

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

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**STUDIES ON THE PREVALENCE, ECOLOGY AND ANTIBIOGRAM OF
STAPHYLOCOCCUS SPECIES ISOLATED FROM CHICKENS SLAUGHTERED AT
SMALL AND LARGE SCALE POULTRY FARMS IN DEBRE ZEIT, ETHIOPIA**

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**A thesis submitted to the School of Graduate Studies of Addis Ababa University in Partial
Fulfillment of the Requirements for Master Degree in Veterinary Medicine**

BY

SIHIN TESFAYE

June 2012

Debre Zeit, Ethiopia

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LIST OF ABBREVIATIONS

µg	Micrograms
µg/kg	Microgram per kilogram
µm	Micrometer
°C	Degree Centigrade
a _w	Water Activity
BAP	Blood Agar Plates
CAD	Carcasses After Defeathering
CAE	Carcasses After Evisceration
CDC	Centers for Disease Control
CFS	Centre for Food Safety
CFU/g	Colony Forming Units Per Gram
CHP	Center for Health Protection
CNS	Coagulase Negative Staphylococci
CPS	Coagulase Positive Staphylococci
CSA	Central Statistical Agency
DNase	Deoxyribonuclease
e.g.	Example
EDTA	Ethylenediaminetetraacetic acid

EFSA	European Food Safety Authority
FAO	Food and Agricultural Organization
FBD	Food Borne Disease
g	Gram
GMP	Good Manufacturing Practices
GP2	Gram positive 2
H ₂ O ₂	Hydrogen peroxide
HACCP	Hazard Analysis Critical Control Point
IgG	Immunoglobulin G
ISO	International Standard Organization
MHA	Muller-Hinton Agar
MRCNS	Methicillin-Resistant Coagulase-Negative <i>Staphylococcus</i>
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
MSA	Mannitol Salt Agar
NaCl	Sodium Chloride
NAP	Nutrient Agar Plates
NCCLS	National Committee for Clinical Laboratory Standards
NIAID	National Institute of Allergy and Infectious Diseases
OIE	Office International Des Epizooties
PAB	Purple Agar Base
PBP	Penicillin-Binding Protein

pH	Power of Hydrogen
PPPs	Poultry Processing Plants
RBCs	Red Blood Cells
SCC	Staphylococcal Cassette Chromosome
SEA	Staphylococcal Enterotoxin A
SEB	Staphylococcal Enterotoxin B
SEC	Staphylococcal Enterotoxin C
SEC1	Staphylococcal Enterotoxin C1
SEC2	Staphylococcal Enterotoxin C2
SED	Staphylococcal Enterotoxin D
SEE	Staphylococcal Enterotoxin E
SEs	Staphylococcal Enterotoxins
SFP	Staphylococcal Food Poisoning
Spp	Species
SOP	Standard Operation Protocols
TSST-1	Toxic Shock Syndrome Toxin-1
VISA	Vancomycin-intermediate <i>Staphylococcus aureus</i>
WHO	World Health Organization

ABSTRACT

A cross-sectional study to determine the prevalence, species involved and antimicrobial profile of *Staphylococcus* species isolated from chicken carcass, equipment and hands of apparently healthy the abattoirs workers was undertaken from November 2011 to April 2012. A total of 384 samples consisting of chicken carcass (n=340), abattoir equipment (n=24) and workers' hands (n=20) were taken from large and small scale poultry farms in Debre Zeit. A total of 187 (47.7%) *Staphylococcus* species were isolated from chicken carcass and immediate slaughter environment and were further identified to species level by important conventional biochemical tests. Furthermore, species identification was made by OmniLog reading. From the total of 187 staphylococci isolated, *Staphylococcus aureus*, *S. intermedius*, *S. hyicus* and coagulase negative staphylococci species contributed 13.8%, 9.6%, 6.8% and 17.4%, respectively. Regarding the origin of the samples, among isolates of the carcass swabs 12.4%, 9.1%, 6.5% and 17.7% were identified as *S. aureus*, *S. intermedius*, *S. hyicus* and coagulase negative staphylococci, correspondingly. While in the immediate slaughter environment the rates of isolation were 25.0%, 13.6%, 9.1% and 15.9%, respectively for the aforementioned species. Consequently, statistically significant association ($p=0.022$) was observed in the prevalence of *S. aureus* between the two types of sample origin. No statistically significant associations of the prevalence of the rest species were detected amongst the carcass and environmental samples. In the large scale farms, the prevalence of *Staphylococcus* species were, *S. aureus* 11.5%, *S. intermedius* 8.3%, *S. hyicus* 6.3% and CNS 16.7%. In the small scale farms the isolation rates of the isolates were *S. aureus* 16.1%, *S. intermedius* 10.9%, *S. hyicus* 7.3% and CNS 18.2%. A higher isolation frequency of all species was noted in the small scale farms; however, these differences were not statistically significant. In this study, varying level of resistance of *S. aureus* (100%) to bacitracin, methicillin, neomycin and polymixin was noticed. The susceptibility pattern indicated *S. aureus* isolates exhibited varying level of resistance to two or more antimicrobial agents. 66.6% of the isolates showed susceptibility to the tested drugs.

Key Words: *Staphylococcus*, poultry, prevalence, carcass, isolation, antimicrobial susceptibility, Debre Zeit.

Meat is considered as the most nutrient source of proteins consumed by humans. It is the most palatable of all important food source to ensure a sufficient nutrient supply to support the growth of organisms. Food-borne zoonotic diseases may be inevitable for as many food products derived as possible each year and are thus an important food safety challenge (Hart et al., 2009). They are the leading cause of illness and death in developing countries owing to lack of control in animal care and animal origin (Pramono et al., 2009). Changes in eating habits, more extensive complex and highly food supply production with increased international movement and globalization practices are major contributing factors (Fisher et al., 2011).

Food-borne diseases (FBDs) are universal public health problems and the transmission and control including health and economic losses (Jain et al., 2003; Krasakopoulou et al., 2007). Food-borne diseases or food poisonings identified by the World Health Organization (WHO) as the illness or disease of microbes or toxic agents caused by the consumption of food or beverages contaminated with bacteria and/or other agents, parasites, viruses, or chemicals (Gyorkos et al., 2005; Hossain et al., 2005). A food-borne outbreak is said to occur if similar illness, often gastroenteritis, in a minimum of two people and evidence of food as the source are confirmed (Jain et al., 2003; Saeed, 2007).

