet within the study area where it was administered to ten food handlers who were not included in the actual test sample. The clarity of the questions and time to fill the questionnaires was determined during the pretesting. This was important, as it should not be perceived as disruptive of normal activities within the premises.

3.5.2 Administration of questionnaires

A modified Sharif and Al-Malki (2010) questionnaire for Knowledge, attitude and practice of the food handlers was adopted. Random cluster sampling method was used between the month of March and May 2011. Questionnaires were distributed among random selected food handlers within the study area. In Githurai and Kahawa Wendani, four questionnaires were administered in three sections that is; 200 meters, 400 meters upto approximately 800 meters away of the Thika Super highway making 12 questionnaires in each case ($12 \times 2 = 24$ questionnaires). This was decided because as one goes away from the main road, the food outlets become rare. In the City market, 5 questionnaires were administered in a radius of approximately 4 meters difference each from the center of the markets that was decided up to 20 meters, making 20 questionnaires. This was because there were no butcheries at the peripheral of the market. A total of Forty-four (44) questionnaires were from Githurai, Kahawa and City market were administered. In addition, 60 questionnaires were also administered to the

food processors (pig factory), 30 during slaughtering and 30 in processing plants making a total of 104 questionnaires. Out of these, four were badly filled and were withdrawn from research hence remaining with 100 questionnaires that were used for the study.

The questionnaire consisted of thirty-five questions divided into three parts. Part one included 15 questions about the knowledge, part two included eight questions about the attitude and part three included 12 questions about the practice (Appendix, 2). All questions about knowledge and attitude were scored on a five-point scale (0 to 4) with options of strongly agrees, agree, not sure, disagree or strongly disagree. However, the questions about practice were scored on a five-point scale (0 to 4) with options of always, most of the times, sometimes, rarely or never. The direction of the scale was (4 to 0) and reversed to (0 to 4) for some questions to check the validity of the responses.

Classification of scores less than 3 were categorized as a negative response, (answering wrong) while the scores 3 and 4 were categorized as a positive response (answering right). Permission was granted by the management of the factory for interviews to be conducted with the food handlers in the sections after a confidentiality agreement was signed (Appendix 3). Questionnaires were filled during working hours (weekdays between 9.00am and 15.00pm) without previous notification. This method of data collection ensured that participants (a) filled out the questionnaires themselves, (b) did not look up the information, (c) were able to answer the questions in their work environment, (d) were able to ask the researcher if they had any queries and (e) could be reassured that other employees/employers would not have access to their responses. To limit variations, the same interviewer conducted the interviews. Care was taken to ensure consistency of approach in the conduct of the survey on each of the food premises

3.6.1.1 Meat products from sellers

Two hundred and twenty (220) samples of meat product (80 beef chunks, 40 pork, 60 fish, and 40 sausages) were purchased from various butcheries and supermarkets within the study area. They were wrapped in sterile polythene bags and packed in a cool box containing ice, before being transported to the laboratory for analysis.

3.6.1.2 Poultry

Sixty (60) poultry carcasses were purchased from various butcheries and supermarkets within the study area. They were wrapped in clean sterile polythene bags and packed into a cool box containing ice, before being transported to laboratories for analysis.

3.6.2 Dairy products

3.6.2.1 Raw milk

A total of 40 raw milk samples each of 200 mls were collected in sterile universal bottles from milk vendors within the study area and packed into a cool box containing ice. They were transported to the laboratory and cultured within one hour after collection.

3.6.2.2 Yoghurt and Pasteurized milk

One hundred (100) samples each of 60 packets yoghurt and 40 packets of pasteurized milk, packed in 500mls were purchased from various supermarkets within the study area. They were packed well in a cool box with ice and transported to the laboratory for analysis.

3.6.3 Samples from meat processing plant

3.6.3.1 Sample from pig carcasses

In the month of March to December 2012, carcasses were randomly selected on a predetermined point on the chain and every fifth count of the carcass was selected (Figure 3.2). Both leading and trailing carcass were given equal chance of being selected during the sampling procedure. A stainless steel template (10cm x 10cm) was pressed on the meat surface and the exposed area was cut with a sterile knife. One hundred and seventy two samples which comprise 84 general body carcasses (with hair, before chlorination, after chlorination, frozen carcass) 54 carcass cuttings (surface forequarter, surface midquarter, surface hindquarter) and 34 carcasses waiting for processing (Conveyor belt, Dixie slicer machine) were removed and the portion placed in a sterile plastic bag. The samples were transported to the laboratory within 4h for analysis (Table 3.2).

Table 3.2: Sample collection per month and category from processing plant

Sample categories	2012										Total	
	March	April	May	June	July	Aug	Sep	Oct	Nov	Dec		
Sampling points												
Carcass general surface	10	10	10	10	8	8	8	8	8	4	84	
Carcass awaiting processing	5	5	5	3	3	3	3	3	2	2	34	
Product	10	10	10	10	10	10	5	5	5	4	79	
Carcass cuttings	10	10	5	5	2	2	5	5	5	5	54	
Overall	35	35	30	28	23	23	21	21	20	15	251	

3.6.3.2 Pork products

A total of 79 samples of finished pork products (19 cooked salami, 20 fresh sausage, 20 uncooked ham, and 20 Hot dog) were collected randomly from within the factory during fabrication processing. All samples were placed in an icebox and transported within 4h to the laboratory for analyses.

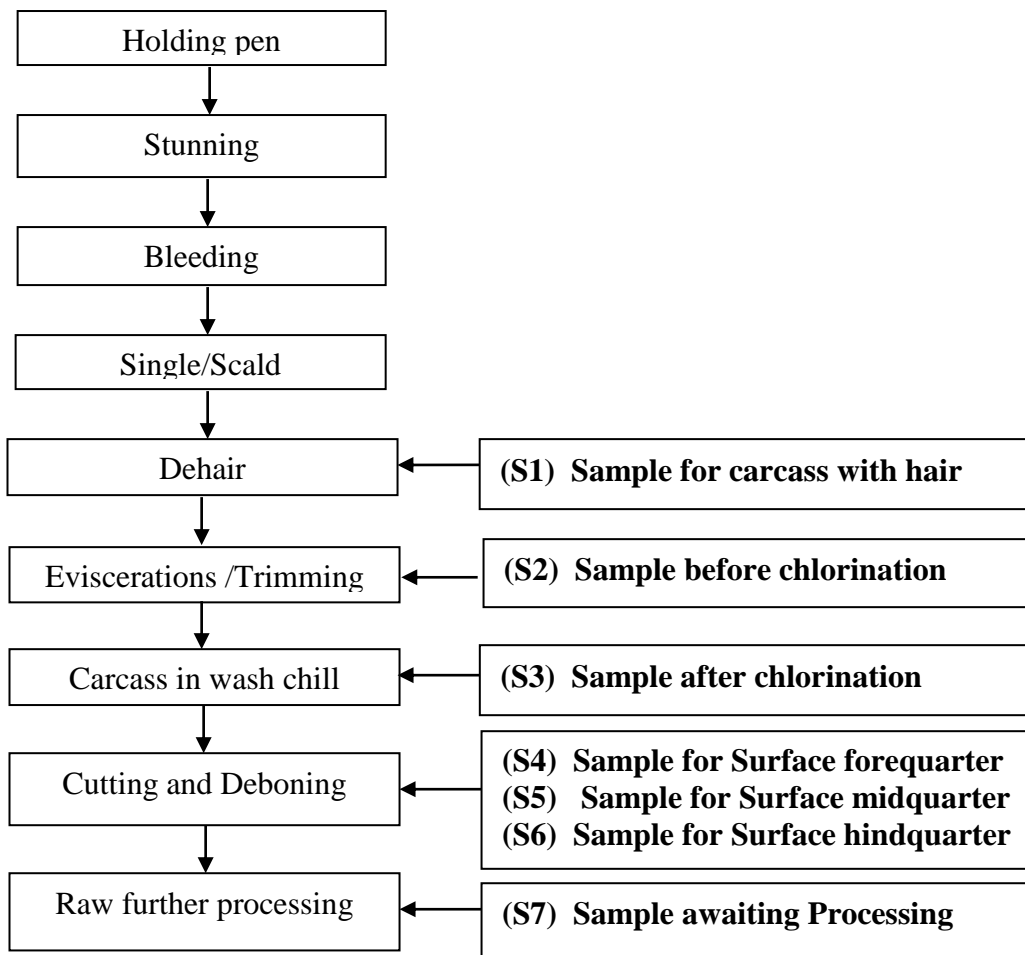


Figure 3.2: Flow chart of sampling point in pig processing plant

3.6.4 Isolation and identification of *Staphylococcus aureus*

This was done according to method described by Bennett and Lacette (2001). Prior to use the surface of agar plate (Baird-Parker agar Oxoid, Basingstoke, and Hampshire, UK) supplemented with egg yolk (Oxoid) was dried at 37°C in an incubator. *S. aureus*

isolates from the raw meat and milk samples were obtained according to the method described in the U.S Food and Drug Administration Bacteriological Analytical Manual (FDA, 1984). In this procedure, 25g of each sample was suspended in 225 ml of tryptic soy broth to which 10% NaCl had been added. The mixture was homogenized under aseptic conditions for 1min and 0.1mls of the homogenite culture were then plated onto agar and incubated at 37°C for 24h. The plates were examined for the typical colonies of *S. aureus*, which shows Dark grey-black shiny convex colonies of 1-1.5 mm diameter (18h) up to 3 mm (48h) narrow white entire margin surrounded by zone of clearing 2-5mm. The negative cultures were re-incubated for further 24h. For each plate, three typical colonies of *S. aureus*, with similar morphologies, were isolated and cultured separately on slants of Brain heart Infusion (BHI, Oxoid).

3.6.4.1 Detection of haemolysis on Blood agar

The test was done according to the method described by Bennett and Lacette (2001). Blood agar was inoculated with an overnight bacterial growth culture and incubated at 37°C for 24h. A clear zone around the colonies was considered as beta haemolytic behavior.

3.6.4.2 Catalase test

The test was done according to the method described by Bennett and Lacette (2001). Two drops of catalase test reagent (3% H₂O₂) were placed on a slide; a growth from the center of a fresh pure colony from nutrient agar plate was mixed with the reagent on the slide. The formation of bubbles indicated a positive result.

3.6.4.3 Oxidase test

The test was done according to the method described by Bennett and Lacette (2001). A piece of filter paper placed in a clean Petri dish and 2-3 drops of freshly prepared oxidase reagent were added to the filter paper. A colony from tested organisms was transferred to the filter paper and rubbed on to the reagent with a sterile wooden stick. The positive result was indicated by a violet or purple color appearance within (10-15) sec.

3.6.4.4 Clumping factor and coagulase test

The test was done according to the method described by Bennett and Lacette (2001). Briefly, an isolated pure colony was emulsified in a drop of saline on a microscopic slide with a minimum of spreading. A similar suspension of control positive and negative strains, of *S. aureus* ATCC 25923, and *S. epidermidis* ATCC 12228 respectively made. One drop of undiluted rabbit plasma was added to each suspension and mixed gently. Result was observed within 10 seconds, for the formation of visible coarse clumping, which indicated a positive result, the negative result or any slow-reacting strain was re-examined by a tube test.

3.6.4.5 Coagulase test (tube test)

The test was done according to the method described by Bennett and Lacette (2001). Citrated rabbit plasma diluted 1:5 times was mixed with an equal volume of overnight bacterial broth culture and incubated at 37°C. The formation of clots in (1-4)h indicates a positive result. Negative results were re-examined for 24h. A tube of plasma mixed with sterile broth was included as a control.

3.6.4. 6 DNase production test

The test was done according to the method described by Bennett and Lacette (2001). DNase agar was heavily streaked with the activated bacteria, and incubated at 35-37°C for 18-24h. Conversion of the medium from a blue color to a yellow colour indicated a positive result.

3.6.4.7 Tolerance of *S. aureus* to different concentrations of salt

The test was done according to the method described by Bennett and Lacette (2001). The tested isolates were grown on the surface of nutrient agar containing 7.5%, 10% and 15% concentrations of sodium chloride salt (NaCl). Appearance of growth indicated a positive result, which meant that the bacteria could tolerate the different concentrations of salt.

3.6.4.8 *Staphylococcus* identification by Analytical Profile Index kit

Species identification was performed by using the API ID Staph system kit (bioMérieux, La Balme les Grottes, Montalieu Vercieu, France). This system consists of strips containing dehydrated substrates in individual micro tubes. These tests were reconstituted by adding to each micro tube an aliquot of API-STAPH medium that has been inoculated with the strain of concern. The strip was then incubated for 8-24h at 35-37°C, after incubation, metabolism produced colour change that was either spontaneous or revealed by the reagents addition. Identification of the staphylococcal species was made by using indicators, and differential charts supplied by the manufacturer. This test system was considered as a confirmatory test for the identification of *Staphylococcus* spp.

3.6.4.9 Storage and preservation of isolated *S. aureus*

For short time preservation, single pure colonies of bacterial isolates were streaked on the nutrient agar culture plate and on the nutrient agar slants, incubated at 37°C for 24h and stored in the refrigerator at 4°C, for one and three months for the plate and slants respectively. Stock cultures of *S. aureus* isolates were maintained in 50% glycerol (w/w) at -20°C for long time preservation. Cultures were thawed and subcultured twice in tryptic soy broth for 24h at 37°C when needed.

3.7 Investigation of enterotoxigenic strains of *S. aureus*

3.7.1 Reverse Passive Latex Agglutination Assay(RPLA)

The reverse passive agglutination (SET RPLA; OXOID) test kit was used to assay for presence of staphylococcal enterotoxins in broth culture (Rosec *et al.*, 1997). The kit consists of polystyrene latex particles sensitized with purified rabbit antiserum raised against staphylococcal enterotoxin A, B, C and D. In the presence of the corresponding enterotoxin, the latex particles agglutinate. Control reagent consists of latex particles sensitized with non-immune rabbit globulins. This test was performed in V-well microlitre plates. The presence of a staphylococcal enterotoxin was indicated by an agglutination that occurs in the form of diffuse, lattice, structure, which upon settling, forms a diffuse layer on the base of the well.

3.7.2 Preparation of enterotoxin for testing

Staphylococcus aureus isolates were inoculated into Bacto-tryptic soy broth and incubated for 37°C for 24h. The broth was filtered through a membrane filter of pore size 0.22µm and the filtrate retained for enterotoxin assay. The v-shaped microtitre plates were arranged such that each row consisted of 8 wells. Five of such rows were used for

each sample and 25µl of the diluents was dispensed into each well of the five rows; and 25µl of the test sample was added to the first well of each of the five rows (Appendix 7).

Doubling dilution was performed along each of the rows using a pipette diluter. The dilution was stopped at the seventh well to leave the last well containing the diluents only. Twenty five microliters of latex sensitized anti-enterotoxin A was added to each well in the first row. Twenty five microlitres of latex sensitized with anti- enterotoxin B to each well of the second row, 25 microlitres of latex sensitized ant- enterotoxin C to each of the wells in the third row, 25 microliters of latex sensitized with anti-enterotoxin D into each of the wells in the fourth row and finally 25 microlitres of control latex into each of the wells in the fifth row. The contents of each well were mixed by rotating the plates in the micromixer. The plates were then covered with a lid to avoid evaporations.

The plates were then left undisturbed in a vibration free surface at room temperature for 20-24h, after which the wells were examined for agglutination using a reflecting mirror. The agglutinations were judged and classified as positive if there was an observable diffuse of layer of agglutination particles at the base of the well. The test was termed as negative if a tight button formed at the base of the well. Results in the row of the control latex were interpreted as negative in all cases, together with all the wells in the 8th column containing the diluents but no test sample (Rosec *et al.*, 1997).

3.8 Assessment of distribution of staphylococcal enterotoxin genes

3.8.1 DNA extraction from bacterial isolates

Total DNA was extracted from 5 mL of a coagulase-positive staphylococcal culture grown at 35°C (± 2°C) for 24h in Brain Heart Infusion (Oxoid) broth. DNA was isolated

using the DNA Purification DNeasy Blood and Tissue Kit (QIAGEN Group, Beckman Instrument, and Icl, USA) and lysozyme 10 mg.mL⁻¹ (Sigma Aldrich) as per manufacturer instructions (Rosec *et al.*, 1997) (Appendix 8). Enterotoxigenic *S. aureus* strains ATCC 13565 (*sea*), ATCC 14458 (*seb*), ATCC 19095 (*sec*), ATCC 23235 (*sed*) and ATCC 27664 (*see*) were used as positive controls and *S. epidermidis* ATCC 12228 as negative control.

3.8.2 Estimation of DNA concentration and purity

The DNA concentration was determined by using spectrophotometer; 5µL of each DNA sample were added to 995µL of distilled water and mixed well. Spectrophotometer was used for measuring the optical density (O.D.) at wavelength of 260 nm and 280 nm. An O.D of one corresponds to approximately 50µg/ml for double stranded DNA. The DNA concentration was calculated using the formula:

$$\text{DNA concentration } (\mu\text{g/ml}) = \text{O.D } 260\text{nm} \times 50 \times \text{dilution factor}$$

The Spectrophotometer was used also to estimate the purity ratio of DNA according to the following formula: DNA purity = O.D 260nm / O.D 280nm, the ratio used for detecting DNA contamination with protein preparation.

DNA quality was assessed by 0.8% agarose gel electrophoresis according to the protocol described by Maniatis *et al.* (1982).

3.8.3 Agarose gel preparation and electrophoresis

Agarose gel was prepared in 0.8 % concentrations for quality of the extracted DNA, by dissolving 0.8 g of agarose powder in 100 ml of 1X TBE buffer, and melted, then the agarose gel was cooled to 50-60°C, 5 µL of ethidium bromide dye was added with

mixing. Agarose was poured out into the gel jar to prevent bubble formation, and then cooled to 20°C. When agarose gel was poured, several wells were carefully made with a comb at one side of the gel about 5 to 10 mm away from the edge of the gel; after final solidification. The comb was carefully removed, and then the jar was put in the electrophoresis tank. Six microliters of the 1 kb DNA ladder were placed in the first left well of the agarose electrophoresis gel. Before loading to the gel wells, DNA samples were first mixed with a loading dye, so that 7 µL of each DNA sample were mixed with 3 µL loading dye. Ten µL of loaded DNA were carefully transferred to a well of the agarose electrophoresis gel, and the electrophoresis tank closed with its special lid, and then an electric current was matched (70 volt for 1 h).

3.8.4 Primers selection

The primers were selected for this study (Table 3.1), and were provided in a lyophilized form. Published DNA sequences of the *S. aureus* genes of oligonucleotides ranging from 18- to 24-mers were selected using Oligo software (version 3.4). Oligonucleotides synthesis was carried out at the International Laboratory Research Institute (ILRI) Segolab. The primers were dissolved in sterile distilled water to give a final concentration of 100 pmol/ µL as recommended by the provider and stored at -20°C until used in PCR amplification. Five genes were selected to be amplified in a sets of multiplex PCR technique. These included *sea*, *seb*, *sec*, *sed* and *see*. The *FemA* gene was used as a specific genomic marker for *S. aureus*.

Table 3.3: Nucleotide sequences, gene locations, and anticipated sizes

Gene	Primer	Oligonucleotide sequence (5-3)	Location within gene	Size of amplified product (bp)
<i>Sea</i>	SEA-1	Ggttatcaatgtgcgggtgg	349–368	102
	SEA-2	Cggcactttttctcttcgg	431–450	
<i>Seb</i>	SEB-1	Gtatggtggtgtaactgac	666-685	164
	SEB-2	Ccaaatagtgacagtagg	810-829	
<i>Sec</i>	SEC-1	agatgaagtagttgatgtatgg	432-455	451
	SEC-2	cacacttttagaatcaaccg	863-882	
<i>Sed</i>	SED-1	Ccaataataggagaaaataaaag	492-514	278
	SED-2	Attggtattttttcgttc	750-769	
<i>See</i>	SEE-1	Aggttttttcacaggtcatcc	237-257	290
	SEE-2	Cttttttcttcggtaac	425-445	
<i>femA</i>	FEMA-1	Aaaaaagcacataacaagcg	1444-1463	132
	FEMA-2	Gataaagaagaaccagcag	1556-1575	

Nucleotide sequence and locations were derived from the published sequences for *sea*, *seb*, *sec*, *sed*, *see*, and *femA* (Costa *et al.*, 2012).

3.8.5 Polymerase Chain Reaction (PCR) technique

Polymerase Chain Reaction assay was performed in a multiplex pattern in order to amplify different fragments of genes under study in a single tube for detecting *S. aureus* (*femA*) and enterotoxin. With slight modifications to the given instructions, Primer mixes were prepared according to the master mixes (Appendix 9) of components from the GeneAmp kit (Perkin-Elmer, Norwalk, Conn). Multiplex primer contained 200 mM deoxynucleoside triphosphates; 5µl of 10 times reaction buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl); 1.5 mM MgCl₂; 20 pmol (each) of *sea*, *seb*, *sec*, *see*, and *femA* primers; 40 pmol of *sed* primer; 2.5 U of Taq DNA polymerase (AmpliAmp DNA polymerase; Perkin-Elmer), and 10 to 1,000 ng of template DNA. Using sterile distilled

water the volume of the mix was adjusted to 50 μ l. To prevent evaporation, additional 100 μ l of sterile mineral oil was added to the reaction. DNA amplification was carried out in a ABI GeneAmp 9700 PCR 96 thermocycler with the following thermal cycling profile: an initial denaturation at 94°C for 5 min was followed by 35 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 7 min. PCR products were separated by electrophoresis at 80 V for 70 min in 2% agarose gel and stained with ethidium bromide. Gels were visualized in a UV transilluminator (Ultra-Lum Electronic Paramount CA, USA) and images were digitalized with a digital camera (Sony DCHX400) (Appendix 6).

3.8.6 Specificity testing

The specificity of the primers was tested by individually analyzing each primer pair and the primer pairs combined in the Multiplex PCR. The DNAs of *S. aureus* (mix of four ATCC strains-13565 *sea*, 14458 *seb*, 19095 *sec* 23235 *sed* and 27664 *see*) were used as a positive control. The specificity of the *FemA* primers was confirmed with the DNA of standard strain of *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, as negative control.

3.8.7 Reproducibility testing

The reproducibility of the Multiplex PCR products was tested by inter-assay analysis of 20 isolates (10 from meat sellers and 10 from meat processing plant), which were tested for five consecutive days.

3.8.8 Staphylococcal enterotoxin production with corresponding genes

This was undertaken using the results obtained from the reversed passive latex agglutination test (RPLA) for the detection of enterotoxin production *in vitro* and multiplex polymerase chain reaction by the *S. aureus* strains isolated from food samples.

3.9 Determination of antibiotic resistance of *S. aureus* isolates

3.9.1 Preparation of Mueller-Hinton plates

Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) was prepared as per the manufacturer's instructions (Appendix 5). The medium was cooled to 45-50°C and poured into the Petri dishes, then allowed to cool and set on a level surface to a depth of approximately 4mm. After solidification, the plates were stored at 4°C until the time of use.

3.9.2 Preparation of inocula for turbidity standard

Colonies from overnight culture of staphylococcal isolates were transferred to 5 ml tube of sterile normal saline to obtain a culture with 1.5×10^8 CFU/ml by adjusting to 0.5 McFarland standards (Appendix 10).

3.9.3 Inoculation of the test plate

A sterile swab was dipped into the inocula and then inoculated into culture plate. Prior to inoculation, care was 'taken to express excess broth from the swab, by pressing and rotating the swab firmly against the side of the tube above the level of the fluid. Rotating the plate through at an angle of 60° the swab was rubbed over the surface of the medium three times after each application. The swab was passed around the edge of the agar surface, and finally left for few minutes to dry at room temperature with the lid closed.

The antibiotic impregnated disks used were Penicillin G (1ug), Minocycline (30ug), Erythromycin (15ug), Methicillin (5ug), Co-trimoxazole (25ug), Chloramphenicol (30ug), Ampicillin (10ug) and Lincomycin (2ug). The methods were done in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2006). Discs were warmed to room temperature, and then dispensed on the agar surface. They were gently pressed down on the surface of the Mueller-Hinton Plates with sterile forceps. The plates were incubated within 30 min for 18-24h at 37°C.

3.9.4 Reading the results of susceptibility test

Plates were incubated and the diameters size of complete zone of inhibition were noted, measured in millimeters and interpreted as per the NCCLS (2006) standards. The results of these tests were recorded after 18-24h of incubation at 37°C. A standard strain of *S. aureus* (ATCC 25923) was used as a control. For typing purposes, a code profile was established based on antibiotic susceptibility of each isolate (NCCLS, 2006). Resistance was coded as “R”, intermediate as “I” and sensitivity as “S” categories by comparing zone of inhibition of growth of the test organism with standard strains.

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3.10 Statistical analysis and presentations

Data were entered using Microsoft Excel® and analyzed by IBM SPSS Statistics® 21.0 (IBM Corporation, New York). Statistical analyses involved computation of appropriate descriptive statistics. Associations between the variables were assessed by use of Chi-square (χ^2) tests. ANOVA and independent t tests were used to test the differences between means of various groups of respondents. The threshold for statistical significance was set at $p < 0.05$.

CHAPTER FOUR: RESULTS

4.1 Levels of Knowledge, Attitudes and Practices

One hundred (100) questionnaires of the recruited food handlers were filled and returned in the current study. The characteristics of the interviewed food handlers are outlined in Table 4.1. Majority of the participants were male (80.0%) and Christians (89.0%). The median [interquartile range (IQR)] age of the participants was 30.5 (26.0-38.0) years. Most of the food handlers (80.0%) who took part in the survey were more than 26 years old and constituted those in the age group of 26 to 35 years (46.0%) and those above 35 years (34.0%). Further, majority of the respondents were married (69.0%).

Table 4.1: Socio- demographic Characteristics of the study respondents

Characteristic	Frequency (n=100)	Percentages
Gender		
Male	80	80.0
Female	20	20.0
Age Category (years)		
25 or less	20	20.0
26-35	46	46.0
>35	34	34.0
Marital status		
Married	69	69.0
Single	31	31.0
Religion		
Christian	89	89.0
Muslim	11	11.0

Analysis of the education of the respondents showed that 36% and 34% had, respectively, attained secondary and post-secondary levels of education (Figure 4.1). Twenty-five of the respondents had primary school as the highest level of education attained. On the other hand, five respondents (5.0%) had no formal education.

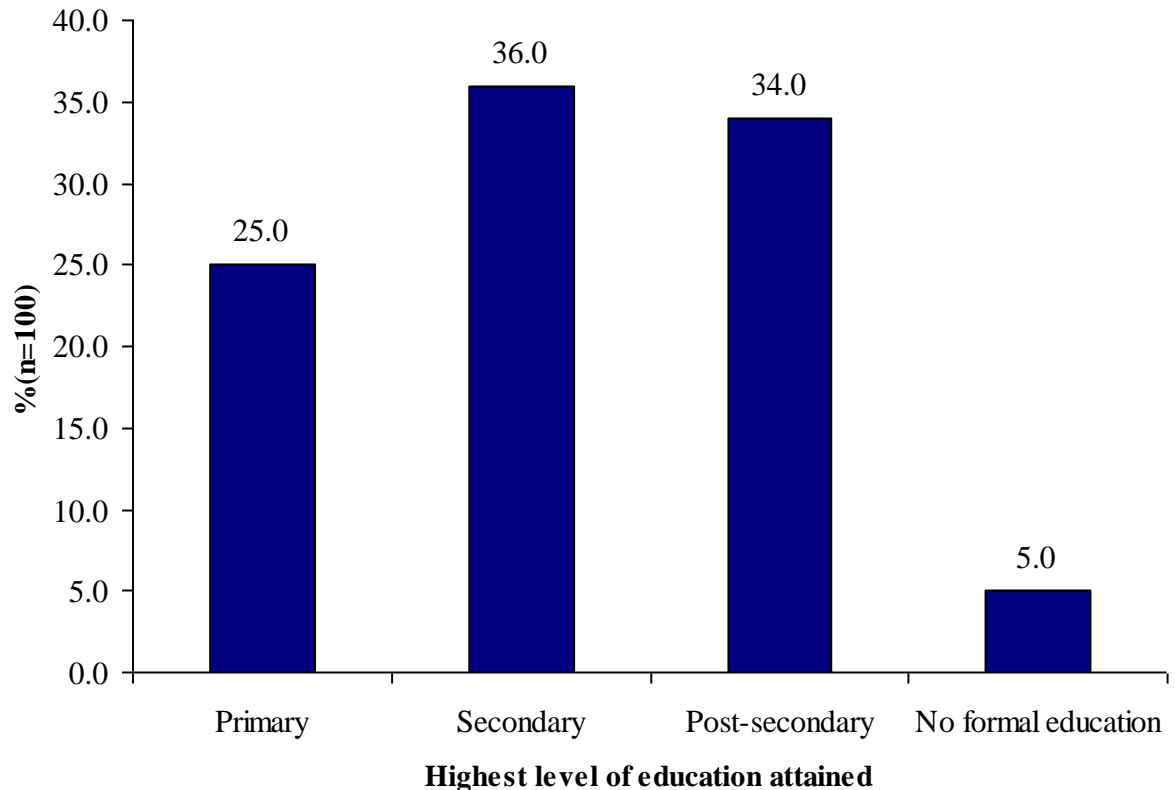


Figure 4.1: Level of education of the respondents

Two categories of food handlers took part in the study, namely, food processors (56) and food sellers (44) (Table 4.2). Examinations of the experience of the participants revealed that a vast majority (74.0%) had worked as food handlers for one year or more. Overall, 64 participants reported in the affirmative on inquiring whether they had ever undertaken a food handling and/or food safety course.

Table 4.2: Professional experience of the respondents

Characteristic	Frequency (n=100)	Percentages
Job description		
Food Processors	56	56.0
Food Sellers	44	44.0
How long have you worked as a food handler?		
Less than 1 year	26	26.0
1 year or more	74	74.0
Ever attended a food handling/safety course		
Yes	64	64.0
No	36	36.0

4.1.2 Knowledge of the respondent

The sampled food-handlers were subjected to a set of fifteen questions aimed at assessing their knowledge on various aspects of hygiene and food handling. The findings are presented in Table 4.3.

Table 4.3: Assessment of knowledge of the respondents

Attribute	*Frequency (n=100)	Percentages
Hands should be washed before handling food and after visiting the bathroom	100	100.0
Food poisoning could cause severe diseases that end in hospitalization and sometimes death	76	76.0
Apparently healthy food handlers might carry food borne pathogens	61	61.0
Eating raw or half -cooked meat is highly risky for food poisoning	95	95.0
The habit of fingering the nose raises the risk to cause food poisoning	76	76.0
Food handlers with unhygienic practice could be the source for food contamination with food poisoning pathogens	94	94.0
Eating covered leftover cooked food, kept at room temperature for more than 6 hours, is at high risk to cause food poisoning	85	85.0
Keeping food at refrigerator temperature helps to prevent food poisoning	91	91.0
Contacting ready to eat food with bare hands cause food contamination with food poisoning pathogens	85	85.0
Skin infections can contaminate food	87	87.0
The correct method for thawing frozen meat is to keep them overnight at room temperature	81	81.0
Insects such as cockroaches and flies might transmit food borne pathogens	99	99.0
Harmful bacteria multiply quickly at room temperature	95	95.0
Vegetables should be placed on higher shelf in refrigerator than meat	95	95.0
Raw and cooked meat should be kept separately	97	97.0

***Frequency represented respondents who gave a yes answer gave informative**

Table 4.3 shows the variation of the scores on knowledge by question. All respondents knew that hands should be washed before handling food and after visiting the bathroom. Additionally, an overwhelming majority of the respondents knew that insects such as cockroaches and flies could transmit food borne pathogens (99.0%) while raw and cooked meat should be kept separately (97.0%). In each case 95 food handlers (95.0%) responded correctly to the following questions; 1. ‘Eating raw or half cooked meat is highly risky for food poisoning?’ 2. ‘Harmful bacteria multiply quickly at room temperature?’ and 3. ‘Vegetables should be placed on higher shelf in refrigerator than meat?’ On the contrary, only 61 respondents were aware that apparently healthy food handlers might carry food borne pathogens. Further, 24 food handlers (24.0%) were unaware that food poisoning could cause severe diseases that may result in hospitalization and sometimes death. An equal proportion of respondents were not aware that the habit of fingering the nose raises the risk of causing food poisoning. Overall, all participants achieved 10 scores or more in the knowledge questions with the mean (standard deviation) score being 13.0 (1.3). Twelve respondents achieved the maximum knowledge score Table 4.4.

Table 4.4: Distribution of overall knowledge scores of the respondents

Overall scores (out of 15)	Frequency (n=100)	Percentages
10	2	2.0
11	15	15.0
12	16	16.0
13	26	26.0
14	29	29.0
15	12	12.0

4. 1.3 Attitudes of the respondents

Majority of the study participants (86.7%) either agreed or strongly agreed with the statement; ‘Food borne outbreaks are natural life event’ (Table 4.5). The surveyed food handlers had a favourable attitude towards training on food handling and food safety with most of them (88.0%) agreeing or strongly agreeing to the statement that; ‘Learning more about food safety through training courses is important to me’. Furthermore, most respondents (92.9%) were willing to change their food handling behaviors on realization that it was hazardous to the health of the customers and/or themselves.

Table 4.5: Assessment of attitude of the respondents

No.	Attribute	N	Strongly agree	Neutral/Strongly disagree
	Food borne outbreaks are natural life event	98	86.7	13.3
	Learning more about food safety through training courses is important to me	100	88.0	12.0
	I am willing to change my food handling behaviors when I know they are incorrect	99	92.9	7.1
	Sanitation and safe food handling are an important part of my job responsibilities	100	100.0	0.0
	It is important to regularly check the temperature of the refrigerator	99	89.9	10.1
	I believe that adequate employee hygiene can prevent food borne illness	100	97.0	3.0
	I believe being medically examined every six months is important for a food handler’s health and that of the customers	98	89.8	10.2
	I think that it is my responsibility as a food handler to ensure that meat I sell is safe	100	100.0	0.0

A substantial proportion of the respondents were in (strong) agreement with the facts that ‘adequate employee hygiene can prevent food borne illnesses’ (97.0%), ‘It is important to regularly check the temperature of the refrigerator’ (89.9%) and ‘being medically

examined every six months is important for a food handler's health and that of the customers' (89.8%). All the respondents were of the opinion that sanitation and safe food handling were an important part of their responsibilities as food handlers and that it was their responsibility to ensure that the meat/food sold is safe.

The overall distribution of the scores on attitude is presented in Table 4.6. The scores ranged from five to eight with the mean \pm SD score being 7.4 ± 0.8 . Out of eight questions assessed from the 100 participants, 3(3%) scored five, 7(7%) scored six while 38(38%) scored seven. Slightly over half (52%) of the respondents had the maximum possible score in the assessment of attitude.

Table 4.6: Overall attitude scores of the respondents

Scores	Frequency (n=100)	Percentages
5	3	3.0
6	7	7.0
7	38	38.0
8	52	52.0

4.1.4 Practices of the respondents

The samples of the food handlers were subjected to a set of twelve questions aimed at assessing practices of the food handlers. This was gauged based on a three-point rating scale (always, sometimes and never) (Table 4.7). The practice of washing hands was reportedly highly prevalent with most of the respondents always washing hands with water and soap before handling meat/food (94.0%) and after visiting toilet and/or micturition (90.0%). Moreover, 96.9% of the respondents said they always dried their

hands with towel after washing them. Quite a sizeable proportion of respondents (13.3%) admitted to not always trimming their fingernails and not cleaning the meat contact surfaces including the chopping boards always (16.0%). Furthermore, 11.3% reportedly did not always ensure that they had valid medical licenses as they at times failed to go for the biannual medical checkups. Of the food handlers interviewed only 86.9 % said that they always ensured that cooked meat was not kept at room temperature for more than 4 hours while only 90.0% always separated raw meat from ready to eat foods. All the respondents responded on the negative ('never') on inquiring whether they ate raw or half-cooked meat.

Table 4.7: Assessment of practices of the respondents

No.	Attribute	Frequency	Sometimes/Never	Always
	Wearing protective gear when handling meat	99	10.1	89.9
	Wash your hands with water and soap before handling meat/food	100	6.0	94.0
	Wash your hand with water and soap after visiting toilet and/or micturition	100	10.0	90.0
	Drying hands after washing them with towel	98	3.1	96.9
	Ensuring you go for a medical checkup every six months	97	11.3	88.7
	Checking the temperature of the refrigerator	93	8.6	91.4
	Stop working when you have 'simple' ailments, e.g., cold or lesions on your hands	100	21.0	79.0
	Trimming of finger nails	98	13.3	86.7
	Ensuring that cooked meat is not kept at room temperature for more than 4 hours	100	10.0	90.0
	Cleaning of meat contact surfaces including the chopping boards	100	16.0	84.0
	Separate raw meat from ready to eat foods	99	13.1	86.9
	Eat raw/half-cooked meat (inside is pink)	100	100.0*	0.0

***All answered never**

The overall variations in practices scores are listed in Table 4.8. The scores ranged from a minimum of 8 to a maximum of 12. The mean \pm sd score was 10.6 ± 1.1 . Out of the twelve questions assessed, 5(5%) respondents scored eight, 10(10%) scored nine, while

29(29%) scored ten and eleven respectively. Twenty-seven respondents scored the highest possible marks of 12 scores.

Table 4.8: Overall practices scores of the respondents

Score (out of 12)	Frequency(n=100)	Percentages
8	5	5.0
9	10	10.0
10	29	29.0
11	29	29.0
12	27	27.0

4.1.5 Overall knowledge, attitude and practice performance (KAP)

The total numbers of questions on assessing KAP were thirty-five (Table 4.9). The mean \pm SD score for the overall performance was 31.0 (1.9) with the range being 26 and 35 scores. One (1%) of the respondent scored twenty-six out of thirty-five while 3(3%) scored the highest possible scores of 35.

Table 4.9: Overall knowledge attitude and practice scores

Overall score (out of 35)	Frequency (n=100)	Percentages
26	1	1.0
27	4	4.0
28	5	5.0
29	10	10.0
30	19	19.0
31	21	21.0
32	13	13.0
33	19	19.0
34	5	5.0
35	3	3.0

4.1.6 Association of KAP scores by selected attributes

On conducting the independent t-test, the overall KAP scores was found not to vary significantly by marital status and religion as shown in Table 4.10. Gender wise comparisons revealed that the mean KAP scores for men and women who were working as food handlers were similar statistically ($p=0.265$). The study participants who had one year of experience as a food handler or more performed slightly better than their counterparts who had less experience as food handlers. However, this was not statistically significant ($p=0.544$).

The food handlers who had ever attended a food handling and/or food safety course had a statistically significantly higher KAP mean score than their counterparts who had reported that they had never attended a food handling and/or food safety course (respectively, 31.5 ± 0.2 versus 30.3 ± 0.3 , ($p=0.265$)). Besides, the overall KAP scores were varied significantly by the type of work the respondent was undertaking ($p=0.011$); processors were statistically significantly more knowledgeable than the sellers (31.5 ± 0.2 against 30.5 ± 0.3 respectively).

Table 4.10: Variation of knowledge attitude and practice scores

Characteristic	Frequency (n=100)	Mean	Std. Error	P-value
Marital Status				
Married	69	30.9	0.2	
Single	31	31.4	0.4	0.939
Gender				
Male	80	31.0	0.2	
Female	20	31.0	0.4	0.265
Religion				
Christian	89	31.0	0.2	
Muslim	11	31.4	0.6	0.549
Experience				
> 1 year	26	31.2	0.3	
≥ 1 year	74	31.0	0.2	0.544
Ever attended a food handling and/or safety course				
Yes	64	31.5	0.2	0.003
No	36	30.3	0.3	
Job Description				
Processor	56	31.5	0.2	
Sellers	44	30.5	0.3	0.011
Age category (years)				
≤25	20	31.4	0.4	
26-35	46	31.2	0.3	
<35	34	30.6	0.3	0.304
Education				
No formal education/Primary	5	29.6	0.9	
Secondary	61	31.1	0.2	
Post-secondary	34	31.2	0.4	0.241

Analysis of variance (ANOVA) of the overall KAP scores by age and education was also performed. There was a decline in performance with age with the performance of food handlers aged above 35 years being the lowest. Nevertheless, these variations by age were not significant statistically. Similarly, overall KAP performance seemed to improve along with the level of education though not significantly ($p=0.241$).

4.2 Levels of occurrence of *S. aureus* strains isolated

4.2.1 Total samples collected

A total of 671 samples comprising of products of food of animal origin were collected in the present study (Table 4.11). Of these, 531 samples (79.1%) were meat and meat products while the rest were dairy products (140, 20.9%). Disaggregation of the samples by source showed that most of the samples were collected from establishments that sell animal products (420, 62.6%). The remaining samples (251, 37.4%) were sourced from a processing plant dealing with pork and pork products.

Table 4.11: Characteristics of food samples of animal origin

Characteristic	Numbers of samples	Percentages
Type of animal product		
Meat	531	79.1
Dairy	140	20.9
Source		
Sellers	420	62.6
Processor	251	37.4
Total	671	100

Overall, a total of 140 (33.3 %) and 280 (66.7 %) samples of dairy products and meat (products) were collected (Table 4.12).