In recent years, food-borne infections and intoxications have assumed significance as a health hazard. Epidemiological studies suggest that poultry meat is still the primary source of human food poisoning. Bacterial contamination of these foods depends on the rearing level of the poultry, carcasses used as the raw product, the hygienic practices during transportation and on the time and temperature of storage (Hossain et al., 2005; Othman and Davies, 2009). Poultry meat is more popular in the consumer market because of advantages such as easy digestibility and acceptance by the majority of people. However, the presence of pathogenic and spoilage microorganisms in poultry meat and its by-products contains a high level of concern for the public, consumer and public health officials worldwide (Vandana et al., 2009).

1. INTRODUCTION

Meat is considered as the most nutritive source of protein consumed by humans. It is the most perishable of all important foods since it contains sufficient nutrient needed to support the growth of microorganisms. Food-borne microbiologic hazards may be responsible for as many cases of illness as possible each year and are thus an important food safety challenge (Rao *et al.*, 2009). They are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs (Fratamico *et al.*, 2005). Changes in eating habits, mass catering complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors (Huber *et al.*, 2011).

Food-borne diseases (FBD) are universal public health problems and the implications are great including health and economic losses (Loir *et al.*, 2003; Kerouanton *et al.*, 2007). Food-borne diseases or food poisonings are defined by the World health Organization (WHO) as an illness or disease of infectious or toxic nature caused by the consumption of foods or water contaminated with bacteria and/or their toxins, parasites, viruses, or chemicals (Aycicek *et al.*, 2005; Bania *et al.*, 2006). A food-borne outbreak is said to occur if similar illness, often gastrointestinal, in a minimum of two people and evidence of food as the source are confirmed (Loir *et al.*, 2003; Shah, 2003).

In recent years, food-borne infections and intoxications have assumed significance as a health hazard. Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning. Bacterial contamination of these foods depends on the bacterial level of the poultry carcasses used as the raw product, the hygienic practices during manipulation and on the time and temperature of storage (Bania *et al.*, 2006; Gündoğan and Devren, 2010). Poultry meat is more popular in the consumer market because of advantages such as easy digestibility and acceptance by the majority of people. However, the presence of pathogenic and spoilage microorganisms in poultry meat and its by-products remains a significant concern for suppliers, consumers and public health officials worldwide (Yashoda *et al.* 2001).

Food-borne pathogens, though, usually cause self-limiting gastroenteritis, complications may occur, resulting in more severity (Zhao *et al.*, 2001). Staphylococcal food poisoning (SFP) is one of the most common FBD worldwide with high occurrence second to salmonellosis (Acco *et al.*, 2003; Aycicek *et al.*, 2005). *S. aureus* is one of the most common agents in bacterial food poisoning outbreaks (Adwan *et al.*, 2005) and symptoms of staphylococcal food intoxication generally occur one to six hours after the food is ingested and the common symptoms are nausea, vomiting, abdominal cramps, and diarrhea (Ash, 2008; Ben-David *et al.*, 2008).

Poultry meat and egg products could be the common sources of *Staphylococcus aureus*, posing a potential health risk. During processing of poultry meat products, broiler carcasses come in contact with many solid surfaces. Bacteria from the carcasses can attach to wet equipment surfaces, and provide a source of cross-contamination for subsequent carcasses. An increased understanding of bacterial attachment formation will assist in the development of interventions to counteract these processes and, thereby, enhance plant sanitation and pathogen control (Arnold and Silvers, 2000; Kumar and Saravanan, 2011).

Staphylococcosis, an infectious bacterial zoonosis of global significance is caused by *S. aureus* which is Gram positive, non-capsulated, non-motile, catalase positive and non sporulated organism, 0.8-1 micrometer in diameter (Pal, 2007). *S. aureus* is important in relation to poultry meat, because it can produce enterotoxins which may cause food poisoning in humans (Busani *et al.*, 2006; Quinn *et al.*, 2002).

In addition to being a major disease-producing microorganism for poultry, approximately 50% of typical and atypical *S. aureus* strains produce enterotoxins that can cause food poisoning in human beings. Poultry associated food poisoning can occur due to enterotoxin producing *S. aureus* strains, which contaminate poultry carcasses at processing. *S. aureus* strains from processed poultry are thought to be human strains endemic to the processing plant or from the hands of workers in the plant (Capita *et al.*, 2002; Andreasen, 2003).

Epidemiological reports suggest that meat product is one of the major causes of diarrheal illness which account for 36% of mortality cases in developing countries (FAO/WHO, 2002). *S. aureus* has been tested in meat and poultry products to assess microbiological safety, sanitation conditions during processing, and storage quality of products. *S. aureus* is a frequent etiological

agent of food poisoning. Therefore its presence in poultry emphasizes the need for laboratory surveillance for this bacterial pathogen (Lee, 2003; WHO, 2007).

However, not all *S. aureus* strains present in processed poultry carcasses are a cause for public health concern. The animal strains make a very small contribution to human food poisoning (WHO, 2007). On the other hand, *S. aureus* isolates from human sources may be considered the most dangerous strains of public health significance. In fact, poultry meat has been frequently associated with food-borne illness in which initial contamination is traceable to food handlers (Van de Venter, 2000; Pal, 2007).

The large amount of *S. aureus* found in meat and poultry meat products, represents a health hazard to the consumers, and emphasizes the need for improved hygiene practice at levels in the food industry, due to their ability to display lipolytic and proteolytic activity under psychrotrophic conditions (Gündoğan and Devren, 2010). Researchers reported that the presence of *S. aureus* in foods commonly indicates contamination that may be directly introduced into the food by workers who have skin lesions containing *S. aureus*, or sneezing or coughing (Rho and Schaffner, 2007; Gündoğan and Devren, 2010).