Table 4.12: Description of samples from selling points

Characteristic	Frequency (n=420)	Percentages
Samples from sellers		
Meat (products)	280	66.7
Beef	80	19.0
Fish	60	14.3
Sausage	40	9.5
Pork	40	9.5
Poultry	60	14.3
Dairy products	140	33.3
Milk (Raw & pasteurized)	100	23.8
Yoghurt	40	9.5

From the processing plant, samples were obtained from general body surfaces 84 (33.5%), Carcass waiting processing 34 (13.5%), Carcass Cuttings 54 (21.5%) and products 79 (31.5%) as shown in Table 4.13.

Table 4.13: Description of samples from processing plant

Characteristic	Number of samples	Percentages
Samples from processing plants		
General body surface	84	33.5
After chlorination	21	8.4
Before chlorination	21	8.4
Carcass with hair	20	8.0
Frozen carcass	22	8.8
Carcass awaiting processing	34	13.5
Conveyor table	17	6.8
Dixie slicer machine	17	6.8
Products	79	31.5
Cooked salami	19	7.6
Fresh pork sausage	20	8.0
Hot dog	20	8.0
Uncooked ham	20	8.0
Carcass Cutting	54	21.5
Surface fore quarter	18	7.2
Surface hind quarter	18	7.2
Surface hind quarter	18	7.2
Total	251	100

4. 2. 2 *Staphylococcus aureus* contamination in foods samples

Of the 671 samples examined, 251 (37.4%) were contaminated with *S. aureus*. The distribution of *S. aureus* contamination by source and type of animal product is presented in Table 4.14. Overall, 36.2% and 39.4% of the samples collected from, respectively, the sellers and processor were contaminated with *S. aureus*. The proportions of contamination of animal products from the two sources were not significantly different ($p=0.400$).

Analysis of *S. aureus* contamination by type of animal product revealed that statistically significantly more contamination was observed in meat and meat products (40.7%) as compared to dairy products (25.0%) ($p=0.001$).

Table 4.14: Distribution of *S. aureus* contamination

Characteristic	Total number of samples	<i>S. aureus</i>		p-value
		-ve (n=420)	+ve (n=251)	
Source				
Seller	420	268(63.8%)	152(36.2%)	0.400
Processor	251	152(60.6%)	99(39.4%)	
Type of animal product				
Meat (products)	531	315(59.3%)	216(40.7%)	0.001
Dairy products	140	95(67.9%)	35(25.0%)	

4.2.3 Recovery of *S. aureus* from samples by locations

Samples from Githurai had the highest contamination in terms of proportion (37.5%) followed by City Market (36.2%) while lowest proportion of *S. aureus* contamination was observed in Kahawa (35.4%) as shown in Table 4.15. Nevertheless, contamination was not significantly associated with the sampling area from where the samples were collected. There were statistically significant differences in the levels of contamination between the types of food of animal products collected from the selling outlets with meat products (40.7%) being more contaminated with *S. aureus* as compared to the dairy products 25.0% ($p=0.001$).

The variations in *S. aureus* contamination observed between various meat and meat products sampled from sellers were, however, not significantly different ($p=0.374$). Similarly, no significant differences were seen on assessment of the levels of

contamination of dairy products with *S. aureus* (yoghurt (27.5%) vs. milk (24.0%). Further investigations of contamination of dairy products with *S. aureus* revealed that pasteurized milk (5.0%) was less contaminated than raw milk (52.5%) ($p < 0.001$).

Table 4.15: Analysis of contamination of samples from sellers

Characteristic	All	<i>S. aureus</i>		p-value
		+ve	-ve	
Area sampled(n=420)				
City Market	170	61(35.9%)	109(64.1%)	
Githurai	120	45(37.5%)	75(62.5%)	
Kahawa	130	46(35.4%)	84(64.6%)	0.936
Overall	420	152(36.2%)	268(63.8%)	
Type of animal product (n=420)				
Meat	280	117(41.8%)	163(58.2%)	
Dairy	140	35(25.0%)	105(75.0%)	0.001
Overall	420	152(36.2%)	268(63.8%)	
Meat (products) (n=280)				
Beef	80	29(36.3%)	51(63.8%)	
Fish	60	29(48.3%)	31(51.7%)	
Sausage	40	13(32.5%)	27(67.5%)	
Pork	40	18(45.0%)	22(55.0%)	
Poultry	60	28(46.7%)	32(53.3%)	0.374
Overall	280	117(41.8%)	163(58.2%)	
Dairy products (n=140)				
Milk	100	24(24.0%)	76(76.0%)	
Yoghurt	40	11(27.5%)	29(72.5%)	0.666
Overall	140	35(25.0%)	105(75.0%)	
Milk (n=100)				
Raw milk	40	21(52.5%)	19(47.5%)	
Pasteurized milk	60	3(5.0%)	57(95.0%)	<0.001
Overall	100	24(24.0%)	76(76.0%)	

Among the meat and meat products collected from various selling points, fish, poultry and pork were found to be the most contaminated products (48.3% , 46.7% and 45.0% respectively) (Figure 4.2). On the other hand, the lowest contaminations were observed in sausage (32.5%) and beef (36.3%).

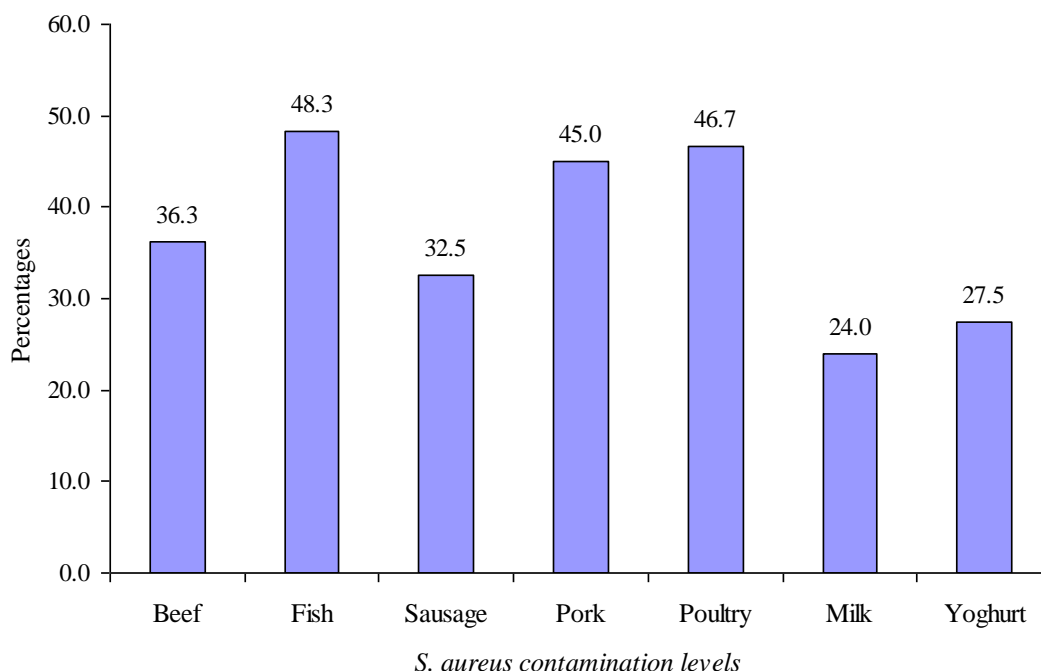


Figure 4.2: *S. aureus* contamination in meat and dairy products from sellers

4.2.4 Recovery of *S. aureus* from meat processing plant

The highest prevalence of contamination was found in samples from carcass surfaces (general body surface) (56.0%) followed by cutting surfaces (carcass cutting) (35.2%) and products (31.6%) (Figure 4.3). Machine surfaces (carcass awaiting processing) had the lowest prevalence of contamination when the samples were examined for *S. aureus* (23.5%).

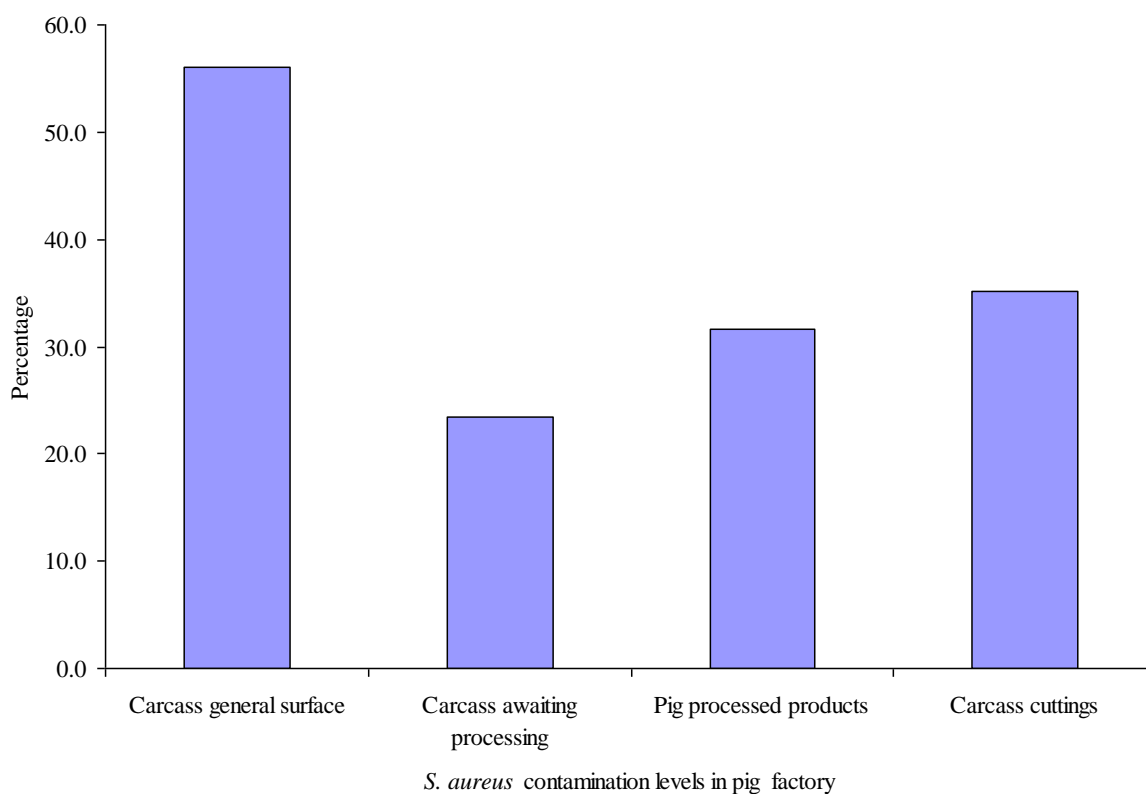


Figure 4.3: Prevalence of *S. aureus* contamination at pig processing plant

There were statistically significant differences in the proportion of contamination in samples taken from various points in the plant ($p=0.001$) (Table 4.16). Further investigations on the particulars of the *S. aureus* contamination at various sampling points are presented in Table 4.16. Highest proportion of contamination in the samples collected from carcass surfaces (general body surface) was observed in before chlorination stage (71.4%) followed by after chlorination (57.1%). Respectively, 54.5% and 40.0% of the samples collected from frozen carcass and carcass with hair were found contaminated with *S. aureus*. However, the observed variations were not significant statistically ($p=0.247$). There were no differences in the levels of contamination observed in the two area for carcass awaiting processing which were sampled, that is Conveyor table and Dixie slicer (17.6% against 29.4%, respectively) ($p=0.419$).

Likewise, no significant differences were observed on the rates of contamination based on the various carcass cuttings sampled ($p=0.348$).

Significant variations were found on examining the samples of various products and the presence of *S. aureus* ($p=0.005$). Majority of the uncooked ham samples were contaminated with *S. aureus* (60.0%). Further, 35.0% and 21.1% of the samples obtained from fresh pork sausage and cooked salami respectively were positive for *S. aureus*. Of the 20 samples of hot dog analyzed only two (10.0%) were found to be harbouring *S. aureus*.

Table 4.16: Occurrence of *S. aureus* in samples from processing plant

Sample categories	Number of samples	<i>S. aureus</i>		p-value
		+ve (n=99)	-ve(n=152)	
Sampling points (n=251)				
Carcass general surface	84	47(56.0%)	37(44.0%)	0.001
Carcass awaiting processing	34	8(23.5%)	26(76.5%)	
Product	79	25(31.6%)	54(68.4%)	
Carcass cuttings	54	19(35.2%)	35(64.8%)	
Carcass general Surface (n=84)				
Carcass with hair	20	8(40.0%)	12(60.0%)	0.247
Before chlorination	21	15(71.4%)	6(28.6%)	
After chlorination	21	12(57.1%)	9(42.9%)	
Frozen carcass	22	12(54.5%)	10(45.5%)	
Carcass awaiting processing (n=34)				
Conveyor table machine	17	5(29.4%)	12(70.6%)	0.419
Dixie slicer machine	17	3(17.6%)	14(82.4%)	
Products (n=79)				
Fresh pork sausage	20	7(35.0%)	13(65.0%)	0.005
Uncooked ham	20	12(60.0%)	8(40.0%)	
Cooked salami	19	4(21.1%)	15(78.9%)	
Hot dog	20	2(10.0%)	18(90.0%)	
Carcass cutting (n=54)				
Surface fore quarter	18	8(44.4%)	10(55.6%)	0.348
Surface middle quarter	18	7(38.9%)	11(61.1%)	
Surface hind quarter	18	4(22.2%)	14(77.8%)	

4.3 Occurrence of enterotoxigenic strains of *S.aureus*

A total of 251 *S. aureus* strains were assessed for the production of various enterotoxins (Table 4.17). Overall, 187 (74.5%) strains were found to be enterotoxigenic producing at least one type of toxin. More than a half of the enterotoxigenic *S. aureus* strains (57.8%) were found to produce a single type of toxin. The rest were either producing two (36.9%) or three (5.3%).

Table 4.17: Distribution of staphylococcal enterotoxins

Enterotoxins	Frequency	Percent
At least one enterotoxin (n=251)		
Yes	187	74.5
No	64	25.5
No. of enterotoxins (n=187)		
1	108	57.8
2	69	36.9
3	10	5.3

Investigations of specific enterotoxins produced by *S. aureus* were done (Figure 4.4). The SEs most frequently detected from the 187 enterotoxigenic strains was staphylococcal enterotoxin A (SEA) 90 (48.1%), followed by a combination of SEA and staphylococcal enterotoxin C (SEC 22) (11.8%). Sixteen strains (8.6%) were found to synthesize a combination of SEA and staphylococcal enterotoxin B (SEB) while fifteen strains (8.0%) synthesized SEA and staphylococcal enterotoxin D (SED). Additionally, nine strains (4.8%) were synthesizing SEB and SED.

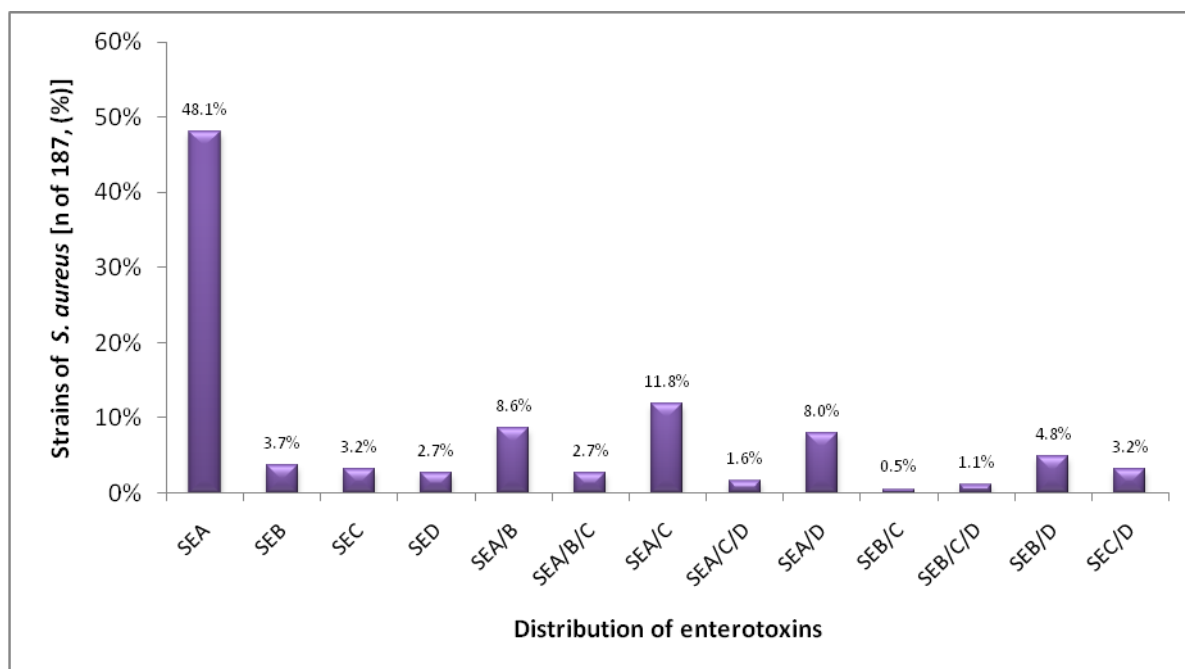


Figure 4.4: Distribution of staphylococcal enterotoxins in *S. aureus* isolates

4.4 Distribution of staphylococcal enterotoxin genes

4.4.1 Specificity and reproducibility testing by multiplex PCR

The reaction with each individual primer pair resulted in the amplification of products when DNA from each reference strain was used as a template (Figure 4 .5). Enterotoxigenic *S. aureus* strains ATCC 13565 for *sea*, ATCC 14458 for *seb*, ATCC 19095 for *sec*, ATCC 23235, ATCC 27664, *see* together with ATCC 25923 specific for *femA* gene, which is unique to *S. aureus*, were amplified with specific primers for each SEA-SEE. *S. epidermidis* ATCC 12228 (N) was used as a negative control. The bands for each PCR amplification products in the agarose gel electrophoresis was done with the specific standards strain of *S. aureus* positive controls that were used as template in the multiplex PCR are shown in figure 4.5.

Amplification of six bands were obtained when a mixture of extracted DNA from representative of each standard strain and the primers set used. Sizes of each amplicon

from the amplified product corresponded with the expected sizes (Table 3.1). DNA template from the negative standard organisms used did not yield amplification product. Although there were variations with bands intensity, their presence and sizes were the same. To test for *femA* (131pb) which is an internal control for *S. aureus*, overwhelming majority of the isolated strains (247, 98.4%) examined were found to harbour the gene of interest, which in this case was used for confirming the presence of *S.aureus*. Only four isolates of staphylococci (1.6%) examined were found to be coagulase positive by biochemical methods but did not amplify *femA* gene.

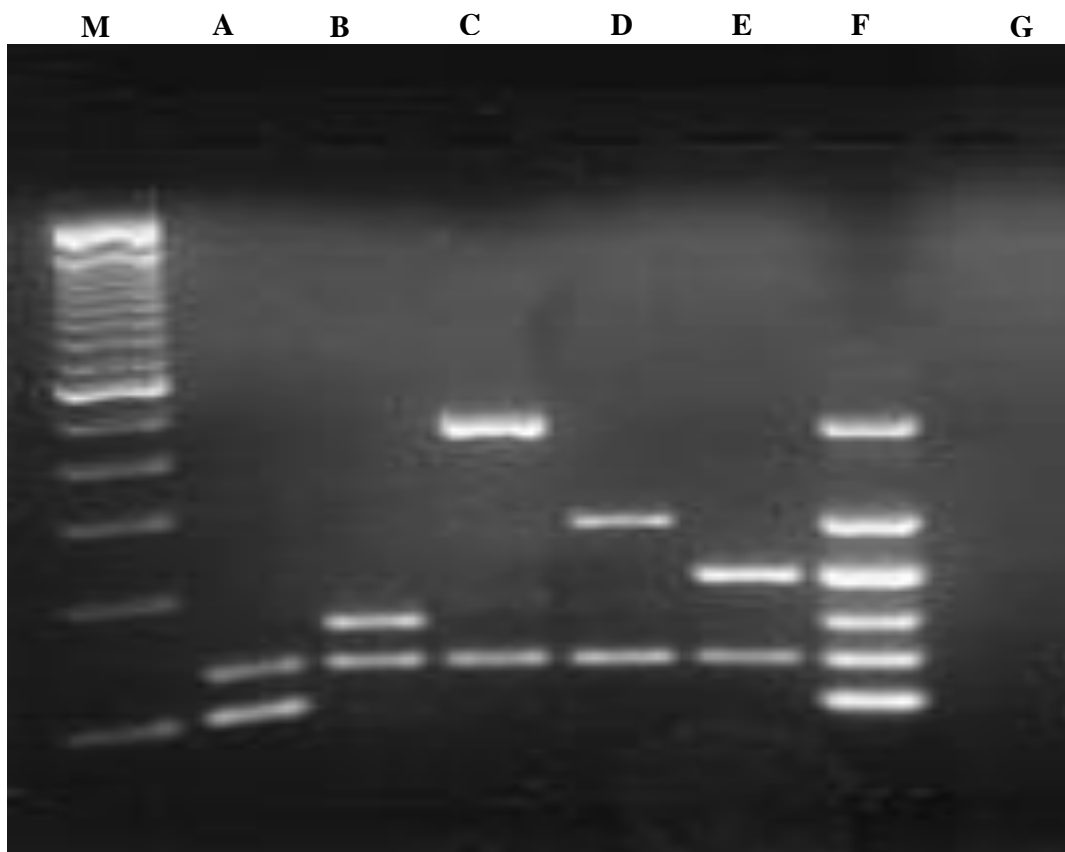


Figure 4.5: Agarose gel Electrophoresis showing amplification of staphylococcal enterotoxins standards by mPCR

Lanes M, DNA molecular size marker (100-bp ladder; lanes A to G, PCR amplicons. Lanes: A, *sea* plus *femA*; B, *seb* plus *femA*; C, *sec* plus *femA*; D, *sed* plus *femA*; E, *see* plus *femA*; F, *sea*, *seb*, *sec*, *sed*, *see*, and *femA* simultaneously; G, negative control.

4.4.2 Genetic analysis of *S. aureus* isolates by multiplex PCR

Using DNA extracts of the *S. aureus* isolates from sellers demonstrated that primers pairs produced amplification products similar with the predicted sizes (Figure 4.6). Amplification from samples that produced classical SEA, SEB, SEC and SED generated bands indicative of *sea* (102bp), *seb* (164bp), *sec* (451bp), *sed* (278bp) and *see* (164pb). Similar amplification of DNA from *S. aureus* isolates from processing plant produced PCR products consistent with those observed from the reference strains (Table 3.1 and Figure 4.7). The isolates as expected amplified *femA* (132bp) gene, which is unique for all *S. aureus*.

The DNA extracted from *S. epidermidis* used as a negative control when subjected to multiplex PCR in both gel electrophoresis did not produce any band. Some isolates were found to simultaneously produce multiple enterotoxin genes in a single reaction, which was represented by various bands formation.

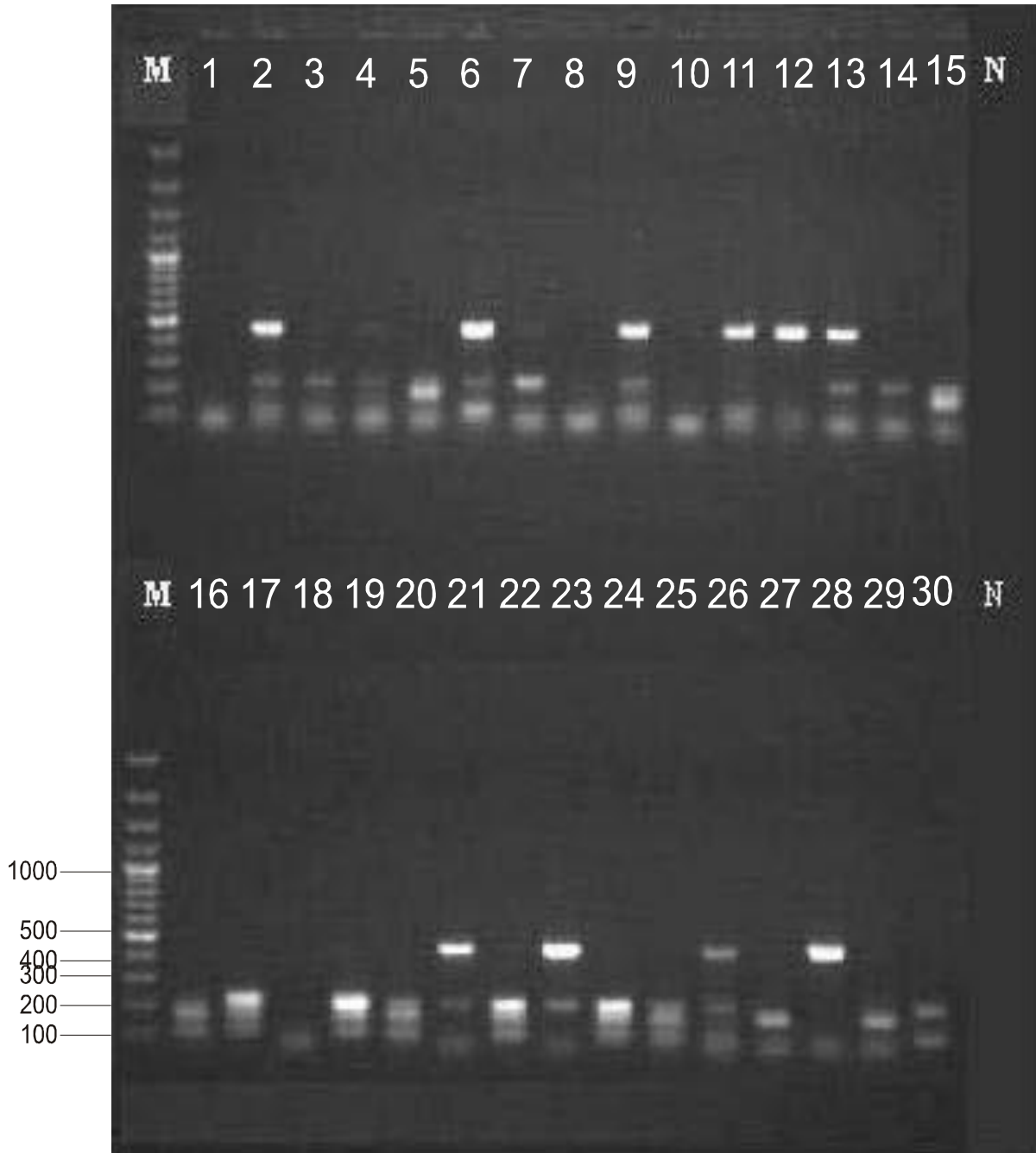


Figure 4.6: Agarose gel showing the results of amplification of staphylococcal enterotoxins by mPCR from *S. aureus* isolates from sellers

Lane M: GeneRuler 100 bp, Lane 1-30: Some examples of isolates amplifying the enterotoxin genes examined from sellers, N: Negative control.

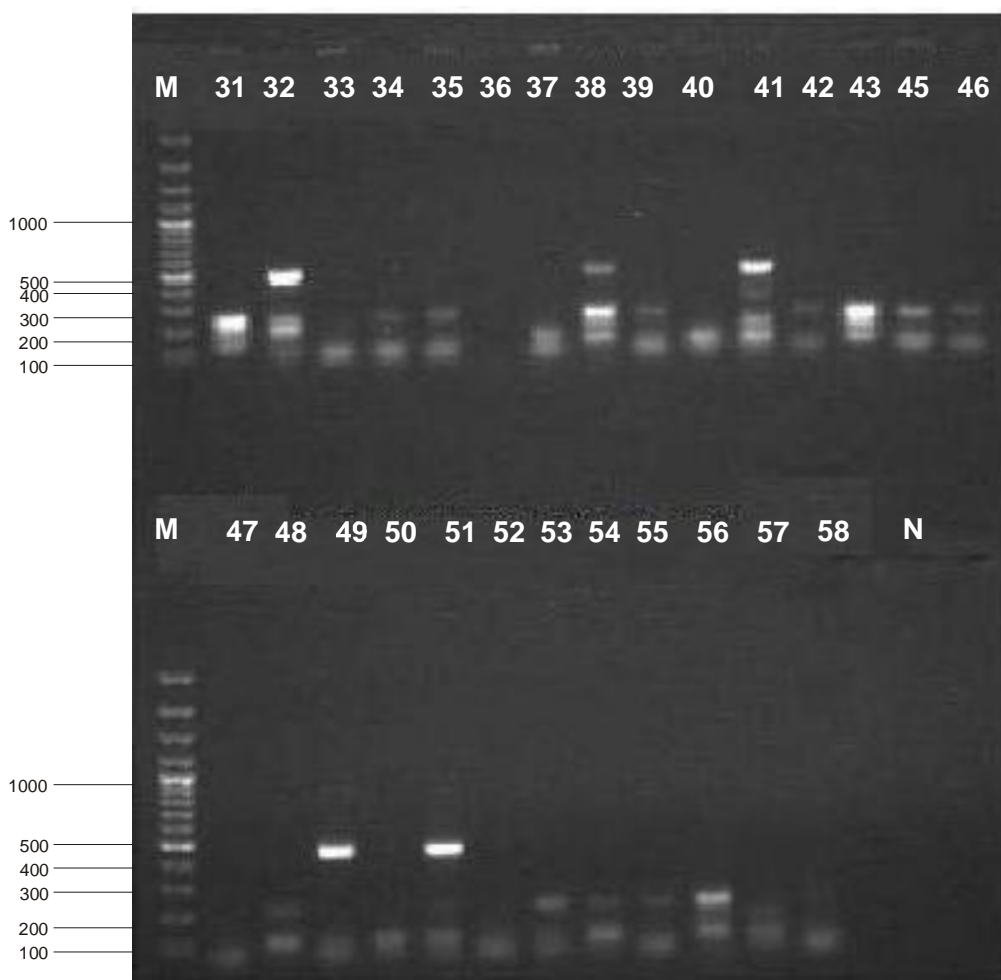


Figure 4.7: Agarose gel showing the results of amplification of staphylococcal enterotoxins by mPCR from *S. aureus* isolates from processing plant
 Lane M: GeneRuler 100bp, Lane 31-58: Some examples of isolates amplifying the enterotoxin genes examined from processing plant, N: Negative control.

4.4.3 Distribution of classical enterotoxin genes

Tabulation and analysis from the gel images revealed that out of the 251 *S. aureus* isolates, 193 (76.9%) were found to have had one or more genes encoding for enterotoxins (Table4.18). Analysis further revealed that about half of the strains 96(49.7%) coded for two genes while 65(25.1 %) and 31(16.1%) coded for one and three genes respectively. Only one strain was found to have four genes. The most frequent gene was *sea* (61.8%) followed by *see* (33.1%), *sed* (17.5%) and *sec* (15.9%) respectively. Staphylococcal enterotoxin B (*seb*) was the least occurring gene in the *S. aureus* isolates examined (13.9%).

Table 4.18: Gene coding for *S. aureus* enterotoxins.

Characteristic	Frequency	Percentage
Genes coding for toxins (n=251)		
Absent	58	23.1
Presence of one or more genes	193	76.9
No. of genes coding for toxins(n=193)		
1	65	33.7
2	96	49.7
3	31	16.1
4	1	0.01
Specifications of the genes for enterotoxins (n=357)		
<i>Sea</i>	155	61.8
<i>Seb</i>	35	13.9
<i>Sec</i>	40	15.9
<i>Sed</i>	44	17.5
<i>See</i>	83	33.1

The specifications of the combinations of genes encoding for various enterotoxins are listed in Table 4.19. Some isolates had only one gene encoding for enterotoxins, which included *Sea* (27.5%), *Seb* (2.6%), *Sed* (0.5%) and *See* (3.1%). Genes occurring in pairs included *Sea/See* (21.2%), *Sea/Sed* (9.8%), *Seb/Sed* (5.7%), *Sea/Sec* (4.7%) *Sed/See* (2.1%) and *Seb/Sec* (0.5%). The highest triple combination was *Sea/Sec/See* (8.3%) among others. Least coding combination was *Sea/Sec/Sed/See* accounting for 0.01%.

Table 4.19: Specifications of the combinations of genes encoding for toxins

Genes	Frequency (n=193)	Percentage
<i>Sea</i>	53	27.5%
<i>Seb</i>	5	2.6%
<i>Sed</i>	1	0.5%
<i>See</i>	6	3.1%
<i>Sea/Seb</i>	8	4.1%
<i>Sea/Sec</i>	9	4.7%
<i>Sea/Sed</i>	19	9.8%
<i>Sea/See</i>	41	21.2%
<i>Seb/Sec</i>	1	0.5%
<i>Seb/Sed</i>	11	5.7%
<i>Seb/See</i>	1	0.5%
<i>Sec/See</i>	2	1.0%
<i>Sed/See</i>	4	2.1%
<i>Sea/Seb/Sec</i>	2	1.0%
<i>Sea/Seb/See</i>	4	2.1%
<i>Sea/Sec/Sed</i>	1	0.5%
<i>Sea/Sec/See</i>	16	8.3%
<i>Sea/Sed/See</i>	1	0.5%
<i>Seb/Sec/Sed</i>	1	0.5%
<i>Seb/Sec/See</i>	2	1.0%
<i>Sec/Sed/See</i>	4	2.1%
<i>Sea/Sec/Sed/See</i>	1	0.0.1%

4.5 Staphylococcal enterotoxin production with corresponding gene

High levels of concordance between the genotypic and phenotypic expressions of the genes in the isolates were tested. Of the 193 isolates which were found to be harbouring genes of classical SEs (sea to see), 4 (2.1%) were found to be discordant where by the *sed* genes were detected by PCR but the corresponding toxins were not detected by the RPLA test (Table 4.20).

Table 4.20: Enterotoxin production and presence of corresponding genes

Number of Isolates				
SE type	Enterotoxin production	Percentage	Corresponding gene	Percentages
A	155	61.8	155	61.8
B	35	13.9	35	13.9
C	40	15.9	40	15.9
D	44	17.5	40	15.9
E	N/A*		83	33.1

N/A* Not tested because anti-SEE sensitized latex has not been produced in the kit.

4.6 Antibiotic resistance of *Staphylococcus aureus*

All the *S. aureus* strains isolated were subjected to antibiotic susceptibility test. Eight antimicrobial agents were selected on the bases on various studies showing resistance. Antibiotic most commonly used for veterinary and human health were also taken into consideration. Overall, 247 out of 251 strains tested positive for antimicrobial resistance to at least one antibiotic. A total of 104 (42.1%), 60 (24.3%) and 56 (22.7%) *S. aureus* isolates were resistant to three, four and two antibiotics, respectively (Table 4.21). Additionally, seven (2.8%) and nineteen isolates (7.7%) were resistant to one and five antibiotics, respectively. One isolate was resistant to six antibiotics.

Table 4.21: Resistance of *S. aureus* isolates to commonly used antibiotics

Resistance to at least one antibiotic (251)	Frequency	Percentage
Yes	247	98.4
No	4	1.6
Antibiotics resistance profiles (n=247)		
1	7	2.8
2	56	22.7
3	104	42.1
4	60	24.3
5	19	7.7
6	1	0.4

Antibiotic resistance pattern of *S. aureus* isolated showed various results with the highest percentage of resistance to Penicilin G (246; 99.6%) followed by Ampicillin (230; 93.1%) (Figure 4.8). Lower than 50% were Cotrimoxazole (89; 36%), Methicilin (67; 27.1%), Minomycin (60; 24.3%), Lincomycin (39; 15.8%) and Erythromycin 31(12.6%). The least resistance was observed in Chloramphenical (10; 4.0%). Low levels of resistance were also observed with only 10(4.0%), 31(12.6%) and 39(15.8%) *S. aureus* isolates being resistant to, Chloramphenical, Erythromycin and Lincomycin respectively. Methicillin resistance was found in 67(27.1%) of *S. aureus* isolates.

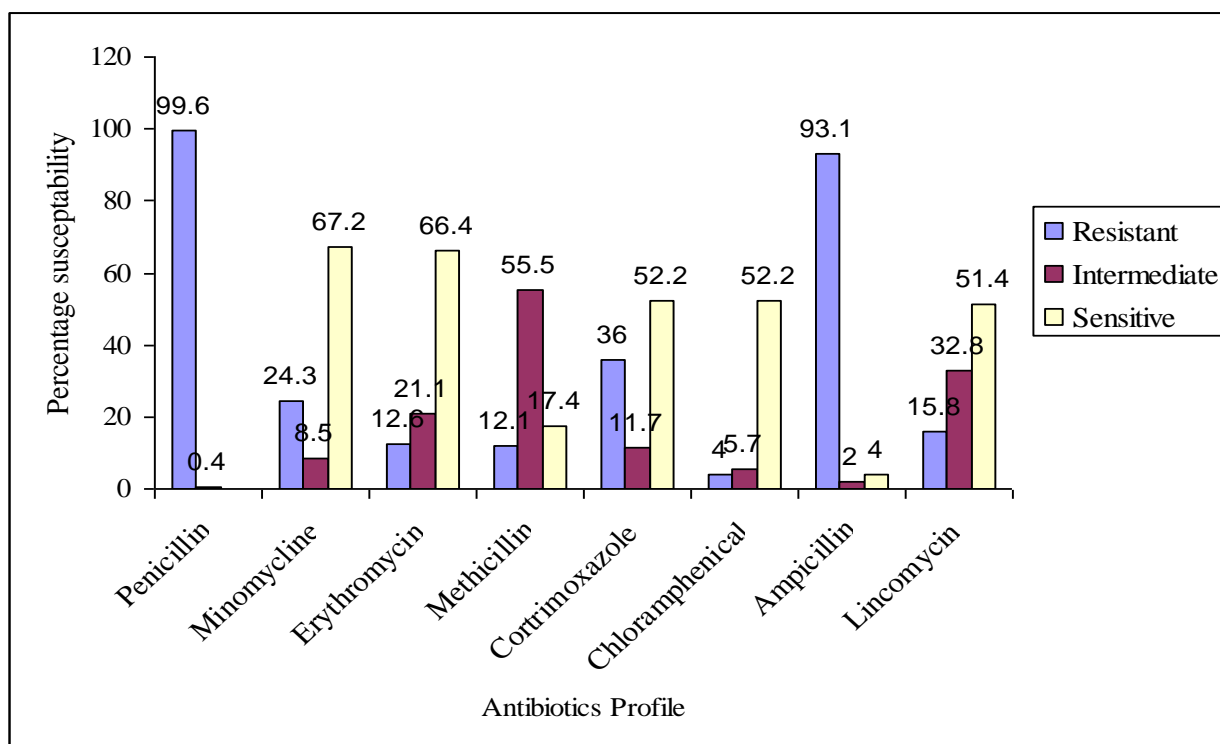


Figure: 4.8. Antibiotic resistance pattern of *S. aureus* isolates

Table 4.22 gives a summary of Multi antibiotic resistance (MAR) of *S. aureus* phenotype isolated from samples of food of animal product from the study area. The most predominant multi antibiotic resistance phenotype were Pen/Amp and Pen/Cot/Amp in 49 (19.8%) and 32 (32%), respectively. Other phenotypes include Pen/Met/Amp, Pen/Min/Amp with 11.75% each and Pen/Cot/Met/Amp with 10.5%. Only one phenotype Pen/Min/Ery/Cot/Amp/Lin (1; 0.4%) was observed from the study.

Table 4.22: Multiple antibiotic resistant phenotypes for *S. aureus*.

Phenotype	No. of strains (n=247)	Percentage
<i>Met</i>	1	0.4
<i>Pen</i>	6	2.4
<i>Pen/Amp</i>	49	19.8
<i>Pen/Amp/Lin</i>	13	5.3
<i>Pen/Cot</i>	2	0.8
<i>Pen/Cot/Amp</i>	32	13.0
<i>Pen/Cot/Amp/Lin</i>	3	1.2
<i>Pen/Cot/Chl/Amp</i>	2	0.8
<i>Pen/Cot/Chl/Amp/Lin</i>	1	0.4
<i>Pen/Cot/met/Amp</i>	26	10.5
<i>Pen/Ery/Cot/Amp</i>	9	3.6
<i>Pen/Ery/Cot/Amp/Lin</i>	1	0.4
<i>Pen/Ery/Met/Cot/Amp</i>	5	2
<i>Pen/Lin</i>	3	1.2
<i>Pen/Met/Amp</i>	29	11.7
<i>Pen/Met/Amp/Lin</i>	1	0.4
<i>Pen/Met/Cot/Chl/Amp</i>	3	1.2
<i>Pen/Min</i>	2	0.8
<i>Pen/Min/Amp</i>	29	11.7
<i>Pen/Min/Amp/Lin</i>	6	2.4
<i>Pen/Min/Chl/Amp</i>	3	1.2
<i>Pen/Min/Cot/Amp</i>	2	0.8
<i>Pen/Min/Ery</i>	1	0.4
<i>Pen/Min/Ery/Amp/Lin</i>	7	2.8
<i>Pen/Min/Ery/Amp</i>	6	2.4
<i>Pen/Min/Ery/Cot/Amp/Lin</i>	1	0.4
<i>Pen/Min/Ery/Cot/Lin</i>	1	0.4
<i>Pen/Min/Lin</i>	1	0.4
<i>Pen/Min/Met/Amp</i>	1	0.4
<i>Pen/Cot/Met/Amp/Lin</i>	1	0.4

Pen , Pencilin ; Min, Minomycin ; Ery, Erythromycin; Cot, Cotrimoxazole; Amp, Ampicillin; Lin, Lincomycin; Met, Methicilin; Chl, Chloramphenical.