After coagulase-negative *Staphylococcus* spp. (Sandel and McKillip, 2004), *S. aureus* is the most widespread *Staphylococcus* spp. that can survive, colonize, and persist at various processing stages in commercial poultry processing plants due to its expression of various key properties, including adhesion and chlorine resistance. In addition, *S. aureus* associated with poultry processing are also notorious as reservoir microorganisms of multiple antibiotic resistance traits (Khan *et al.*, 2000; Smith, 2007).

An important characteristic that differentiates *S. aureus* from most *Staphylococcus* spp. is its ability to produce coagulase, an enzyme that clots blood plasma. Other coagulase - positive species such as *S. hyicus* and *S. intermedius* have been also identified (Alişarlı *et al.*, 2003). These and several coagulase - negative species including *S. epidermidis* and *S. xylosum* have been shown to produce low levels of enterotoxins. Among them, *S. epidermidis* and *S. intermedius* were reported to be the causative agents in food-borne outbreaks (Çepoğlu *et al.*, 2010).

Therefore, while a high correlation between coagulase production and enterotoxigenicity has been reported, the ability to produce coagulase should not be considered the only indication for enterotoxin production (Soriano *et al.*, 2002). In addition, very little is known about the growth of coagulase-negative *Staphylococcus* in foods. Consequently, it is important to note that coagulase negative staphylococci can produce enterotoxins and they can also cause food poisoning (Çepoğlu *et al.*, 2010).

There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (Van Looveren *et al.*, 2001). Excessive use of medication during poultry production, or disinfectants used in the food-processing industry, can give rise to the problem of resistance. This adds to the problem of food hygiene (Heath, 2006). Antibiotics are still used as growth promoters even in many developed countries. The antibiotics are used in order to enhance the production qualities of poultry and other livestock. The non-regulated use of antibiotics as veterinary treatment might be a link to the appearance of resistant strains of pathogens posing a threat to human health. The antibiotics used for veterinary treatment can in some cases overlap with crucial ones used to treat human illnesses (FAO, 2004; Antunes *et al.*, 2006).

Most food industries in developing countries are not well aware of food safety issues, and knowledge of modern technologies, good manufacturing practices (GMP), hygiene, hazard analysis critical control point (HACCP) system, and quality control is often limited or absent. Cold storage facilities are inadequate and quality of water used for food processing may not be suitable. The vast numbers of laborers that handle food in factories, as well as on farms, are illiterate and untrained. In developing countries, including Ethiopia, food-borne illnesses are perceived as mild and self-limiting diseases. Their severe and chronic health consequence is often overlooked, as are their consequences on trade and the economy. In such countries, lack of information leads to lack of appreciation of the health significance of unsafe food and this, in turn, leads to low priority and sometimes no resources assigned to food safety (Van de Venter, 2000; Randolph *et al.*, 2007).

The prevalence of food-borne diseases in developing countries is alarmingly high, and action is needed especially with regard to consumer awareness. To achieve this, there is a need for more information to be gathered about the conditions in individual countries (Stamoulis *et al.*, 2004; Singer *et al.*, 2007). In spite of the abovementioned prevailing situation and the presence of a number of public health problems due to FBDs resulting from the consumption of different food items in Ethiopia, there is a paucity of well documented information on the occurrence of *Staphylococcus* species in chicken meat.

The ultimate goal of the current research work is to partly contribute towards the development of national food safety strategies, which aim to protect the consumer from food borne diseases. This is through provision of basic data on the situation of *Staphylococcus* species in poultry carcass, equipments and personnel.

General objective:

The general objective of this research undertaking was to conduct microbiological study of *Staphylococcus* species from poultry meat, immediate slaughter environment and its potential implications as health hazard to consumers.

Specific objectives:

- To determine the prevalence and distribution of *Staphylococcus* species from chickens slaughtered in large and small scale poultry farms in Debre Zeit, Ethiopia.
- To assess the prevalence of *Staphylococcus* species among abattoir equipment and personnel's hands; and establish their association with contamination of the chicken carcasses.
- To investigate the susceptibility of *Staphylococcus* isolates to antimicrobial agents used in veterinary and human medicine for the treatment of bacterial diseases including staphylococcosis.

2. LITERATURE REVIEW

2.1. The General Characteristics of *Staphylococcus* Organisms

The staphylococci were first described by the Scottish surgeon, Sir Alexander Ogston as the cause of a number of pyogenic (pus forming) infections in humans. In 1882, he gave them the name *Staphylococcus* (Greek: *staphyle*, bunch of grapes; *coccus*, a grain or berry), after their appearance under the microscope (Adams and Moss, 2005).

The four special characteristic of staphylococci are virulence- causing severe disease in normal hosts, difference- causing different diseases in different sites by different mechanisms and involving different strains, persistence- both in the environment, and on humans, who are frequently asymptomatic carriers and resistance- to many antibiotics that were previously effective (Shah, 2003; Todar, 2008).

Staphylococci do not produce endospores but are highly resistant to drying, especially, when associated with organic matter such as blood, pus and other tissue fluids. They are quite resistant to desiccation and high osmotic conditions. These properties facilitate their survival in the environment and growth in food products. Staphylococci organisms are usually readily killed at cooking, pasteurization temperatures and survives frozen storage. On the other hand, staphylococcal enterotoxins (SEs) are extremely heat stable (Silva *et al.*, 2000; Quinn *et al.*, 2002; Ash, 2008).

Staphylococci frequently isolated from poultry include *S. aureus* and *S. epidermidis*. Another species, *S. gallinarum*, has been isolated from processed poultry (Andreasen, 2003). The principal habitat of the staphylococci is the skin, skin glands and the mucous membranes of warm blooded animals (Pal, 2007). Several species are associated with particular hosts, for example *S. hyicus* with pigs, and *S. gallinarum* with chickens. *S. aureus* is more widespread but occurs most frequently on the skin of higher primates. In humans, it is particularly associated with the nasal tract where it is found in 20-50% of healthy individuals (Adams and Moss, 2005).

The *Staphylococcus* genus is classified into two groups based on their ability to produce coagulase: coagulase positive staphylococci (CPS) and coagulase negative staphylococci (CNS). *S. aureus*, *S. intermedius*, *S. hyicus* are the species that belong to CPS (Turutoglu *et al.*, 2005; Morrison, 2008).