Table 4.23 outlines the resistance profiles of *S. aureus* isolates from samples collected from sellers to individual antibiotic. An overwhelming proportion of the isolates from different products were resistant to Penicilllin with the overall resistance being 96.7%. Likewise, most of the isolates were resistant to Ampicillin (139, 91.4%).

On the other hand, no isolate was found to be resistant to Lincomycin. Resistance of isolates to Chloramphenicol and erythromycin was low (5.9% and 9.2%, respectively). Resistance of *S. aureus* isolates to Minocycline, Methicillin and Cotrimoxazole showed significant variations across the range of products samples ($p < 0.001$, $p < 0.001$ and $p = 0.133$ in each case).

Table 4.24 outlines the resistance profiles of *S. aureus* isolates from samples collected from processor to individual antibiotic. All the *S. aureus* isolates were resistant to Penicillin. Majority were also resistant to Ampicillin (91.9%). Only one isolate derived from cooked *salami* showed resistance to Chloramphenicol. A total of 39 (39.4%) and 37 (37.4%) isolates were resistant to Lincomycin and Minocycline respectively. There were statistically significant differences in the resistance profiles of isolates from different samples to various antibiotics including Minocycline, Erythromycin, Methicillin, Cotrimoxazole and Lincomycin $p < 0.05$.

Table 4.24: Resistance profiles of *S. aureus* isolates from processing plant

Antibiotic	Carcass general surface (n=84)						Carcass awaiting processing (n=34)			Products (n=79)		Carcass cutting (n=54)				Total (n=251)	P-value		
	Carcass general surface	Carcass awaiting processing	Product	Carcass cuttings	Carcass with hair	Before chlorination	After chlorination	Frozen carcass	Conveyor table machine	Dixie slicer machine	Fresh pork sausage	Uncooked ham	Cooked salami	Hot dog	Surface fore quarter			Surface mid quarter	Surface hide quarter
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%			%	%
Penicillin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	1.000
Minocycline	44.7	50.0	48.0	0.0	12.5	75.0	60.0	16.7	40.0	66.7	71.4	41.7	25.0	50.0	0.0	0.0	0.0	37.4	<0.001
Erythromycin	8.5	25.0	28.0	21.1	12.5	0.0	13.3	8.3	40.0	0.0	71.4	8.3	25.0	0.0	12.5	0.0	42.9	17.2	0.026
Methicillin	17.0	12.5	0.0	57.9	50.0	0.0	13.3	16.7	0.0	33.3	0.0	0.0	0.0	0.0	37.5	50.0	85.7	20.2	<0.001
Cotrimoxazole	21.3	12.5	16.0	57.9	62.5	0.0	6.7	33.3	0.0	33.3	14.3	8.3	50.0	0.0	75.0	25.0	57.1	26.3	<0.001
Chloramphenicol	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	1.0	0.954
Ampicillin	95.7	100.0	80.0	94.7	87.5	100.0	93.3	100.0	100.0	100.0	85.7	66.7	100.0	100.0	87.5	100.0	100.0	91.9	0.222
Lincomycin	14.9	87.5	100.0	0.0	0.0	0.0	0.0	58.3	100.0	66.7	100.0	100.0	100.0	100.0	0.0	0.0	0.0	39.4	<0.001

CHAPTER 5: DISCUSSION

5.1 Knowledge, Attitude and Practice

In developing countries, Kenya included, food handlers play a major role in transmitting pathogens from contaminated sources. Knowledge, attitude and practice on food hygiene are three factors that play major roles in the occurrence of food poisoning associated with food handlers (Sharif and Al-Malki, 2010). The findings from the present study indicated a high level of knowledge, attitudes and practices among the food handlers. The studies revealed that majority of the participants were male (80.0%), Christians (89.0%) and married (69.0%). This result agrees with a study in Nagpar, India and Hail, Saudi Arabia where majority of respondents were 62.7% and 62% male, respectively (Kasturwar and Shafee, 2011). These finding are in contrast with studies conducted in Slovenia and Nigeria where majority food handlers were females, that is 88.8% and 65.1%, respectively (Jevsnik *et al.*, 2007). In their study, it was noted that employers mostly employ females because they maintain proper personal and food hygiene. The difference in proportions could be attributed to the fact that the current study concentrated on food establishments dealing with animal products including butcheries, which entails handling of heavy carcasses, thus a preference for males.

This study revealed overall the food handlers' knowledge score was high. All participants achieved 10 scores or more out of 15 in the knowledge questions with the mean (standard deviation) score being 13.0 (1.3). Twelve respondents achieved the maximum knowledge score. This is in contrast with that performed in small and micro-enterprises in South Africa, to assess food handlers' knowledge on food hygiene and the average percentage of correct answers, which was 46.0% (Marais *et al.*, 2007).

The mean food safety knowledge score of food handlers in Ankara, Turkey was 43.4% (Çakiroglu and Ucar, 2008). The food handlers expressed excellent knowledge in all the categories including high risk foods, food borne diseases, food storage temperatures, and sources of food contamination.

Nevertheless, lower levels of knowledge was demonstrated on enquiring about the proper method of thawing frozen food, where 81% of the food handlers reported that the correct method for thawing frozen meat is to keep them overnight at room temperature. To prevent the growth of harmful bacteria in meat and poultry, it is essential to keep the meat cold while it is defrosting. Hence, the best way to safely thaw meat is in the refrigerator since there is greater danger of bacterial growth and food spoilage when food is thawed at room temperature. Defrosting meat more rapidly can also be done using a microwave. Clean cold water in a sink or in a container may also be used to thaw food (Çakiroglu and Ucar, 2008). The present study is in concordant with a survey done in Accra, Ghana by Annor and Baiden, (2011). On posing the same question to the food handlers, they found that 90% of the food handlers thought that the correct method for thawing frozen meat and broiler is to keep them overnight at room temperature. A substantial proportion of the respondents (39%) did not know that apparently healthy food handlers might carry food borne pathogens.

Generally, just like in the levels of knowledge, the food handlers' practice towards food safety and handling was high. On comparison, higher scores in attitudes were found in a study conducted in Jordan than the current study (Sharif *et al.*, 2013). The high mean score of food handlers in the Jordan can be explained by the way the two research settings were conducted.

The survey done in Jordan was conducted in a military hospital and the “obeying of orders” and attitude of military workers could justify the discordance of the results between the two studies. Indeed, the study reported significant differences ($p < 0.05$) in the mean percentage between the military (91.1) and the civilian (78.3) food handlers with the civilian score being significantly lower than those of the military food handlers.

The practice of washing hands was reportedly highly prevalent. Majority of the respondents always washed hands with water and soap before handling meat/food (94%) and after visiting the toilet /or micturition (90%). Additionally, 97% of the respondents always dried their hands with a towel following hand washing. On the contrary, other studies showed that the extensive knowledge of the correct practices for hand hygiene do not necessarily translate into practice. For instance, an observation study carried out by Green *et al.*, (2006) showed that workers removed their gloves, if worn, then placed their hands under running tap water. The most appropriate way of hand washing is running water with soap and drying hands with paper or cloth towels. Workplace pressures and/or lack of structural, staff, facilities and accessibility to supplies of soap and towel are some of the contributing factors.

In this study, regular cleaning of meat contact surfaces, including the chopping boards and utensils was done regularly by 84% of the sampled food handlers. This is a crucial step in the food establishments as it drastically reduces the risk of cross-contamination of food. A vast majority of the food handlers sampled from the military hospitals (99%) in Jordan practiced proper cleaning and disinfecting procedure of premises, surfaces and utensils. This could be attributed to the high levels of discipline associated with the armed forces. In discordance with present study, a survey carried out in Ireland showed

that 21.5% of all the chefs interviewed suggested that the use of disinfectants in sanitizing worktops is not an essential step (Bolton *et al.*, 2008). According to Garbutt, (1997) unless chopping boards or knives, which have been used to prepare raw meat, are washed thoroughly, ready-to- eat foods must never be prepared using these items. In line with this, a substantial proportion of the food handlers interviewed (87%) said that they always ensured that they separated raw meat from ready to eat foods.

Enquiries on the storage position of a vegetable salad with respect to meat, most of the study participants (95%) also knew they were to place the vegetables in higher shelves in refrigerators than meat. This ensures that contamination does not arise from the drips of the meat. An almost similar proportion (92%) was reported in a study conducted in the United States of America (USA) by the Environmental Health Specialists Network (EHS-Net), a network of environmental health specialists focused on the investigation of contributing factors to food borne illness, including food preparation practices and hand washing practices (Green *et al.*, 2006). On the other hand, research involving food handlers working in food businesses in Accra, Ghana, found that 23.8% of the respondents reported that they would place the salad and the meat side by side. Besides, 4.8% were not certain where to place the vegetable salad (Annor and Baiden, 2011).

A sizeable proportion of the respondents (21%) reported that they do not stop working when they had 'simple' illnesses such as cold or lesions on their hands. A hand-to-mouth existence prevails for most food handlers, their families, and the greater consideration to loss of income if they do stop working may perhaps override the effect of knowledge. Additionally, some food handlers who consciously overlook the safety risks associated with food handling during cases of ailments may perhaps believe that the risks are

outweighed by their culinary prowess or that they have sufficient knowledge and experience with foods to control the degree of risk. These lines of reasoning have been cited by some studies (Azanza *et al.*, 2005) as justifications used by food handlers who were identified as a cause of food borne diseases by taking short cuts in food handling to the detriment of food wholesomeness.

The food handlers' attitudes towards food safety was found to be favourable which is in concordance with the findings from a survey by Sharif *et al.* (2013) where the mean score for the practice assessment was high (90%). The surveyed food handlers had a favourable attitude towards training on food handling and food safety with most of them (88.0%) agreeing or strongly agreeing to the statement that; '*Learning more about food safety through training courses is important to me*'. Similar findings among food handlers at a residential college and canteen with a majority of the food handlers (72.3%) stating that learning more about food hygiene was imperative for them was reported in Malaysia (Nee and Norrakiah, 2011).

All the respondents in the current study were of the opinion that it was their responsibility as food handlers to ensure that the meat they sold was safe. The overall KAP scores in this study did not significantly differ for marital status and religion. The overall mean KAP scores for men and women were similar statistically ($p=0.265$). This is in disagreement with a survey done in hospitals in Jordan where females had a higher mean KAP percentage score as compared to their male counterparts (Sharif *et al.*, 2013).

Further, Sanlier and Konaklioglu, (2012) found a statistically significant difference between male and female participants on the total food safety knowledge, attitude and practice. The latter study dealt with University students in Turkey who may have not

been subjected to any formal training on food handling as compared to the participants in this study.

The study participants who had one or more years of experience as food handlers performed slightly better than their counterparts who had less experience as food handlers. However, this had no statistical significance ($p=0.544$). Though there was an upward trend in the overall KAP scores by levels of education the association of education and overall KAP scores was not significant ($p=0.241$). Conversely, a survey conducted involving military hospital food handlers revealed that the overall KAP scores were significantly ($p<0.05$) affected by the levels of education, in which the average scores increased with the education level. No statistically significant differences were found between participants of different experience levels just like in the study by Sharif *et al.*, (2013). Similar study also found that the educational level of respondents did not influence respondent's knowledge and practice of food hygiene (Annor and Baiden, 2011). In this study, age was not significantly associated with the overall KAP scores.

The study also revealed that overall KAP scores varied significantly by the type of work the respondent was undertaking in which the respondent working in the processing factory were statistically significantly more knowledgeable than the sellers in other outlets ($p=0.011$). The food handlers who had attended a food handling and/or food safety course had a statistically significantly higher KAP mean score than their counterparts ($p=0.003$) who had reported that they had never attended a food handling and/or food safety course.

5.2 Occurrence of *Staphylococcus aureus* isolate

Staphylococci are common inhabitants of healthy humans, domestic, food animals, and they are isolated from several sites of their bodies, including the nose, throat, skin, hairs, and stool (Hanselmann *et al.*, 2006). *Staphylococci* may also exist in food products of animal origin or those that are handled directly by humans (Jay *et al.*, 2005). In this study, 671 samples comprising of food products of animal origin were collected. Of these, 531 samples (79.1%) were meat and meat products while the rest were dairy products (140, 20.9%). Overall, 36.2% and 39.4% of the samples collected from, the sellers and processors respectively were contaminated with *S. aureus*.

The proportions of contamination of animal products from the two sources were not significantly different ($p=0.400$). In the survey conducted previously in Italy on several kinds of foodstuffs revealed a total prevalence of coagulase positive *Staphylococci* of 17.3%, with contamination rates ranging from 17.1% to 48.1% in meat products (Normanno *et al.*, 2005). In another three year survey (2003- 2005) on the occurrence of *S. aureus* in meat and dairy products, 209 (12.8%) were contaminated with *S. aureus* (Normanno *et al.*, 2007). High occurrence of *S. aureus* from bovine, caprine bulk milk (75% and 96.2%, respectively) and in raw milk products (37.8%) were reported in Norway (Jørgensen *et al.*, 2005). This could be attributed to differences in hygienic practices of milk during handling.

Out of the 413 different food samples consumed in Eskisehir and Kutahya provinces in Turkey, 138 (33.4%) were contaminated with *S. aureus* (Kiymet *et al.*, 2010). The study found that the rate of contamination of meat and meat product were higher (48.1%) than milk and dairy products (23.2%) which are in agreement with the finding reported in the

current study. This study disagrees with work conducted in Egypt, which noticed that contamination rate of raw milk, was higher than that of raw meat (58% vs 18%) (Khalifa *et al.*, 2015). Reports suggested that humans are the source of *S. aureus* that contaminates milk (Zadoks *et al.*, 2002; Matyi *et al.*, 2013). Milk acts as a good medium for the growth of many microorganisms, especially bacterial pathogens and is also nutritious food for human beings (Chye *et al.*, 2004). Bustron *et al.* (2010) indicated that milk and its products can also be an important sources of foodborne pathogens.

Identification of enterotoxigenic *S. aureus* in raw milk poses a potential health hazard to consumers and such strains should be used as part of a risk analysis of milk and milk products (Zouharova and Rysanek, 2008). According to Normanno *et al.* (2007), 100 (10%) of the meat product samples and 109 (17%) dairy samples were found contaminated with *S. aureus* with milk and dairy products being significantly higher ($p=0.001$) contaminated than meat products. Higher levels of contamination of meat and chicken samples (53.3%) and milk and ice cream samples (61.1%) were reported in Ankara, Turkey (Gundogan *et al.*, 2006). High level of *S. aureus* contamination in current study may be attributed to the unhygienic handling of food of animal origin by sellers and processors, which expose consumers' health risk in Nairobi, Kenya.

According to this study, Githurai had the highest contamination by *S. aureus* in terms of proportion (37.5%) followed by City Market (36.2%) while lowest proportion of *S. aureus* contamination was observed in Kahawa (35.4%). Contamination was not significantly ($p=0.936$) associated with the area from which the samples were collected. There was statistically significant difference ($p=0.001$) in the levels of contamination

between the types of animal products collected from the selling outlets with meat (products) being more contaminated with *S. aureus* compared to the dairy products (41.8% against 25.0%). Among the meat and meat products sampled, no statistically significant differences in *S. aureus* contamination were observed ($p=0.374$). Similarly, no significant differences were observed in the levels of contamination of dairy products with *S. aureus* (yoghurt 27.5% vs. milk 24.0%) ($P=0.666$). Further investigations of contamination of dairy products with *S. aureus* revealed that pasteurized milk (5.0%) was less contaminated than raw milk (52.5%).

A total of 44% of the farm bulk milk samples and 72% of the milk collection centers were contaminated with *S. aureus* with no isolate from pasteurized milk samples (Fanta *et al.*, (2013). High contamination rate at the milk collection centers might be attributed by cross contamination and poor handling across the dairy value chain. Pasteurization of commercially distributed milk has greatly reduced the risk of infection resulting from the consumption of contaminated milk (Jayarao and Henning, 2001). This shows that *S. aureus* are inactivated during the pasteurization process, which can reduce contamination. This might only be effective if maintenance of pasteurization temperature and proper packaging are carried out well. It should also be noted that enterotoxins produced by the *S. aureus* retains their biological activity even after pasteurization, which is a hazard for consumers (Asao *et al.*, 2003).

Out of 250 food samples examined in Egypt, 127(50.8 %) isolates were identified as *Staphylococcus* species (EI-Jakee *et al.*, 2013). The highest isolation rate was observed in raw milk samples (56%) followed by yoghurt samples (22%), chicken products (6%), white soft cheese samples and pasteurized milk samples (4% each) then meat and meat

products (2%). The results are higher than those indicated in this study. In China, raw meat, milk and dairy products, frozen products and cooked foods have been found as major food types contaminated by *S. aureus*, taking up 38%, 20%, 16% and 14%, respectively.

The processed meat products are public health hazard due to the possible presence of food borne pathogenic bacteria, which cause toxicities and outbreaks (Rajic *et al.*, 2007). The contamination of food by *S. aureus* may directly occur due to skin lesions of workers containing bacteria during sneezing and coughing as approximately 50% of the human population carries *S. aureus* as commensals. Other contamination sources of *S. aureus* are soil, water, dust and air (Hanson *et al.*, 2011; Kitai *et al.*, 2005).

Among the meat and meat products collected from various selling points in current study, fish and poultry were found to be the most contaminated products (48.3% and 46.7%, respectively). The lowest contaminations were observed in sausage (32.5%), beef (36.3%) and pork (45.0%). The variations in *S. aureus* contamination observed between various meat and meat products sampled from sellers were, however, not significantly different ($p=0.374$). Of the 444 samples of raw chicken meat that retailed in different supermarkets in 47 prefectures in Japan were found contaminated with *S. aureus*. The results showed no significant difference in the detection rate of *S. aureus* according to the type of meat examined. High contamination rate was found in retail raw chicken (65.5%) compared with pork (26.0%) and beef (20%) (Jiang *et al.*, 2001).

Safety of fish products and their quality assurance is one of the main problems of food industry today. The presence or absence of foodborne pathogens in a fish product is a

function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment (FDA, 2001; Huss, 2003). The handling of fish products during the manufacturing process involves a risk of contamination by *S. aureus*, (Shena and Sanjeev, 2007). Fish contains large amount of proteins and their breakdown into amino acids support the growth of *S. aureus*. *Staphylococcus spp.* may be isolated from newly caught fish, especially in warm waters (Gram and Huss, 2000). Contamination of fish products through contaminated surfaces has also been revealed in many cases. According to Basti *et al.* (2003), some kinds of salt smoked fish may be considered as risk of *L. monocytogenes* and *S. aureus* infection and intoxication for Iranian consumers respectively. Contamination of the surface of carcasses in the process of pig slaughter has a wide range of potential pathogens, such as *Salmonella spp* and *L. monocytogenes* (Korsak *et al.*, 1998; Berend *et al.*, 1997; Duffy *et al.*, 2001; Akier *et al.*, 1989). Many opportunities for carcass contamination to occur are during slaughter, but main emphasis of control is applied at the end of evisceration in the form of washing. Scalding and singeing steps that are performed in the initial to de-hair carcasses have demonstrated to remove a substantial proportion of the carcass surface microflora and can be considered to act as barriers to minimize the transfer of pathogens through the processing line (Borch *et al.*, 1996; Sorquist and Danielssen, 1986).

When considering pig processing plant which was included in this study for comparison purposes, the highest prevalence of contamination with *S. aureus* was found in samples from carcass surfaces (56.0%) followed by cutting surfaces (35.2%) and then products (31.6%). Machine surfaces had the lowest levels of contamination of *S. aureus* (23.5%). Reports have been published showing the potential for carcass contamination during dehairing and evisceration operations (Nesbakken *et al.*, 1994; Gill and Bryant, 1993

Rivas *et al.*, 2000). Such studies have been based on enumerating total aerobic and indicator organism counts from samples recovered from carcasses. However, although such methods permit the gross changes in carcass microflora it does not provide sufficient data to elucidate the origins of pathogens. Occurrence in low number of pathogen does not reflect an increase in bacterial counts.

Investigations on the particulars of the *S. aureus* contamination at various sampling points in this study revealed that the highest proportion of contamination in the samples were collected from carcass surfaces before chlorination stage (71.4%), followed by after chlorination (57.1%). The study also revealed that 54.5% and 40.0% of the samples collected from frozen carcass and carcass before dehairing were respectively contaminated with *S. aureus*. However, the observed variations were not statistically significant ($p=0.247$). There were no differences in the levels of contamination observed in the two machines which were sampled, that is, Conveyor belt (17.6%) and Dixie slicer (29.4%) respectively. Likewise, no significant differences were observed on the rates of contamination based on the various cutting surfaces. Significant variations ($p=0.05$) were found on examining the samples of various products on the presence of *S. aureus*. Re-contamination of carcasses is reported to occur during scraping and polishing (Rivas *et al.*, 2000; Gill and Bryant 1993). It is therefore probable that within the slaughter line, additional cross contamination sites are present during sampling.

Increased surface contamination during evisceration is well documented where bacteria present within the intestinal tract, in addition to mouths can contaminate the carcass (Autio *et al.*, 2000; Gill and Jones, 1995). Despite the carcass being washed through a pressure hose *E. coli* and Enterobacteriaceae load on post-eviscerated carcasses are

relatively high. Previous reports indicate that carcass washing primarily re-distributes bacteria across the surface as opposed to removing contamination (Dickson and Anderson, 1992; Rivas *et al.*, 2000). Therefore, the high variation in counts recovered from post-eviscerated carcasses was not only due to the efficiency by which the process was performed but also as a result of re-distribution of bacteria due to the water wash.

Contamination at an early period of processing may be unexpected considering the relatively high level of wet scraper blade that the carcasses would have been subjected to singeing where the surface temperature would reach in excess of 100 °C. *E. coli* genotypes originally present on the scraper and polisher blades found by the end of processing had been transferred to the band saw in addition to the butcher's hands (Warriner *et al.*, 2002). Although good manufacturing practices are employed and segregation of dirty/clean areas was applied, this clearly illustrates that the transfer of *E. coli* and other pathogens could still occur. Persistence of certain strains over others remains unclear but may be attributed to the inherent resistance of the cell that adapts to the processing environment. Bolton *et al.* (2008) previously observed that endemic *S. aureus* was isolated from poultry processing plants that had enhanced resistance to sodium hypochlorite disinfectant used during hygiene operations.

In the current study, majority of the uncooked ham samples were contaminated with *S. aureus* (60.0%). Further, 35.0% and 21.1% of the samples obtained from fresh pork sausage and cooked salami respectively were positive for *S. aureus*. Of the 20 samples of hot dog analyzed only two (10.0%) were found to be harbouring *S. aureus*. From 2004 to 2006, *S. aureus* was recovered from 8.8%, 11.3%, and 4.3% of pork carcass samples, respectively, collected at 53 slaughterhouses in Taiwan.

During 2003 to 2005, it was recovered from 0.3%, 0.4%, and 7.8% of rinse fluids from chicken carcasses, respectively, collected at 17 meat processing plants (Lin *et al.*, 2009). Charlene *et al.* (2013) analysed 100 samples each of retail pork products and retail beef products, which were collected for testing. *S. aureus* was isolated from 100 retail meat samples (54%; 95% confidence interval, 52.2% to 55.8%). Significantly ($p < 0.05$), more *S. aureus* bacteria were isolated from retail beef than from retail pork. Overall, 63% of the beef and 45% of the pork products were positive for *S. aureus* with only pork stomach and pork liver being negative. In the retail meat samples other staphylococcal species were detected including, *S. epidermidis*, *S. saprophyticus*, *S. sciuri*, *S. intermedius* group *S. xylosus*, and *S. caprae*, from retail pork. From retail beef, *S. sciuri*, *S. xylosus*, *S. saprophyticus*, and *S. warneri* were similarly detected. Every portion of retail meat tested was positive for either *S. aureus* or some other staphylococcal species (Charlene *et al.*, 2013).

5.3 Occurrence of enterotoxigenic strains of *S.aureus*

To test presence of staphylococcal enterotoxins, reverse passive latex agglutination (RPLA) was used in this study (Schumacher *et al.*, (1995). A RPLA method has several advantages at present, including (i) high specificity and sensitivity, (ii) simplicity (no need for complicated procedures or expensive equipment), and (iii) economy (Igarashi *et al.*, 1986).

In this study, 187 (74.5%) strains were found to be enterotoxigenic producing at least one type of toxin. More than a half of the enterotoxigenic *S. aureus* strains (57.8%) were found to produce a single type of toxin. The study also revealed that some strains produced two (36.9%) and three (5.3%) enterotoxins. The SEs most frequently detected

was SEA 90 (48.1%), followed by a combination of SEA and SEC 22 (11.8%). Sixteen strains (8.6%) were found to synthesize a combination of SEA and SEB while fifteen strains (8.0%) synthesized SEA and SED. Additionally, nine strains (4.8%) were synthesizing SEB and SED. The relatively high percentage of classical enterotoxin forming *S. aureus* strains from meat samples found in this study is confirmed by previous findings (Jay *et al.*, 2005; Le Loir *et al.*, 2003; Pereira *et al.*, 2009). According to results obtained in the current study, consumers of foods of animal especially meat and dairy products are at risk of staphylococcal food poisoning if strict hygienic and preventive measures to void SEs products are not considered.

From 223 *S. aureus* isolates, from Iran, 30 (13.3%) were found to be enterotoxigenic (Ebrahim *et al.*, 2013). A study in Italy of 125 enterotoxic strains from the positive samples was screened, by the RPLA (SEA to SED) and a single PCR (*sea* to *see*). The result showed that most of the isolated strains produced SED (33.6%), followed by SEA (18.4%), SEC (15.2%), and SEB (6.4%) respectively. Thirty (24%) of the enterotoxic strains synthesized two SEs, while only one strain (0.8%) produced three SEs (SEB, SEC and SED) which is contrary to finding of this study. These findings highlight high potential risk for consumers of meat and dairy products with staphylococcal food poisoning. According to Balaban and Rasooly, (2000) the SEs most commonly involved in cases of SFP are SEA and SED, followed by SEB.

The result obtained by Morandi *et al.*, (2007) by two immunoassay approach of *S. aureus* from milk and dairy products revealed that SEA is the most common (31 positive strains) followed by SED (24) and SEC (14). Only one of the stains produced SEB and none produced the SEE toxin.

These findings are also in agreement with Normanno *et al.*, (2005) who reported frequency enterotoxin A and D in many Italian cow dairy products. Holeckova *et al.* (2002) reported 39% enterotoxigenic-positive strains for different ready-to-eat foods, which are similar to the finding of these results. Out of 87 processing factory workers examined, 54 (62%) were found to be carriers of *S. aureus*. Of the 54 strains tested for enterotoxigenicity, 15 (28%) were found to be positive. This further emphasizes the need for proper implementation of hygienic and sanitary measures. However, some of the findings differed from those reported by other researchers in other countries. In Egypt, Kamal *et al.*, (2010) showed that 68 out of 106 *S. aureus* isolates were enterotoxigenic with an incidence of 64.2% distributed as follows: enterotoxin C was the highest detected (32.1%) followed by enterotoxin A (17.9%) but enterotoxin B (14.2%) was the least detected.

Previous results by Jorgensen *et al.*, (2005) found similar results with SEC being the most common enterotoxin detected in *S. aureus* isolates from bovine mastitis. Samah, (2003) also showed similar results of 16.6% isolates of 106 *S. aureus* isolates obtained from milk producing SEC. The frequency of detection of enterotoxigenic *S. aureus* strains in the retail raw chicken ranged from 11.4 to 31.3% according to previous reports (Jiang *et al.*, 2001). According to Satoru *et al.*, (2005) 21.7% of the isolates examined were enterotoxigenic, SEB being the most frequently found in the isolates from the raw chicken meat regardless of the type of meat. It has been shown that 95% of staphylococcal food poisoning outbreaks are caused by enterotoxins SEA through SEE. Balaban and Rasooly, (2000) previously reported that SEA is the most common enterotoxin recovered from food poisoning outbreaks followed by SED and SEB.

5.4 Distribution of staphylococcal enterotoxin genes

Detection and differentiation of pathogens have gained more and more importance in food hygiene using modern molecular biological techniques (Pimbley and Patel, 1998; Wang *et al.*, 1997; Olsen *et al.*, 1995). Identification methods of *S. aureus* have been described (Brakstad *et al.*, 1993; Johnson *et al.*, 1991). According to Martineau *et al.* (1998), staphylococcal strains harbouring copies of enterotoxin genes can be detected rapidly by PCR. In this study, 247 (98.4%) strains of *S. aureus* analysed were found to harbor the *femA* gene, which was used as an internal control for confirming the presence of *S. aureus*.

The *femA* gene product, a 48-kDa protein, is implicated in cell wall metabolism, which is found in large amounts in actively growing cultures (Geha *et al.*, 1994; Vannuffel *et al.*, 1995). Other researchers have used 16S rRNA, *nuc*, or IS4311 as internal positive control (Barski *et al.*, 1996; Brakstad *et al.*, 1993). The failure of fragment amplification by the specified primer does not necessarily exclude the presence of the gene. It is possible that insufficient intact target DNA may have been extracted from the suspected food source, or co-purification of inhibitors of the hybridization reaction could have occurred (McLauchlin *et al.*, 2000).

In this study, 194 samples (77.3%) were found to have had one or more genes encoding for enterotoxins. The most frequent gene was *Sea* (61.8%) followed by *See* (33.1%) and *Sed* (17.5%) and then *sec* (15.9%). The least occurring gene in the *S. aureus* isolates examined was *seb* (13.9%). Further analyses revealed that about half of the strains (96, 49.5%) had two genes that code for enterotoxins. Besides, 65 (33.5%) and 32 (16.5%)

strains of *S. aureus* had one and three genes coding for enterotoxins respectively. The result revealed that one strain coded four genes.

In Brazil, Jamaira *et al.* (2008) found that of the 30 selected isolates, 21 were found to harbor enterotoxin genes where 38% amplified *sea*, 29% *seb*, and 24% both *sea* and *seb*. Genes for *sec* and *sed* (either alone or concomitantly) were infrequently detected. The result by Rall *et al.*, (2008) with regard to the genes encoding enterotoxins, 39 (68.4%) out of 57 strains of *S. aureus* were positive for at least one enterotoxin gene. The most frequently observed gene was *sea*, observed in 16 (41%) isolates, followed by *sec* (8 strains, 20.5%), *sed* (5 strains, 12.8%), *seb* (3 strains, 7.7%), and *see* (2 strains, 5.1%). This is in agreement with the present study, which found *sea* as the most frequent gene coding for enterotoxin. Ingestion of skim milk and yogurt prepared using powdered milk contaminated with 0.38 ng/ml and 3.7 ng/g of SEA was reported in Japan (Asao *et al.*, 2003). The minimal amount of enterotoxin that is required to cause the disease is not known, but the ingestion of at least 1 ng of toxin per 100 g of food is enough to induce the symptoms.

Samples of goat and sheep milk in Switzerland were positive (65.2%) for the presence of genes that encode for enterotoxins, a frequency very close to that observed in the present work (68.4%). Katsuda *et al.* (2005) reported 67.8% of *S. aureus* isolates being positive for the presence of genes coding for one or more enterotoxins in Japan. Similar frequency values (67%) were observed of the *S. aureus* strains isolated from milk and dairy products in Italy (Morandi *et al.*, 2007). The perceived percentage of enterotoxigenic, or potentially enterotoxigenic *S. aureus* strains have increased due to the discovery of the new enterotoxins. Thirty nine (68.4%) strains of *S. aureus* were found

to be positive for the presence of at least one SE gene. However, considering only the classic enterotoxins (*sea* to *see*), the number would drop to 31 (52.5%) (Rall *et al.*, 2008). Rosec and Gigaud, (2002), also observed an increase in the number of enterotoxigenic strains due to discovery of the new SEs.

In their study, the frequency of the isolates found to be coding genes of classic toxins was 30% but increased to 57% when the new SEs were taken into account. Simultaneous occurrence of these two genes in *S. aureus* strains isolated from milk of cows with mastitis has also been observed (Zschock *et al.*, 2005). Of the 61 strains analyzed in that study, 36 were shown to carry *seg* and 22 (61%) were found to be positive for *sei*. Lammler *et al.* (2000) and Omoe *et al.*, (2002) have reported that *seg* and *sei* are frequently found together in cows with mastitis and in raw milk. With regard to the genes encoding enterotoxins the study revealed that some isolates had only one gene encoding for enterotoxigenic genes. These included *Sea* (27.5%), *Seb* (2.6%), *Sed* (0.5%), and *See* (3.1%). Genes occurring in pairs included *Sea/See* (21.2%), *Sea/Sed* (9.8%), *Sed/See* (2.1%), *Sea/Sec* (0.7%), and *Seb/Sec* (0.5%) among others. The data is in agreement with finding by Rall *et al.*, (2008) that the most frequently observed gene was *sea*, observed in 16 (41%) isolates, but disagrees with the frequencies of the other enterotoxins genes. One (0.5%) isolate simultaneously was positive for genes encoding four enterotoxins *sea/sec/sed/see*.

Strains of the isolates producing a combination of three genes, whose individual genotypes are *sea + seg + sei + sej*, *sea + sed + seg + sei* and *seb + seg + seh + sei* have been revealed (Rall *et al.*, 2008). Nashev *et al.*, (2004) identified genetic profiles comprising of multiple genes in *S. aureus*. In their study, 9.1% and 4.5% of the strains

tested were found to coding four (*seb* + *seg* + *seh* + *sei*) and five (*sea* + *sed* + *seg* + *sei* + *sej*) enterotoxin genes respectively.

Several authors have also reported that enterotoxin Genes *Sea* and *sed* are the most common in *Staphylococci* isolated from foodstuffs (Araujo *et al.*, 2002; Portocarrero *et al.*, 2002). In Brazil, Carmo *et al.*, (2002) investigated the presence of *Staphylococci* in minas fresh cheese and raw milk and observed that the isolates were able to produce enterotoxins A, B and C. The difference in these results may be due to multiplicity of parameters described as intrinsic and extrinsic factors and the processing effects. Such includes pH, the size of inoculum, the type of growth medium, the NaCl concentration (a_w), the temperature and the atmosphere (Genigeorgis, 1989). Cheese produced in Serra da Canastra, M G, also contained *S. aureus* isolates able to produce enterotoxins B and C genes (Borelli *et al.*, 2006). In Poland, Weronika and Jacek (2014) found that 20(11.9%) strains were positive for one or more enterotoxin genes tested. Eighteen (90.0%) *S. aureus* harboured one of five classical enterotoxin where two (10.0%) isolates possessed *sea* and *seb* genes. The result showed that genes encoding enterotoxins *sec* and *sea* were most frequently found among *S. aureus* strains tested, which accounted for nine (45.0%) and six (30.0%) isolates respectively. In their analysis, no strains harboured the *see* gene, which disagrees with the current study. Variation in the results reported in current study may be as a result of different sampling techniques employed, seasonal effects, and/or laboratory methodologies employed from other studies.

Many researchers have developed attraction in the detection and identification of *S. aureus*. Using multiplex PCR (9.3%), *S. aureus* isolates obtained from milk samples of cows with subclinical mastitis were found to be positive for one or more enterotoxin

genes (Karahana *et al.*, 2009). Thirty two percent of dairy products (cream 18%, cheese 10%, and milk 4%) were found contaminated by *S. aureus* (Fooladi *et al.*, 2010). Results of their PCR showed that *S. aureus* isolates possessed the SEA gene (15.6%), SEB gene (9.3%), and both SEA and SEB genes (6.2%).

In Ankara, Turkey, 41 enterotoxigenic Staphylococci isolated from white, tulum, and kashar cheese were reported producing SEA (25), SEB (4), SEC (6), SEA+SEC (5), and SED (1) (Kuplulu *et al.*, 2004). Rivas *et al.* (2000) reported that SEA as the most common produced enterotoxin gene in ice cream, followed by SEB and SEA + SED. According to the analysis by Gucukoglu *et al.* (2011), seven isolates from the raw milk samples (13.7%) were found to enterotoxigenic where five produced SEA gene (71.4%), one produced SEB gene (14.2%), and one produced SEA+SEB gene (14.2%). From the white cheese samples, four isolates (19%) produced the SEA (25%), SEC (25%), SED (25%), and SEA+SED (25%) enterotoxins genes respectively. In kashar cheese samples, two isolates (50%) were found to be enterotoxigenic, one producing SEA (50%) while the other produced SED (50%). One isolate showed enterotoxigenic characteristic from the butter samples (25%) producing enterotoxin SEB (100%). Of the analyzed samples, the products were found to be potentially hazardous to public health because of the fact that levels of contamination were higher than 10^5 – 10^6 cfu/g/ ml. Detection of the enterotoxin genes in the Coagulase Positive *S. aureus* (CPS) isolates coming from food is not an indication that the toxins are effectively present in the food.

5.5 Staphylococcal enterotoxins production and gene expression

In this study, a comparison of the RPLA test for the detection of classical enterotoxin A-D with corresponding genes on PCR revealed a correspondence of 98.4%. Complete

correspondence for SEA, SEB, and SEC was observed. On the contrary, four of the 193 strains (2.1%) were found to be discordant whereby the SED genes were detected by multiplex PCR but the corresponding toxins were not detected by RPLA test.

This discrepancy could be explained by the production of enterotoxin in quantities that were below the limit of detection of the RPLA test or its non-expression. In summary, for the toxins detectable using RPLA, a good level of correlation was observed between the two methods, as also reported from other authors (Zschock *et al.*, 2005). In this study, the detection rate of SE-encoding genes was almost in agreement with PCR and SET-RPLA, except for SED.

Structurally and biological properties SEs are known to be similar but differ in amounts produced. SEB and SEC are expressed in greater quantities than SEA and SED (Bergdoll, 1979). The frequency of detecting SEB and SEC production were consistent with detection of their corresponding genes, while using PCR techniques the detection levels of *sea* and *sed* were higher (Klotz *et al.*, 2003). From 67% of *S. aureus* isolates from milk only 52% produced detectable amount of the classical SEs (Morandi *et al.*, 2007). Reverse Passive Latex Agglutination should be regarded as the 'gold standard' for confirmation of the results within the range of SEs detected by this method (McLauchlin *et al.*, 2000). Discrepancies of less than five were obtained between the results of PCR and the RPLA assays (McLauchlin *et al.*, 2000; Jørgensen *et al.*, 2005).

Morandi *et al.* (2007) noted discrepancy of 15%. Zouarova and Rysanek, (2008), found higher (32%) discrepancy level in a study. Cases of SEA positive isolates have been described earlier where individual genes were not detected by the PCR method,

indicating the existence of sequence variations in *sea* or a toxin, not yet described, that cross reacts immunologically with SEA (Jørgensen *et al.*, 2005; McLauchlin *et al.*, 2000). In their study, isolates harbouring the *sea* were found but production of SEA by the same isolates was not confirmed. Inability of the gene involved to express an intact and biologically active toxin, or production of very small amount (<0.5 ng/ml) of the toxin explains why PCR results obtains higher detection level of enterotoxigenic strains (McLauchlin *et al.*, 2000).

Gene's mutation may be a second explanation whereby the toxin genes are converted to silent genes with a consequent expression defect (Okoji *et al.*, 1993). Reactivated of the silent genes could be due to a single mutational event and be expressed under appropriate conditions (Hall *et al.*, 1983). Therefore, not only production of enterotoxin but also detection of enterotoxin genes must be taken into account. In Italy, Carfora *et al.* (2015) revealed complete correspondence (100%) for *seb* gene with SEB. Production and expression differed with the other enterotoxins where *sea*, *sec* and *sed* had 16(51.6%), 20(62.5% 23(06.5%) discrepancies respectively.

5.6 Antibiotic resistance profiles of *S. aureus* strains

Ability to develop and expand resistance to a broad array of antimicrobial classes by *S. aureus* has been demonstrated (Chamber, 2001). *S. aureus* is a prominent pathogen in both the hospital and the community settings. The bacteria is emerging as a multi-drug resistance pathogen worldwide, but wide variations in incidence exist regionally (Peles *et al.*, 2007; Pesavento *et al.*, 2007; Normanno *et al.*, 2007; Gündoğan *et al.*, 2006).

Use of antibiotics for both prophylaxis and as growth promoters in animals, contributes to the development of antibiotic resistant bacteria (Xu *et al.*, 2014). Acquisition of new genetic material from other resistant organisms through transformation, transduction and conjugation makes bacteria resistant to antimicrobial agents and the indiscriminate use of these antimicrobial agents might account for high resistance (Zouhairi *et al.*, 2010). Resistance genes for single or multiple antimicrobial agents may be contained in the Plasmids and have been reported to transfer this resistance from one bacterium to another (Lockley *et al.*, 1982). *Staphylococci* resistance to several therapeutically useful antibiotics, including, Novobiocin, Fusidic acid, Rifampicin, and Streptomycin is thought to be derived from chromosomal mutation (Lacey, 1984).