Three species of staphylococci are human pathogens: *S. aureus*, *S. epidermidis*, and *S. saprophyticus*. Of the three, *S. aureus* is by far the most important. *S. aureus* produces a carotenoid pigment that imparts a golden color to its colonies. This pigment enhances the pathogenicity of the organism by inactivating the microbicidal effect of superoxides and other reactive oxygen species within neutrophils. *S. epidermidis* does not synthesize this pigment and produces white colonies. The virulence of *S. epidermidis* is significantly less than that of *S. aureus*. Two other characteristics further distinguish these species, namely, *S. aureus* usually ferments mannitol and hemolyzes red blood cells, whereas the others do not (Levinson, 2008).

2.1.1. Taxonomy of *Staphylococcus* Species

Clinically the most important genus of *Staphylococcaceae* family is *Staphylococcus* (Rho and Schaffner, 2007). To date over 40 species and 24 subspecies of the genus *Staphylococcus* that are collectively referred to as staphylococci are described (Jay, 2000; Todar, 2008). According to Shah (2003) and Todar (2008) the scientific classification of *Staphylococcus* is as follows:

Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	<i>Staphylococcaceae</i>
Genus	<i>Staphylococcus</i>

The pathogenic staphylococci, *S. aureus*, *S. intermedius* and *S. hyicus* (most strains) are coagulase-positive (Morrison, 2008). Enterotoxin production is principally associated with the species *S. aureus*, although it has also been reported in others including *S. intermedius* and *S. hyicus* (Adams and Moss, 2005). The coagulase test correlates well with pathogenicity. Two commonly isolated coagulase-negative staphylococci, *S. epidermidis* and *S. saprophyticus*, occur as commensals and in the environment. They cause opportunistic infections in humans and, very occasionally, in animals although they are usually regarded as non-pathogenic (Quinn *et al.*, 2002; Walderhaug, 2007).

Some strains of coagulase positive *S. aureus* are endowed with the genetic capacity to produce certain extracellular proteins which, when ingested, cause a severe gastrointestinal disturbance. These proteins are known as staphylococcal enterotoxins. There are five distinct, major, serological types of enterotoxins currently recognized as significant and they are designated as serotypes A, B, C (C1, C2, C3), D and E (Loir *et al.*, 2003).

2.1.2. Morphology and staining reaction

S. aureus is the only staphylococcal species in poultry considered to be pathogenic. Typical pathogenic *S. aureus* strains are Gram-positive, coccoid in shape, and found in clusters when grown on solid media. In liquid media, they may occur in short chains. Older cultures (>24 hours) may stain Gram-negative (Andreasen, 2003).

They have unique peptidoglycan structure that is highly cross-linked with bridges of amino acids (Shah, 2003; Hein *et al.*, 2005). In addition, to the usual peptidoglycan, staphylococci have two unique components that is protein A and teichoic acids in their cell wall. Protein A is linked to the peptidoglycan with an outer end protecting the microbe from phagocytosis (opsonisation) and the teichoic acids are glycerophosphates which are involved in complement activation and attachment to mucosal surfaces as they bind to fibronectin (Smith, 2007; Walderhaug, 2007; Todar, 2008).

The staphylococci are Gram-positive cocci that tend to be arranged in irregular clusters or 'bunches of grapes' formation (Figure 1). The average diameter of the cocci is 1.0 μm . Cell division occurs in more than one plane so that cells form irregular clumps resembling bunches of grapes. They are facultative anaerobes (fermentative) catalase-positive, oxidase-negative, non-spore forming, non-capsulated and non-motile (Quinn *et al.*, 2002; Pal, 2007).

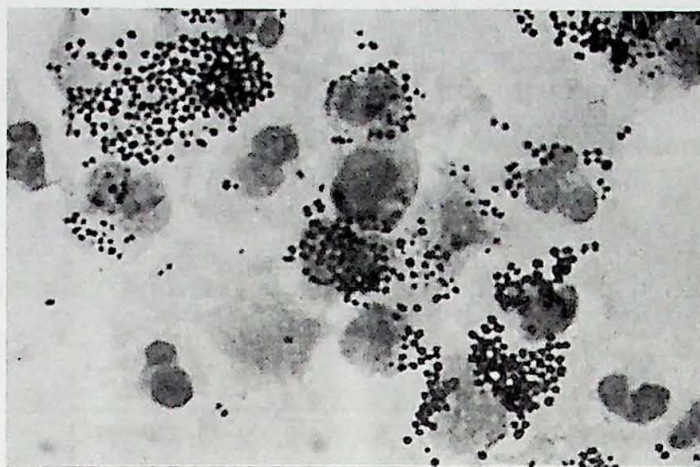


Figure 1: *Staphylococcus aureus* in a Gram-stained smear showing the characteristic 'bunches of grapes' and other arrangement of cells ($\times 1000$) (Todar, 2008).

The most obvious morphological character is their marked tendency to occur in masses of cells in grape-like clusters. This happens as a consequence of the geometry of cell division. Staphylococci grow in clusters because cell division occurs in more than one plane (Quinn *et al.*, 2002).

2.1.3. Growth requirement and cultural characteristics

Staphylococci are not highly fastidious in their nutritive requirements and grow readily on the usual meat extract peptone mediums. Growth of *Staphylococcus* is most profuse on sheep blood agar medium (Jay, 2000; Quinn *et al.*, 2002). They are facultative anaerobes that grow best by aerobic respiration or fermentation that yields principally lactic acid (Silva *et al.*, 2000).

The optimum temperature for growth is 35 $^{\circ}\text{C}$ to 37 $^{\circ}\text{C}$ (they can grow at temperatures ranging from 6 $^{\circ}\text{C}$ to 48 $^{\circ}\text{C}$ (Adams and Moss, 2005; Aycicek *et al.*, 2005; Ash, 2008). Foods with a pH around 7 are ideal for bacterial growth and most animal food products including meat, fish,

poultry, eggs and milk have a pH around 7 (Sandel and Mc Killip, 2004; Rho and Schaffner, 2007).