This study observed the resistance patterns of *S.aureus* to eight antimicrobial agents. Of 251 strains tested 247 (96%) were positive for antimicrobial resistance to at least one antibiotic. A total of 104 (42.1%), 60 (24.3%) and 56 (22.7%) *S. aureus* isolates were resistant to three, four, and two antibiotics respectively. The analysis revealed that additionally, seven (2.8%) and nineteen isolates (7.7%) were resistant to one and five antibiotics respectively. One isolate was resistant to six antibiotics. In Iran, the resistance pattern of *S. aureus* isolated from commercial dairy products to 12 antimicrobial agents showed that most of the *S. aureus* isolates (95.0%) were resistant to one or more antimicrobial agent. Three isolates (15.0%) were resistant to single antibiotic and seven isolates (35.0%) showed resistance to two antimicrobial agents. Multi resistance, which was defined as resistance to three or more of antimicrobial agents tested, was found in 45.0% of *S. aureus*, isolated (Rahimi, 2013).

The results of a study carried in Turkey by Arslan and Özdemir, (2012) indicated that a total of 23.9% of 71 clinical *S. aureus* isolates were resistant to at least one or more antimicrobial agents, but 9.9% of clinical isolates showed multiple resistances (≥ 3 antibiotics). Among 222 isolates of food origin, 8.6% were seen individual resistance (≥ 1 antibiotics). Of all the *S. aureus* isolates analysed, in this study, the highest percentage of resistance was to Penicilin G (246; 99.6%), followed by Ampicillin (230; 93.1%).

Lower than 50% were Cotrimoxazole (89; 36%), Methicilin (67; 27.1%), Minomycin (60; 24.3%), Lincomycin (39; 15.8%) and erythromycin 31(12.6%). Aarestrup *et al.* (1995) found penicillin resistance in 75% of the 20 antibiotic resistant strains from bovine milk in Denmark. Kiyet *et al.* (2009) in Turkey found that of the 138 strains isolated *S. aureus* from meat and dairy products, 92.7% was resistant to Penicillin G that is similar to the present study.

This study is also in agreement with other finding obtained in many studies (Soares *et al.*, 1997; Acco *et al.*, 2003; Gundogan *et al.*, 2005, 2006; K rouanton *et al.*, 2007; Nitzsche *et al.*, 2007). Foodborne *S. aureus* isolates from Shanghai had the highest frequency of resistance to Penicilin (74.4%), followed by Erytromycin (59.0%), Clindamycin (44.9%), Ciprofloxin (37.2%), Tetracyclin (24.4%), and Oxacylin (23.1%). Few of the isolates showed intermediate resistance to Cotrimoxazole (20.5%) and Clinadmycin (20.5%), followed by Gentamycin (11.5%) and Ciprofloxin (9.0%) (Xu *et al.*, 2014). Results obtained from this study were more or less comparable with those from India where high resistant toward penicillin G (86.04%), Ampicillin (74.42%), and Methicillin (13.95%) was reported (Sudhanthirakodi *et al.*, 2015).

Alian *et al.* (2012) revealed that resistance (resistance and intermediate resistance) to Ampicillin was the most common finding (54.3%), followed by Oxacillin (28.3%), Tetracycline (26.1%), Penicillin G (23.9%), Erythromycin (23.9%), Trimethoprim-sulfamethoxazole (17.4%) and Cephalotin (2.2%) and all isolates tested for antibiotic sensitivity were susceptible to Methicillin, Vancomycin, Chloramphenicol and Ciprofloxacin. As reported by other investigators, the resistance of *S. aureus* isolates to β -lactams such as Ampicillin, Penicillin, Tetracycline and Oxacillin was evident (Pere *et al.*, 2007; Pereira *et al.*, 2009; Pesavento *et al.*, 2007 and Gundogan *et al.*, 2006). Results from the current study demonstrated that the most predominant multi antibiotic resistance phenotype were Pen/Amp and Pen/Cot/Amp in 49 (19.8%) and 32 (32%) respectively with only one phenotype Pen/Min/Ery/Cot/Amp/Lin (1; 0.4%) obtained.

Deresse *et al.* (2012) demonstrated that the most predominant multi antibiotic resistance phenotypes for *S. aureus* were PG/AP/AC/E/CRO/Ox and PG/AP/Ox in 19.2% and 20.5% of the isolates, respectively. Shitandi and Mwangi (2004) reported the most frequent resistance was penicillin (72.2 %) followed by Trimethoprim + sulfamethazin (59.2 %), tetracycline (57.9 %), Chloramphenicol (46.8 %), Erythromycin (21.3 %), and Methicillin (7.8 %). Multi antibiotic resistance to Penicillin and at least two other non- β -lactam classes of antimicrobials were also observed in 76.9 % of isolates. Multiple resistances to more than four antimicrobials were 13.4 % while 1.9 % was susceptible to all six antimicrobials tested. The finding that a large number of *S. aureus* were resistant to Ampicillin, Penicillin, Tetracycline and Oxacillin are, however, a cause for concern and should be investigated further as these drugs are used in veterinary medicine in Kenya.

Erythromycin resistance was low in comparison with the other antibiotics tested. Despite the fact that 31(12.6%) of *S. aureus* isolated from foods of animal origin samples was resistant to erythromycin, it was evident from our results that this antibiotic was not frequently used in animals by large-scale farmers. Gundogan *et al.* (2005) observed that few of the strains obtained from chicken (33.3%), minced calf meat (14%) and chicken carcass were resistant to erythromycin. The occurrence of Erythromycin resistance among poultry *S aureus* was 24% and that the *ermA* gene was found in most Erythromycin resistant *Staphylococci* in Denmark (Aaresstrup *et al.*, 2000).

Their results suggest that *eamA* genes might be introduced into the community through food chain. Chloramphenicol was the drug to which small proportion of the isolates were resistant 10 (4.0 %). Similarly, a previous study reported that only a small percentage (15.7% to 23.8%) of *S. aureus* isolated from meat and dairy products was resistant to Sulfamethoxazole (Normanno *et al.* , 2007; Aarestrup *et al.*, 2000). This drug is not used in dairy cattle farms in the sampled area of Kenya. The results presented herein are similar to previous studies in which Gram-positive bacteria were generally susceptible to Vancomycin, Methicillin, Chloramphenicol, Cephalotin and Ciprofloxacin (Pereira *et al.*, 2009; Gundogan *et al.*, 2006). These drugs are no longer used in veterinary medicine in many countries, which may account for the results reported (Pace and Young, 2006; Ateba *et al.*, 2010).

According to this study, the distribution of antimicrobial resistance differed among *S. aureus* strains from the sellers and processor. Results from the pig-processing factory showed that all the *S. aureus* isolates were resistant to Penicillin. Resistance to

Ampicillin was also high in isolates (91.9%). The study showed statistically significant differences in the distribution of resistances of isolates from different foods of animal origin to various antibiotics including Minocycline, Erythromycin, Methicillin, Cotrimoxazole and Lincomycin. An overwhelming proportion of the isolates from different meat and milk products also showed resistance to Penicillin with the overall resistance at 96.7%. The results indicated that no isolate from sellers was resistant to Lincomycin.

Resistance of *S. aureus* isolates to Minocycline, Methicillin and Cotrimoxazole showed significant variations across the range of meat and dairy products. In Korea, the patterns of antimicrobial resistance differed significantly among the *S. aureus* isolates from poultry, cattle, and swine sources. A lower proportion of isolates from raw beef (16) and pork samples (17) were resistance to Gentamicin and tetracycline. A greater proportion of these showed resistance to Penicillin, Ampicillin, and Erythromycin as compared to the isolates obtained from raw chicken. This may be due to the diverse selective pressure from the different usage of antibiotics within the three species.

The current study disagrees with investigation done in Italy, in three for the major meat species types of raw meat (beef, poultry, and pork) in which strains of *S. aureus* presented higher resistance levels for all the tested antimicrobial substances, except for Ampicillin and Penicillin G. Resistances were similar in the three kind of meat except for Oxacillin, where resistance was more frequently in isolates from poultry as compared with pork and beef (Pesavento *et al.*, 2007). In Kenya, Ombui *et al.*, (2000) found a higher proportion (44.9%) of isolates from meat resistant to antimicrobial agents

compared to 36% of milk isolates. Resistant to Penicillin (90.5%), Ampicillin (61.9%), and Methicillin (42.9%) was higher in meat compared to milk isolates.

The study also revealed a higher proportion of isolates from milk resistant to Cotrimoxazole (56%), compared to meat isolates but there was no significant difference in resistance to Chloramphenicol, Erythromycin, Minocycline, and Lincomycin between the isolates from meat and from milk. The main route of dissemination of resistance into the human populations is through contact with animals or consumption of food products with resistance strains (Spike *et al.*, 1987; Holmberg *et al.*, 1984).

Of concern to this study is the resistance to Methicillin (27.1%). This is an indicator that in Kenya, Methicillin Resistance *S. aureus* (MRSA) is increasing in food of animal origin. In the United States and some European countries, high levels of MRSA have been reported over the last few decades (Mark *et al.*, 2003). *S. aureus* strains resistant to the antibiotic methicillin (MRSA strains), particularly in nosocomial settings has increased (Haley, 1982). Antibiotics resistance is attributed to the presence of *mecA* gene, a product of 78-kDa protein called penicillin binding protein 2a (Utsui and Yokota, 1985). Identification of *mecA* in such MRSA strains has led to some knowledge regarding the use of the antibiotic Vancomycin (Chambers, 1997; Zhang *et al.*, 2005). *S. aureus* strains from food of animal origin in Italy (3.75%) were found to harbor the *mecA* gene (Normanno *et al.*, 2007). Each *mecA* positive strain was derived from a different food sample. Four strains were from bovine milk, one from pecorino cheese, and one strain from mozzarella cheese. It has also been documented that MRSA isolates that are resistant to beta-lactam antibiotics may develop induced resistance to Vancomycin.

The results of this study generated remarkable similarity in antibiotic resistance and staphylococcal enterotoxin productivity among the *S. aureus* strains originating from meat and dairy in Nairobi Kenya, which shows a possibility of clonal transmission of antibiotic resistance and enterotoxigenic *S. aureus* from one food source to another. The data showed that for the commonly used antibiotic used to treat human and animals, no drug achieved 100% susceptibility, and overwhelming majority of *S. aureus* expressed genes that code for enterotoxin production. If the necessary action is not taken regarding food hygiene practices and indiscriminate use of antibiotics, prevalence of enterotoxigenic and multi antibiotic resistance strains may increase in Kenya. This may lead to serious health hazards within the population.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- i. Overall knowledge attitude and practice performance seemed to improve along the level of education and work experience.
- ii. The result of this study clearly indicated relatively high frequency of occurrence of *S. aureus* in food of animal origin. High levels of enterotoxigenic *S. aureus* (74.5%) was obtained with more half (57%) producing a single toxin. Staphylococcal enterotoxin A (SEA) (48%) was the most prevalence while some isolates produced a combination of two enterotoxin SEA and SEC (11.8%) and three SEA/SEB/SEC (2.7%).
- iii. *Staphylococcus aureus* genes were detected in 76.9% of the examined sample. Large proportional of the isolates amplified enterotoxin gene was *sea* (61%), followed by *see* (33.1%), *sed* (17.5%) and *sec* (15.9%). Analysis revealed that some strains coded for two enterotoxin (49.5%), three (33.5) while only one coded four genes.
- iv. Many of the strains in this study showed multi antibiotics resistances with almost 100% resistance to Penicillin G (99.6%) followed by Ampicillin (93.1%), and Cotrimoxazole (36%). High levels of multi antibiotic resistance to two drugs (22.7%), three (42.1%) and six (0.4%) was obtained. Based on this, it can be concluded that spread of drug resistant strains in the foods of animal origin in Nairobi, Kenya is likely to occur.

6.2 Recommendations

- i. Education, training, acquired experience, incentive should be provided to encourage food handlers to acquire more Knowledge, develop positive attitudes

and observe good practices for prevention of cross-contaminations of foods for human consumption.

- ii. Public health Department in Nairobi should ensure strict hygienic and preventative measures are adhered to in order to reduce food contamination by *S. aureus* during processing and the distribution to the consumers.
- iii. The high frequencies of enterotoxigenic *S. aureus* in foods of animal suggest that the pathogenic potential of these bacteria is more than had been before.
- iv. Indiscriminative use of antibiotics should be minimised to prevent development of resistance strains of *S. aureus*.

6.3 Suggestions for further research work

- i. Further research should be done on whether pre employment training and interactive media influence behavioral changes in food handlers.
- ii. Identification and assessment of the distribution of staphylococcal enterotoxins in food poisoning outbreaks should be researched further.
- iii. Gene coding antibiotic resistance should be investigated at molecular level to understand resistance dynamic.

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APPENDICES

APPENDIX 1

Abstracts for manuscripts submitted for review and publication

- i) **John M. Mathenge, Michael M. Gicheru, Paul O. Okemo, Paul M. Ng'ang'a, James M. Mbaria.** Food safety knowledge, attitudes and practices among food handlers working in meat-selling establishments in the city of Nairobi.

Abstract

Introduction: Food-borne diseases have been increasing in recent years with a greater impact on the health and economy of developing countries. Good levels of knowledge towards food safety among food handlers and the effective practices in food handling are imperative in reducing such illnesses. The aim of the present study was to evaluate the level of knowledge, attitudes and practices in food safety among food handlers working in meat eating points the city of Nairobi.

Methodology: A cross-sectional study was conducted in the City of Nairobi. Data were collected from food handlers working in food establishments selling meat in the city using an interviewer administered questionnaire.

Results: Majority of the participants were male (80.0%) and Christians (89.0%). The median (interquartile range (IQR)) age of the participants was 30.5 (26.0-38.0) years. The respondents demonstrated above average proficiency in the assessments involving knowledge, attitudes and practices. The overall KAP scores were not associated with age, gender, level of education and marital status. On the other hand, respondents who had ever attended a food handling &/or food safety course had a statistically significantly higher KAP mean score than their counterparts who had never attended (respectively, 31.5 ± 0.2 versus 30.3 ± 0.3 , $p=0.003$).

Conclusion: The level of knowledge, attitude, and practice on food safety and food handling amongst the sampled food handlers were found to be favourable. Training, motivation and initiatives should be provided to encourage food handlers to sustain this positive trend in knowledge, attitude, and practices in food safety

Keywords: *Knowledge, Attitudes, Practices, Food handlers, Safety*

- (ii) **Mathenge. J.M, P. O. Okemo, P.O, Ng'ang'a, P.M, Mbaria, J.M, Gicheru, M.M. (2015).** Identification of Enterotoxigenic *Staphylococcus aureus* Strains from Meat and Dairy Products by Multiplex PCR and RPLA in Nairobi, Kenya. The East and Central Africa Medical Journal 2: (2) , 97-103

Abstract

Background: Foods of animal origin, especially milk and dairy products, are associated with foodborne disease. In many countries, *S. aureus* is considered the second or third most common pathogen responsible for outbreaks of food poisoning. In Kenya, enterotoxigenic staphylococcal food poisoning poses a potential health hazard to the consumers of meat and dairy products since very little is known about the strains involved in food poisoning.

Objective: The objective of this study was to investigate the occurrence of enterotoxigenic *Staphylococcus aureus* strains in meat and milk products. The gene coding classical Staphylococcal enterotoxins was also detected using multiplex polymerase chain reaction (PCR) and reversed passive latex agglutination (RPLA).

Methodology: This survey was a cross-sectional descriptive study conducted in Nairobi County, Kenya. The study was confined to meat and milk outlets in the Central Business District of Nairobi (CBDN) and its surroundings. 420 food samples of animal origin, comprising of meat and meat and dairy products, were randomly collected. Gene coding classical Staphylococcal enterotoxins was detected using multiplex polymerase chain reaction (PCR) and the production by reversed passive latex agglutination (RPLA). 251 samples of raw pork and parked pork products from a nearby processing plant were included in the study.

Result: Of the 671 samples examined, 251 (37.4%) were contaminated with *S. aureus*. Staphylococcal enterotoxin genes (*ses*) were detected in 194 (77.3%) of the total isolates. The most frequent gene was *sea* (61.8%) followed by *see* (33.1%), *sed* (17.5%) and *sec* (15.9%) respectively. Staphylococcal enterotoxin B (*seb*) was the least occurring gene in the *S. aureus* isolates examined (13.9%). Genes occurring in pairs included *Sea/See* (21.2%), *Sea/Sed* (9.8%), *Sed/See* (2.1%), *Sea/Sec* (0.7%) and *Seb/Sec* (0.5%) among others. Toxin production was detected in 187 (74.5%) of the strains of *S. aureus* isolated. A relatively low number 4 (2.1%) of discrepancies between the results of multiplex PCR and RPLA were found where by the *sed* genes were expressed by PCR but the corresponding toxins were not detected by RPLA.

Conclusion: The study clearly indicated that meat and milk products marketed in and around Nairobi, Kenya were contaminated with enterotoxigenic *S. aureus* posing a high risk of food poisoning to the consumers. Equally, these data demonstrated that Multiplex PCR and RPLA are useful methods for detection of enterotoxigenic potential of *S. aureus*.

Keywords: Staphylococcal enterotoxin, PCR, RPLA meat, dairy products

APPENDIX 2**Assessment of Knowledge, attitude and practice****(i) Social demographic information**

Indicate a (*) where appropriate

Ageyears

Gender: Male () Female ()

Marital status: Married () Single ()

Religion: Christian () Muslim () Others ()

Education: Primary () Secondary () Post Secondary ()

Job description: Seller of meat or milk () Processor of meat product ()

(ii) Knowledge questions on food poisoning

In the following questions, indicate whether you

(4) Strongly agree

(3) Agree

(2) Not sure

(1) Disagree

(0) Strongly disagree

No	Attribute
1	Hands should be washed before handling food and after visiting the bathroom
2	Food poisoning could cause severe diseases that end in hospitalization and sometimes death
3	Apparently healthy food handlers might carry food borne pathogens
4	Eating raw or half -cooked meat is highly risky for food poisoning
5	The habit of fingering the nose raises the risk to cause food poisoning
6	Food handlers with unhygienic practice could be the source for food contamination with food poisoning pathogens
7	Eating covered leftover cooked food, kept at room temperature for more than 6 hours, is at high risk to cause food poisoning
8	Keeping food at refrigerator temperature helps to prevent food poisoning
9	Contacting ready to eat food with bare hands cause food contamination with food poisoning pathogens
10	Skin infections can contaminate food
11	The correct method for thawing frozen meat is to keep them overnight at room temperature
12	Insects such as cockroaches and flies might transmit food borne pathogens
13	Harmful bacteria multiply quickly at room temperature
14	Vegetables should be placed on higher shelf in refrigerator than meat
15	Raw and cooked meat should be kept separately

(iii) Attitude questions on food poisoning

In the following questions, indicate whether you

(4) Strongly agree

(3) Agree

(2) Not sure

(1) Disagree**(0) Strongly disagree**

No.	Attribute
1	Food borne outbreaks are natural life events
2	Learning more about food safety through training courses is important to me
3	I am willing to change my food handling behaviors when I know they are incorrect
4	Sanitation and safe food handling are an important part of my job responsibilities
5	It is important to regularly check the temperature of the refrigerator
6	I believe that adequate employee hygiene can prevent food borne illness
7	I believe being medically examined every six months is important for a food handler's health and that of the customers
8	I think that it is my responsibility as a food handler to ensure that meat I sell is safe

(iv) Practice questions on food poisoning

In the following questions, indicate whether you

(4) Always**(3) Most of the time****(2) Sometimes****(1) Rarely****(0) Never**

No	Attribute
1	Wearing protective gear when handling meat
2	Wash your hands with water and soap before handling meat/food
3	Wash your hand with water and soap after defecation and/or micturition
4	Drying hands after washing them with towel
5	Ensuring you go for a medical checkup every six months
6	Checking the temperature of the refrigerator regularly
7	Stop working when you have 'simple' ailments, e.g., cold or lesions on your hands
8	Trimming of finger nails
9	Ensuring that cooked meat is not kept at room temperature for more than 4 hours
10	Cleaning of meat contact surfaces including the chopping boards, utensils etc
11	Separate raw meat from ready to eat foods
12	Eat raw/half-cooked meat (inside is pink)

APPENDIX 3

Research Authorization

APPENDIX 4

Consent Letter and Form

John M.Mathenge
Kenyatta University
School of Pure and Applied Sciences
Dept.Of Zoological Scs
P.O Box 43844-00100 Nairobi
Tel: 254 208710901 Ext 57035

I am a student studying at the Kenyatta University for a PhD Degree, trying to gather information on the knowledge, attitudes and practices towards Food Contact Surfaces in Meat Processing Plants for food safety and hygiene in and around Nairobi City. We would like to you to participate in this study.

Why are we doing this?