The most favorable pH for growth is 7 to 7.5. The organism is resistant to drying and may grow and produce enterotoxins in foods with water activity (a_w) as low as 0.85. They can grow in foods with 25% NaCl but grows well in 7-10% NaCl (Aycicek *et al.*, 2005; Ash, 2008). The optimum temperature for toxin production is 35 °C to 40 °C (range: 10 °C to 40 °C), pH is 5.3 to 7.0 (range: 4.8 to 9.0), a_w is 0.90 (range: 0.86 to 0.99) and greatest toxin production is in the presence of oxygen (Table 1) (Adams and Moss, 2005; Aycicek *et al.*, 2005; Morandi *et al.*, 2007; Ash, 2008).

Table 1: Factors permitting growth and enterotoxin production by *Staphylococcus aureus* (Adams and Moss, 2005).

Factor	Growth		Enterotoxin Production	
	Optimum	Range	Optimum	Range
Temperature	35-37	7-48	35-40	10-45
pH	6.0-7.0	4.0-9.8	5.3-6.8	4.8-9.0
NaCl	0.5-4.0%	0-20%	0.5%	0-20%
Water activity	0.98->0.99	0.83->0.99	>0.99	0.86->0.99
Atmosphere	Aerobic	Aerobic- Anaerobic	5-20%	Aerobic- Anaerobic

A range of selective and diagnostic media have been developed to assist in the detection and enumeration of staphylococci in routine food surveillance programs and food poisoning investigations. Selective bacteriological media containing one or more agents that are inhibitory to microorganisms other than the target pathogen (staphylococci) can be applied. The microorganism of interest is not inhibited by the presence of these components in the medium,

and will therefore, form visible colonies during incubation (Quinn *et al.*, 2002). The two selective agents most commonly used for these pathogens are sodium chloride and potassium tellurite (Pal, 2007).

A common medium used for the isolation of pathogenic staphylococci is mannitol salt agar (MSA). Some organisms cannot tolerate a high osmotic pressure. Media containing higher than normal salt concentrations such as MSA inhibit the growth of these non-tolerant organisms other than the salt tolerant staphylococci. Additionally, MSA contains the sugar mannitol. Staphylococcal organisms can utilize mannitol as a fermentable carbohydrate and will produce acid end products from this metabolism (Jay, 2000; Quinn *et al.*, 2002).

Since this process is invisible, an indicator is added to the medium to detect changes in pH. Phenol red is the indicator used in MSA. It is red at neutral pH but turns yellow if conditions in the media become acidic. Pathogenic staphylococci not only grow on the medium, but they also produce acid from it. This acid production turns the pH indicator from red to yellow. Nonpathogenic staphylococci can grow on the medium but produce no acid from it and the media remains pink (Quinn *et al.*, 2002).

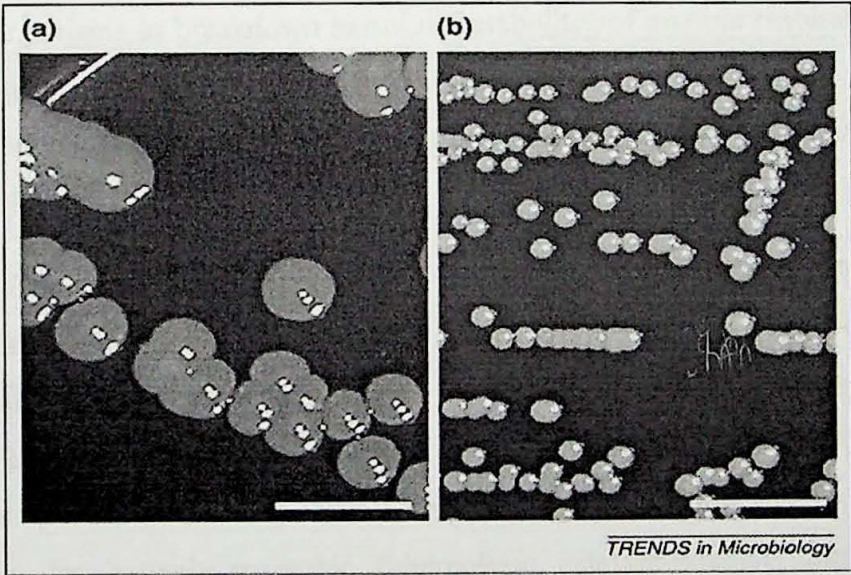


Figure 2: Pictures of *S. aureus* of same clonal origin on sheep blood agar plates after 48hrs incubation. Scale bar = 1 cm. (a) Normal-sized colonies, of 2–3 mm in size, show a typical golden pigmentation. (b) Small colony variants (Sendi and Proctor, 2008).

On agar plates, staphylococcal colonies appear opaque to golden yellow in color, glistening, smooth and in circular form. Blood agar is the medium of choice for isolation of the organism. In 24 hours of incubation, they give good growth of creamy, often deeply pigmented, colonies that are surrounded by narrow zones of clear hemolysis, a broader zone of incomplete hemolysis or none depending on the species (Quinn *et al.*, 2002; Bendahou *et al.*, 2008).

Some species of *Staphylococcus* synthesize the enzyme hemolysin that lyses red blood cells (RBCs). The presence or absence of hemolytic properties, therefore, cannot be used as a definitive identification of *Staphylococcus* spp. as some species and strains of *Staphylococcus* spp. may not demonstrate hemolysis (Quinn *et al.*, 2002; Salandra *et al.*, 2008).

2.1.4. Biochemical characteristics

Catalase test: It is important to distinguish streptococci (catalase-negative) from staphylococci, which are catalase-positive. The catalase test determines if the organism produces the enzyme that breaks down hydrogen peroxide (H_2O_2) to water and oxygen (Shah, 2003; Sandel and Mc Killip, 2004). When mixed with 3% H_2O_2 , catalase-positive organisms will generate bubbles of oxygen, which are visible to the naked eye while catalase-negative organisms do not. This enzyme allows organisms to breakdown harmful metabolites of aerobic respiration, and may be seen in aerobic and facultatively anaerobic organisms. It is preferable to test colonies for catalase production from media without blood since erythrocytes possess catalase activities (Quinn *et al.*, 2002; Todar, 2008).

Coagulase test: The presence of coagulase can be demonstrated using EDTA-treated rabbit plasma in the tube coagulase test. Coagulase is an extracellular substance which coagulates human or animal blood plasma in the absence of calcium. Pathogenic staphylococci produce coagulase, while nonpathogenic strains are coagulase-negative (Turutoglu *et al.*, 2005). Coagulase is not specific to *S. aureus* but is also produced by *S. intermedius* and *S. hyicus*. *S. aureus* is aerobic, facultatively anaerobic, β -hemolytic, catalase-positive, and fermentative for glucose and mannitol, and gelatinase-positive (Andreasen, 2003; Morrison, 2008).

Maltose fermentation: Purple agar base with the addition of per cent maltose is a useful medium to differentiate the pathogenic staphylococci with coagulase positive isolates. This presumptive

identification is based on the fact that *S. aureus* strains rapidly ferment maltose and the acidic metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. *S. intermedius* gives a weak or delayed reaction and *S. hyicus* does not ferment maltose but attacks the peptone in the medium producing an alkaline reaction (a deeper purple) around the colonies (Quinn *et al.*, 2002).

Table 2: Reactions of coagulase-positive staphylococci on purple agar with 1% maltose (Quinn *et al.*, 2002).

Species	Maltose fermentation	Reactions on purple agar with 1% maltose
<i>S. aureus</i>	+++	Diffuse yellow color around colonies. Rapid reaction within 24 hours of incubation.
<i>S. intermedius</i>	±	Little change in the medium, slight yellow zone under colonies may have a yellowish tinge.
<i>S. hyicus</i>	-	Diffuse deep purple (alkaline) zone around the colonies.

+++ = rapid fermentation of maltose; ± = weak and slow fermentation; - = negative

2.2. Methicillin Resistant *Staphylococcus aureus*

Treating *S. aureus* infection has been complicated by emergence of drug-resistant strains. For instance, methicillin resistant staphylococci (MRS) infection is now a serious public health problem in both hospitals and the community (Lin *et al.*, 2009). MRS are a major concern to public and animal health. In veterinary medicine and along the meat and milk production line only very limited data were available on characteristics of Methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS) (Lin *et al.*, 2009; Huber *et al.*, 2011).

Since the introduction of β -lactamase-stable antimicrobial drugs into clinical use, methicillin-resistant *S. aureus* (MRSA) strains have emerged as worldwide important pathogens; their prevalence in the community is increasing substantially. Food may be contaminated by MRSA: eating and handling contaminated food is a potential vehicle for transmission. It can enter the slaughterhouse in or on animals and occurs on raw meat. Where MRSA prevalence is high in food-producing animals, people in direct contact with these live animals (especially farmers and veterinarians, and their families) are at risk of colonization and subsequent infection (EFSA, 2004; Persoons *et al.*, 2009).

It is assumed that methicillin-resistance genes had evolved in CNS and were then horizontally transferred among staphylococci (Barbier *et al.*, 2010). The *mecA* gene is located on a mobile genetic element called staphylococcal cassette chromosome (SCC) and confers resistance to methicillin by encoding an altered penicillin-binding protein (PBP2a), which shows limited affinity to beta-lactam antibiotics. The *mecA* gene is now distributed among both coagulase positive and -negative staphylococcal species (Huber *et al.*, 2011).

S. aureus is a well-known pathogen of humans and animals. Methicillin resistance in this bacterial species represents a threat to human health. MRSA has been isolated from raw chicken meat or carcasses in South Korea (Lee, 2003) and in Japan (Kitai *et al.*, 2005); however, these strains were human-associated and not the livestock-associated strains. Thus, the possibility of human contamination of poultry carcasses by slaughterhouse employees cannot be ruled out (Witte *et al.*, 2007).

Poultry farmers can be colonized by MRSA (Van den Broek *et al.*, 2009). Elevated rates of MRSA carriage were also reported in poultry slaughterhouse workers. In the Netherlands, 0% to 24% of broilers entering abattoirs carried MRSA, with an overall prevalence of 6.9%, and 23% of their flocks of origin were colonized. Within the colonized flocks, the prevalence of MRSA varied from 10% to 100% (Mulders *et al.*, 2010).

2.3. Coagulase-negative *Staphylococcus* Species

Although coagulase-positive *S. aureus* is the main agent responsible for food intoxication, but several staphylococcal species other than *S. aureus* are reported to produce staphylococcal enterotoxins. For example, among the coagulase-negative species *S. cohnii*, *S. xylosus* and *S. haemolyticus* have been found to produce staphylococcal enterotoxins (SEs) (Bania *et al.*, 2006; Kerouanton *et al.*, 2007).

From morphological viewpoints, coagulase-negative staphylococci (CNS) are Gram-positive, catalase positive, immobile and non-encapsulated spherical cocci, arranged in irregular shaped clusters. CNS includes 41 types/subtypes, not all of which were isolated from human materials (Barbier *et al.*, 2010). The species most frequently isolated from human material is *S. epidermidis*. By susceptibility to novobiocin, CNS are classified into two groups: novobiocin susceptible and novobiocin resistant CNS (Longauerova, 2006; Thorberg, 2008).

CNS are normal flora components of various parts of the skin and of respiratory and gastrointestinal system mucosa of man; they may also appear in animals and foodstuff. They may participate, as commensal flora, in the development of infections only when external barriers (e.g. skin) were damaged due to wounds (Longauerova, 2006; Thorberg, 2008).

In addition, their increasing resistance to antibacterial drugs evoked deepening concern of infections involving CNS. From the aspect of their pathogenic potential, the most important property of CNS is their capability to form biofilm on surfaces. In consequence of such formation they are protected in the biofilm against the effect of antibacterial drugs and the immune system (Lourdes *et al.*, 2004; Huber *et al.*, 2011).

Very little is known about the growth of coagulase-negative *Staphylococcus* in foods. These strains have rarely been implicated in food poisoning because they do not grow rapidly in foods. Nevertheless, CNS can contaminate foods because humans are common carriers of these microorganisms and may be related to specific human infection. They are normal abundant colonizers of humans and become pathogenic only in certain conditions. They are commonly isolated in clinical specimens and several species are recognized as important agents of nosocomial infections, especially in neonates (Becker *et al.*, 2001; Waters *et al.*, 2011).