Training and education to prevent food-borne illness has been identified by the health department as an important element of ensuring Food Safety. Despite continuing progress made in food quality and safety, foodborne disease outbreaks continue to be reported in the literature. The most frequently identified factors contributing to the outbreaks are cross contamination, improper cooling, improper storage or holding temperatures of foods held for long duration, inadequate cooking times and temperatures, contaminated raw foods ingredients, and poor personal hygiene by persons who handle food. The aim of this study therefore is to assist us in finding out information of Food Contact Surfaces in Meat Processing in order to identify any gaps in the food quality, safety and training.

Who are the participants?

The participants are employee of Meat Processing Plants, butchers and consumers

What do we expect from the participants in this study?

A trained Enumerator will ask questions about your knowledge, practices and attitudes towards food safety. This will take approximately few minutes. A questionnaire will be completed during the interview. Prior to this your written consent will be obtained. All information collected is confidential and only the researchers will have access to it. The Enumerator will also look at how you prepare and store all meat that processed, sold and consumed.

What can participants expect?

Once we have finalized the research report, a meeting will be held and the results will be presented to you.

Can you withdraw from the study?

Certainly, you may withdraw from the study at any time or refuse to participate. Your participation is entirely voluntary and you do not need to give a reason should not wish to participate. There are no benefits to participating in the study other than the enhancement of the lives of customers and yourselves in your community. Neither your political affiliation, employment nor level of services will be affected by the participation or refusal to participate in the study.

Do you have any further Questions?

More information can be obtained from Mr J.M. Mathenge on **072 1 614144**

If you are willing to participate in the study, please read and sign the consent form.

THANK YOU

Consent Form

Knowledge, attitudes and practices of Food Contact Surfaces in Meat Processing Plants. The study has been described to me in language that I understand and I freely and voluntarily agree to participate. My questions about the study have been answered. I understand that my identity will not be disclosed and that I may withdraw from the study without giving a reason at any time and this will not negatively affect me in any way.

RESPONDENT AGREES TO BE INTERVIEWED 1

Name..... Sign.....

RESPONDENT DOES NOT AGREE TO BE INTERVIEWED 2

END

Respondent remarks: _____

Interviewer remarks: _____

Should you have any questions regarding this study or wish to report any problems you have experienced related to the study, please contact the Researcher **0721 614 144**

Or Lead Supervisor Dr M .M Gicheru: **0722 609 765**

APPENDIX 5**Bacteriological Culture Media****(i)Baird Parker Agar**

Formula / Liter

Enzymatic Digest of Casein	10 g
Beef Extract.	5 g
Yeast Extract	1 g
Lithium Chloride	5 g
Glycine	12 g
Sodium Pyruvate	10 g
Agar	*17 g

*15 - 20 g according to gel strength Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Egg Yolk Tellurite, 100 mL

(Chicken egg yolk and egg, 100%)

(Potassium Tellurite, 0.21 g)

(ii) Blood Agar, Base

Ingredients	Grams/ Litre
Meat extract.....	10.0 g
Peptone	10.0g
Sodium chloride	5.0g
Agar	15.0g

Final pH 7.3+/-0.2 at 25°C

For blood agar, cool to 45-50°C and add aseptically 6% (5-10% is typically) of sterile defibrinated blood

(iii) DNase Test Agar

Formula / Liter

Enzymatic Digest of Casein.....	15 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride.....	5 g
Deoxyribonucleic Acid.....	2 g
Agar.....	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications

(iv) Mueller Hinton Agar

Formula / Liter

Beef Extract.....	2 g
Acid Hydrolysate of Casein.....	17.5 g
Starch.....	1.5 g
Agar.....	17 g

Final pH 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

(v) Brain Heart Infusion Broth

Formula / Liter

Brain Heart Infusion.....	17.5 g
Enzymatic Digest of Gelatin.....	10 g
Dextrose.....	2 g
Sodium Chloride.....	5 g
Disodium Phosphate.....	2.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

(vi) Peptone Water

Formula / Liter

Peptone.....	10 g
Sodium Chloride.....	5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

(vii) Bacto Tryptic Soy Broth (DIFCO)

Formula / Liter

Pancreatic Digest of Casein.....	17.0 g
Enzymatic Digest of Soybean Meal	3.0 g
Sodium Chloride.....	5.0 g
Dipotassium Phosphate	2.5 g
Dextrose	2.5 g

Formula Adjusted and /or supplemented as required to meet performance criteria.

APPENDIX 6**(i) Agarose Gel Electrophoresis**

For a 1% Agarose gel, add 1 gram of Agarose to 100 ml of 1x electrophoresis buffer.
Gel Concentration Required for DNA Separation

Materials needed	<u>Agarose</u>
	TAE Buffer
	6X Sample Loading Buffer
	DNA ladder standard
	Electrophoresis chamber
	Power supply
	Gel casting tray and combs
	DNA stain
	Staining tray
	Gloves
	Pipette and tips

Recipes:

TAE Buffer

4.84 g Tris Base

1.14 ml Glacial Acetic Acid

2 ml 0.5M EDTA (pH 8.0)

- bring the total volume up to 1L with water

Add Tris base to 900 ml H_2O . Add acetic acid and EDTA to solution and mix. Pour mixture into 1 L graduated cylinder and add H_2O to a total volume of 1 L.

For convenience a concentrated stock of TAE buffer (either 10X or 50X) is often made ahead of time and diluted with water to 1X concentration prior to use.

6X Sample Loading Buffer

1 ml sterile H₂O

1 ml Glycerol

Enough bromophenol blue to make the buffer deep blue (~ 0.05 mg)

For long-term storage, keep sample loading buffer frozen.

(ii) Agarose Gel Electrophoresis Protocol

Preparing the agarose gel

- Measure 1.25 g Agarose powder and add it to a 500 ml flask
- Add 125 ml TAE Buffer to the flask. (The total gel volume will vary depending on the size of the casting tray).
- Melt the agarose in a microwave or hot water bath until the solution becomes clear. (If using a microwave, heat the solution for several short intervals - do not let the solution boil for long periods as it may boil out of the flask).
- Let the solution cool to about 50-55°C, swirling the flask occasionally to cool evenly.
- Seal the ends of the casting tray with two layers of tape.
- Place the combs in the gel-casting tray.
- Pour the melted agarose solution into the casting tray and let cool until it is solid (it should appear milky white).
- Carefully pull out the combs and remove the tape.
- Place the gel in the electrophoresis chamber.
- Add enough TAE Buffer so that there is about 2-3 mm of buffer over the gel.
- Gels can be made several days prior to use and sealed in plastic wrap (without combs). If the gel becomes excessively dry, allow it to rehydrate in the buffer within the gel box for a few minutes prior to loading samples.

Loading the gel

- Add 6 µl of 6X Sample Loading Buffer to each 25 µl PCR reaction
- Record the order each sample will be loaded on the gel, including who prepared the sample, the DNA template - what organism the DNA came from, controls and ladder.
- Carefully pipette 20 µl of each sample/Sample Loading Buffer mixture into separate wells in the gel.
- Pipette 10 µl of the DNA ladder standard into at least one well of each row on the gel.
- Note – if you are running multiple gels, avoid later confusion by loading the DNA ladder in different lanes on each gel.

Running the gel

- Place the lid on the gel box, connecting the electrodes.

- Connect the electrode wires to the power supply, making sure the positive (red) and negative (black) are correctly connected. (Remember – “Run to Red”)
- Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on the size of the electrophoresis chamber – **it should not exceed 5 volts/ cm between electrodes.**
- Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
- Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye – this will take a couple of minutes (it will run in the same direction as the DNA).
- Let the power run until the blue dye approaches the end of the gel.
- Turn off the power.
- Disconnect the wires from the power supply.
- Remove the lid of the electrophoresis chamber.
- Using gloves, carefully remove the tray and gel.

Gel Staining

- Using gloves remove the gel from the casting tray and place into the staining dish.
- Add warmed (50-55°) staining mix.
- Allow gel to stain for at least 25-30 minutes (the entire gel will become dark blue).
- Pour off the stain (the stain can be saved for future use).
- Rinse the gel and staining tray with water to remove residual stain.
- Fill the tray with warm tap water (50-55°). Change the water several times as it turns blue. Gradually the gel will become lighter, leaving only dark blue DNA bands. Destain overnight for best results.
- View the gel against a white light box or bright surface.
- Record the data while the gel is fresh, very light bands may be difficult to see with time.

Gels stained with blue stains are stable for long periods. When destaining is complete, remove gel from water and allow the gel to dehydrate. Dark bands can be seen for in a dried gel for weeks or months.

(vi) Ethidium Bromide Staining Procedure after Electrophoresis

1. Place the gel into the appropriate volume of 0.5 µg/ml ethidium bromide (EtBr) stain for 15–30 minutes. Use enough staining solution to cover the entire gel.
2. Destain the gel for 10–30 minutes in dH₂O using the same volume used for staining. Ethidium Bromide can be removed from the DNA with extended destaining. This will cause lower sensitivity of detection. However, insufficient destaining will create higher background fluorescence.
3. Rinse the gel briefly with dH₂O to remove any residual staining solution.
4. Place the gel on a UV transilluminator for nucleic acid visualization and analysis. DNA/Ethidium Bromide complexes may be illuminated with UV light of 254, 302, or 366 nm. Sensitivity decreases with illumination at higher wavelengths. However, nicking of DNA will increase below 302 nm.

Nucleic acids in the gel can be visualized through the UVTP trays. If a UVTP tray is not used, place household plastic wrap between the UV transilluminator and the gel to avoid contaminating the transilluminator with nucleic acids or EtBr.

(vii) Composition of buffers

1x Tris-Acetate-EDTA (TAE) — 40 mM tris (pH 7.6), 20 mM acetic acid, and 1 mM EDTA. 50 xs Stock (1 liter) —dissolve in 600 ml distilled water:

242 g tris base (FW = 121)

57.1 ml glacial acetic acid 100 ml 0.5 M EDTA (pH 8.0).

Fill to a final volume of 1 liter with distilled water.

1x Tris-Boric Acid-EDTA (TBE)—89 mM tris (pH 7.6), 89 mM boric acid, 2 mM EDTA

10 xs Stock (1 liter)—dissolve in 600 ml distilled water:

108 g tris base (FW = 121)

55 g boric acid (FW = 61.8)

40 ml 0.5 M EDTA (pH 8.0)

Fill to a final volume of 1 liter with distilled water.

APPENDIX 7

Staphylococcal Enterotoxin –Reverse Passive Latex Agglutination Kit Toxin Detection Kit

Code: TD0900

Components of Kit

TD901 Latex sensitised with anti-enterotoxin A. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin A.

TD902 Latex sensitised with anti-enterotoxin B. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin B.

TD903 Latex sensitised with anti-enterotoxin C. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin C.

TD904 Latex sensitised with anti-enterotoxin D. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin D.

TD905 Latex control. Latex suspension sensitised with non-immune rabbit globulins.

TD906 Staphylococcal enterotoxin A control

TD907 Staphylococcal enterotoxin B control.

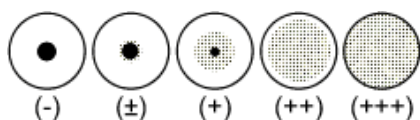
TD908 Staphylococcal enterotoxin C control.

TD909 Staphylococcal enterotoxin D control.

TD910 Diluent. Phosphate buffered saline containing bovine serum albumin and sodium hexametaphosphate.

Interpreation of Test Results

The agglutination pattern should be judged by comparison with the following illustration.



Results classified as (+), (++) and (+++) are considered to be positive.

Limitations of the Test

The sensitivity of this test in detecting the enterotoxins has been reported to be 0.5ng/ml in the test extract

APPENDIX 8

Qiagen DNeasy DNA extraction protocol for bacterial cultures

Adapted from QIAgen DNeasy.

Procedure:

Appropriately label a 1.5 ml tube for each sample.

1. Add 1.75 ml of bacterial culture to a labeled 2 ml tube.
2. Spin tubes at 20,000-x g for 5 minutes in centrifuge. Decant liquid.
3. Add 180 ul of enzymatic lysis buffer to you tube and vortex 10-20 s.
4. Incubate at 37° C for 30 min.
5. Add 25 ul of proteinase K to the tube
6. Add 200 ul of Buffer AL to the tube.
7. Vortex the tube briefly.
8. Incubate at 56° C for 30 min. Now is a good time to label all the tubes you need for the rest of the protocol.
9. Add 200 ul of 100% ethanol to the tube
10. Vortex briefly.
11. Using a micropipette, transfer entire contents (~600 ul) of tube to labeled spin column.
12. Centrifuge column at 10,000 x g for 1 min.
13. Remove column from collection tube. Place column in new collection tube.
14. Add 500 ul of buffer AW1 to the column and centrifuge at 10,000 x g for 1 minute.
15. Remove column from collection tube. Place column in new collection tube.
16. Add 500 ul of buffer AW2 to the column and centrifuge at 20,000 x g for 3 minute.
17. Carefully remove tubes from centrifuge, do not let flow-through contact column. If this happens, spin tube again for 1 min at 20,000 x g.
18. Transfer the column to a 1.5 ml tube and add 200 ul of buffer AE to the column.
19. Let column stand at room temperature for 1 minute.
20. Centrifuge at 10,000-x g for 1 minute. Discard the column and store the DNA appropriately (4° C for short term, -20° C for long term).

Materials required

Qiagen DNeasy Blood and Tissue kit
 200 and 1000 ul pipette tips
 1.5 ml microcentrifuge tubes
 2.0 ml microcentrifuge tubes
 Overnight bacterial cultures

Equipment required

Bench top centrifuge capable of 20,000 x g 200-ul micropipette
 1000 ul micropipette
 Vortexer

APPENDIX 9**Polymerase Chain Reaction (PCR) master Mix**

M7501 10 reactions

M7502 100 reactions

M7505 1,000 reactions

Description:

PCR Master Mix includes Nuclease-Free Water and PCR Master Mix, 2X. PCR Master Mix is a premixed, ready-to-use solution containing Taq DNA polymerase, dNTPs, MgCl₂ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by **PCR**.

PCR Master Mix, 2X: 50 units/ml of Taq DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400 μ M dATP, 400 μ M dGTP, 400 μ M dCTP, 400 μ M dTTP, 3mM MgCl₂.

Storage Conditions: See the Product Information Label for storage recommendations. Minimize the number of freeze-thaw cycles by storing in working aliquots. Product may be stored at 4°C for up to three months. Mix well prior to use.

Quality Control Assays

Activity Assays

Functional Assay: PCR Master Mix is tested for performance in the polymerase chain reaction (PCR) using PCR Master

Mix, 1X, to amplify a 360bp region of the α -1-antitrypsin gene from 100 molecules (0.35ng) of human genomic DNA. The resulting PCR product is visualized on an ethidium bromide-stained agarose gel.

Taq DNA Polymerase Activity Assay: Taq DNA polymerase activity is confirmed before the enzyme is added to the PCR

Master Mix, 2X. The polymerase activity is assayed in 50mM Tris-HCl (pH 9.0); 50mM NaCl; 5mM MgCl₂; 200 μ M each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [3H] dTTP); 10 μ g activated calf thymus DNA and 0.1mg/ml BSA in a final volume of 50 μ l.

Contaminant Assays

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected.

1. Description

PCR Master Mix has been optimized for use in routine PCR reactions for amplifying DNA

template in the range of 0.2–2kb.

2. Product Components

Product Size Cat. No

PCR Master Mix 10 reactions M7501

Each system contains sufficient reagents to perform ten 50 μ l reactions. Includes:

- 250 μ l PCR Master Mix, 2X
- 1.25ml Nuclease-Free Water

Product Size Cat. No

PCR Master Mix 100 reactions M7502

Each system contains sufficient reagents to perform one hundred 50 μ l reactions.

Includes:

- 2 × 1.25ml PCR Master Mix, 2X
- 2 × 1.25ml Nuclease Free Water

Product Size Cat. No

PCR Master Mix 1,000 reactions M7505

Each system contains sufficient reagents to perform one thousand 50µl reactions.

Includes:

- 1 × 25ml PCR Master Mix, 2X
- 1 × 25ml Nuclease Free Water

3. Protocol

1. Thaw the PCR Master Mix at room temperature. Vortex the Master Mix and then spin it briefly in a microcentrifuge to collect the material in the bottom of the tube.

2. Prepare one of the following reaction mixes on ice:

Component	Volume	Final Conc.
For a 25µl reaction volume:		
PCR Master Mix, 2X	12.5µl	1X
upstream primer, 10µM	0.25–2.5µ	1 0.1–1.0µM
downstream primer, 10µM	0.25–2.5µ	1 0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water	to 25µl	N.A.
For a 50µl reaction volume:		
PCR Master Mix, 2X	25µl	1X
upstream primer, 10µM	0.5–5.0µl	0.11.0µM
downstream primer, 10µM	0.5–5.0µl	0.11.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water	to 50µl	N.A.
For a 100µl reaction volume:		
PCR Master Mix, 2X	50µl	1X
upstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
downstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water	to 100µl	N.A.

APPENDIX 10

Preparation of McFarland Standards for antibiotic resistance

Reagents:

1. Sulfuric acid, 1%
2. Barium Chloride, 1.175%

Equipment:

1. 100ml volumetric flasks
2. Vortex
3. Spectrophotometer
4. Magnetic stirrer and stirring rod

Procedure for the Preparation of a 0.5 McFarland Standard:

1. Add approximately 85 ml of 1% sulfuric acid (H_2SO_4) to a 100ml volumetric flask.
2. Using a volumetric pipette, add 0.5ml of 1.175% anhydrous barium chloride ($BaCl_2$) drop wise to the 1% sulfuric acid (H_2SO_4) while constantly swirling the flask.
3. Bring the volume to 100ml with 1% H_2SO_4 .
4. Stir or mix for approximately 3 to 5 minutes while examining visually, until the solution appears homogeneous and free of clumps.

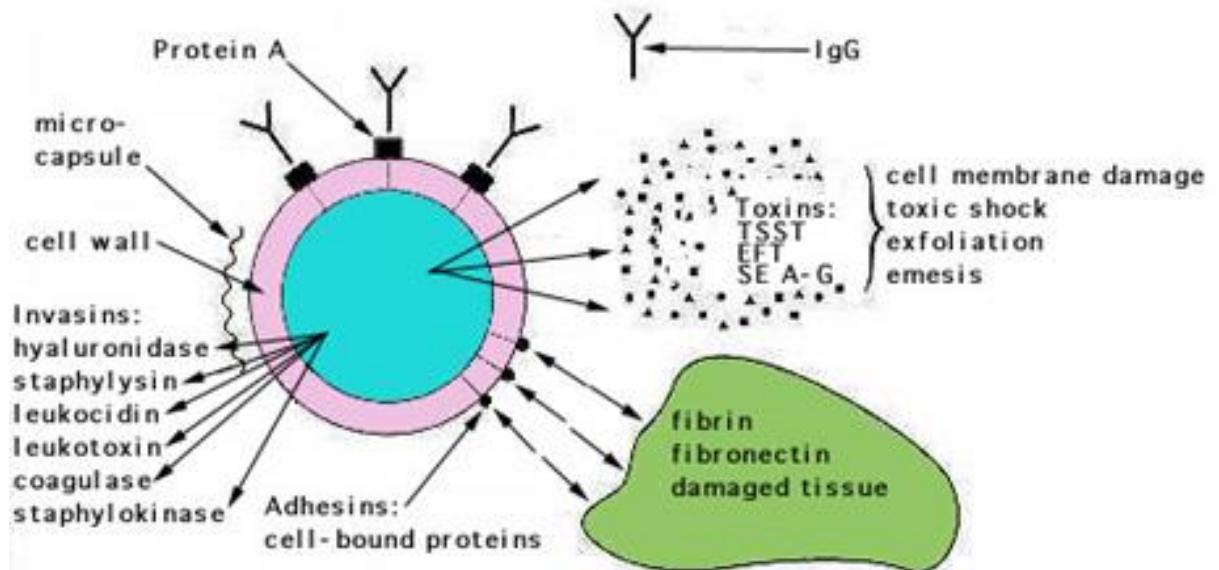
A magnetic stirrer can be used for this step if available. Check optical density (OD) of the McFarland standard at a wavelength of 625nm and record results.

The acceptable range for a McFarland 0.5 standard is 0.08 to 0.1

APPENDIX 11

Virulence determinants of *Staphylococcus aureus*.

S. aureus is known for its capacity to cause a broad range of important infections in humans. Such capacity is related to the expression of an array of factors that participate in pathogenesis of infection, allowing this bacterium to adhere to surfaces/tissues, avoid or invade the immune system, and cause harmful toxic effects to the host. These factors are known as virulence determinants, and can be divided into cell-surface-associated (adherence) and secreted (exotoxins) factors.



APPENDIX 12

General overview of analytical methods used to improve SFPO characterization