There are two coagulase-negative staphylococci of medical importance: *S. epidermidis* and *S. saprophyticus*. *S. epidermidis* infections are almost always hospital-acquired, whereas *S. saprophyticus* infections are almost always community-acquired (Levinson, 2008). *S. epidermidis* is part of the normal human flora on the skin and mucous membranes but can enter the bloodstream (bacteremia) and cause metastatic infections (Lourdes *et al.*, 2004; Levinson, 2008).

S. saprophyticus causes urinary tract infections, particularly in sexually active young women. Most women with this infection have had sexual intercourse within the previous 24 hours. This organism is second to *Escherichia coli* as a cause of community-acquired urinary tract infections in young women (Levinson, 2008). *S. saprophyticus* can be distinguished from other species of coagulase negative staphylococci by their resistance to novobiocin where *S. aureus* and *S. epidermidis* are not (Morrison, 2008; Huber *et al.*, 2011).

Udo and co-investigators (2000) have been able to isolate *S. aureus* and CNS from restaurant workers hand; in which one or a combination of staphylococcal enterotoxins A, B or C and toxic shock syndrome toxin-1 was detected in the isolated. The detection of enterotoxigenic CNS on food handlers suggests that such strains may contribute to food poisoning if food is contaminated by them and held in conditions that allow their growth and elaboration of the enterotoxins. It is recommended that enterotoxigenic CNS should not be ignored when investigating suspected cases of staphylococcal food poisoning (Lourdes *et al.*, 2004; Barbier *et al.*, 2010).

CNS also elaborates a number of important toxins and haemolysins implicated in serious disease conditions. For example, some they produce staphylococcal toxic shock syndrome toxin-1 (TSST-1) which causes toxic shock syndrome (Lourdes *et al.*, 2004). Furthermore, staphylococcal enterotoxins (SE) have been detected in CNS isolates from food, goat's milk and other animal products. Despite these findings, studies that have investigated the sources of staphylococcal food poisoning have focused almost exclusively on the carriage of enterotoxigenic *S. aureus* and rarely on enterotoxin-producing CNS, in food handlers (Udo *et al.*, 2000; Barbier *et al.*, 2010).

As the human nares and fingers are the important sources of *S. aureus* contaminating food in processing plants and because CNS inhabit the human skin and mucous membranes, they can also contaminate food from the hands of food handlers if proper care is not taken when preparing

and serving food. Therefore, enterotoxigenic CNS strains may contribute to staphylococcal food poisoning, as any enterotoxigenic staphylococcal strain can be involved in food poisoning (Lourdes *et al.*, 2004; Longauerova, 2006).

2.4. Protease and Lipase Activity of *Staphylococcus aureus*

S. aureus is considered the third most important cause of disease in the world amongst the reported food-borne illnesses. *S. aureus* is present on the skin and mucosae of humans and animals, and in the environment (Pal, 2007). Some strains, so-called “endemic strains”, are present in some processing plants, such as poultry processing lines. As a consequence, food products may originally become contaminated during or after processing (Gündoğan and Devren, 2010).

It is well known that the organism produces various extracellular active substances, such as coagulase, hemolysins, nuclease, acid phosphatase, lipase, proteases, fibrinolysin, enterotoxins, and toxic shock syndrome toxin. These active substances are thought to contribute to the pathogenicity of the organism (Tamarapu *et al.*, 2001).

Many researchers reported that strains of *S. aureus*, which had strong proteolytic activity, isolated from chickens suffering from edematous and necrotic dermatitis. In addition, they conjectured that there might be a relationship between the protease and the dermatitis in the chickens, because dermatolysis was observed in young chickens and mice when they were inoculated subcutaneously with more than 10^7 cells of protease positive strains (Sandel and McKillip, 2003).

The importance of staphylococcal lipases, like other microbial lipases, results from their significance in bacterial lipid metabolism and their involvement in pathogenic processes (Rosenstein and Götz, 2000). Most of the known staphylococcal lipases are produced by pathogenic members of the genus, i.e., *S. aureus* and *S. epidermidis*. While it is possible that lipases might support the persistence of these strains in the fatty secretions in mammalian skin and thus have an indirect influence on their pathogenic potential, a direct involvement of lipases in pathogenesis remains to be demonstrated (Alvarez-Astorga *et al.*, 2002).

Investigations for proteolytic and lipolytic activity of *S. aureus* under psychrotrophic conditions (+4 °C and +20 °C) associated with meat spoilage; demonstrated that *S. aureus* isolates showed no proteolytic and lipolytic activities at +4 °C, but showed proteolytic and lipolytic activity under psychrotrophic conditions at (+20 °C). Both proteolytic and lipolytic bacteria can change the quality of raw meat and decrease the shelf-life, resulting in spoilage (Gündoğan and Devren, 2010).

2.5. Association of *Staphylococcus* Species with Poultry Meat and Poultry Processing Plants

Food-borne diseases are major health problems throughout the world. According to statistics from WHO, 1.8 million people died of diarrheal diseases in 2005 (WHO, 2007). It is estimated that up to 70 per cent of such cases are associated with contaminated food. One of the most common food-borne pathogens is *Staphylococcus* spp. It is easily spread during the trade and processing of poultry products, specifically non-processed and non-heat handled products (EFSA, 2006).

In foods that provide a satisfactory medium they can grow to sufficient numbers to produce enterotoxin if the foods are not properly refrigerated. These organisms can be transferred to equipment; if the equipment is then not adequately cleansed before use, the organisms can be transferred to foods (Arnold and Silvers, 2000).

Healthy chickens ready for processing harbor a tremendous amount and variety of bacteria. These bacteria are present on the surfaces of feet, feathers, skin and also in the intestines. Live poultry can carry staphylococci in bruised tissues, infected lesions, nasal sites, and arthritic joints and on skin surfaces. The carcass becomes recontaminated with *S. aureus* particularly from the defeathering machines at the slaughterhouses (Mead, 2000).

During processing, a high proportion of these organisms will be removed, but further contamination can occur at any stage of the processing operation. Evisceration and chilling are also processing stages which have been incriminated in contaminating carcasses with *S. aureus* (Acco *et al.*, 2003; Iroha *et al.*, 2011).

PPPs are favorable environments for the survival and transmission of various commensal, spoilage, and potentially pathogenic bacteria throughout the human food chain. In addition, they are prone to indoor air contamination, and microorganisms settle on and contaminate working surfaces, equipment, and the hands of workers (Whyte, 2002). As one of the most predominant groups along the slaughtering and processing line of poultry, staphylococci have been recovered from air samples (Khan *et al.*, 2000), neck skin of chicken carcasses, and equipment and machinery surfaces (Huys *et al.*, 2005; Randolph *et al.*, 2007).

Poultry meat and egg products could be the common sources of *S. aureus*, posing a potential health risk. In poultry, the highest percentage of MRSA positive animals was found in Belgium; with 2 of 14 randomly selected broiler farms, (14.3%) being positive (Persoons *et al.*, 2009). There are also, descriptions of many outbreaks of food borne disease due to MRSA. This study suggested that food contaminated by the food handler was likely to have caused the case of MRSA septicemia which was subsequently transmitted to other persons (Jones *et al.*, 2002).

Among coagulase-positive species, *S. aureus* is well known as an enterotoxin producer. A large number of *S. intermedius* and *S. hyicus* strains are also coagulase positive, and it appears that some produce enterotoxins. Occasionally, the possession of enterotoxins has been reported for *S. intermedius*, and its involvement in an outbreak of SFP has been reported. Hence, in addition to the role of *S. aureus* in the etiology of SFP, we may assume a causative role particularly for canine *S. intermedius* isolates via canine human contact. Therefore, *S. intermedius* should not be disregarded when screening food products for contamination with enterotoxin-producing pathogens or studying outbreaks of SFP (Becker *et al.*, 2001).

Prevention of SFP from the infected food handlers may be difficult as carriers are asymptomatic (Schmid *et al.*, 2007). Other studies also reported high prevalence of enterotoxin-producing *S. aureus* in food handlers. A cross-sectional study conducted among 127 food handlers working in cafeterias in Ethiopia indicated that 16.5% of fingernail contents of the food handlers were cultured positive for *S. aureus* (Andargie *et al.*, 2008; CFS, 2008).

2.6. Epidemiology of *Staphylococcus* species

Per capita demand for meat and fish products in developing countries has grown a rate of 3.7% over the last 20 years (FAO, 2003). Food borne diseases can also be related to demographic movements from rural areas to the cities, which cause overcrowding and, therefore, problems with hygiene, sanitation, housing conditions, etc., particularly in developing countries (Heath, 2006). In developing countries, the source of infection is mainly the contaminated environment and water source. The two most important sources to foods are nasal carriers and individuals whose hands and arms with boils and carbuncles, and are permitted to handle foods (Acha and Szyfers, 2001; Nel *et al.*, 2004).

Staphylococci are ubiquitous in the environment and often represent part of normal bacterial flora of the skin and mucosal surfaces of the respiratory, upper alimentary and urogenital tract of mammals and birds. Thus staphylococci are easily spread between animals and under certain conditions to humans as well as by skin to skin contact, but also by contact with excretions which contain staphylococci, such as saliva, or aerosols released during sneezing and coughing. Moreover, staphylococci may be spread by animal products (El-Jakee *et al.*, 2008).

Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans and animals. Humans and animals are the primary reservoirs. Staphylococci are present in the nasal passages and throats and on the hair and skin of 50% or more of healthy individuals (Forsythe, 2000).

Entry of the microorganisms into the food processing plant occurs through worker's shoes and clothing, transporting equipment, raw foods of animal origin and healthy human carriers (Acha and Szyfers, 2001). *S. aureus* is carried on the mucous membranes of the nose by a significant percentage of the human population and some strains of this species can produce a powerful toxin capable of eliciting a vomiting response. Animals may also act as a source of *S. aureus*. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with *S. aureus* (Adams and Moss, 2005).

Whatever the source of food poisoning bacteria, fault lies in preparation and storage and this lead to the multiplication of organisms in the cooked foods, which is immediate cause of the outbreak. The organisms spread from raw to cooked foods or from person to cooked food during the food preparation (Mosupe and Holy, 2000).

2.6.1. Mode of transmission

Humans are the reservoir for staphylococci. The nose is the main site of colonization of *S. aureus* and approximately 30% of people are colonized at any one time of these 50% carry food poisoning strains (Atanassova *et al*). Chronic nasal carriage increases the risk of infection by *S. aureus* (Levinson, 2008). Transmission of the agents can take place by direct contact between individuals or through the environment by indirect contact with contaminated surfaces. Transmission via the air, water, food and feed or by mechanical or biological vectors also occurs (EFSA, 2006). Factors responsible for whether a person becomes a persistent nasal carrier or not, are poorly understood (Peacock *et al.*, 2001).

Furthermore, some lineages of *S. aureus* show host specificities, and are therefore associated with animals more than humans and vice versa (Sung *et al.*, 2008). The skin to skin contact is probably the main route of transmission between humans, humans to animals, animals to human and between animals, however contaminated materials, surfaces, food or dust can play also a role in transmitting the agent (Lee, 2003; Asoh *et al.*, 2005). Dust is identified as a vehicle for the airborne transmission of *S. aureus* and may play a role in the spread of infections; therefore the presence of MRSA in air of a positive farm is likely (Shiomori *et al.*, 2001).

In humans, the organisms are most frequently present on the mucous membranes of the nose and throat and in the pores of hair follicles of normal skin, particularly in damp areas such as axillae and perineum. Breaks in the skin and mucous membranes allow entrance of these organisms into the body where they may cause disease (Lourdes *et al.*, 2004; Walderhaug, 2007).

Infection and epidemics are usually traceable to various food products derived from meat, eggs, milk and poultry. Other means of infection is derived from food and water contaminated with wild birds and rodent feces, infected food handlers and contaminated equipment and utensils.

Most common hazard arises through cross-contamination from raw to cooked meat or other food (Berynestad and Granums, 2002).

As with other zoonotic agents, people in contact with animals colonised or infected with MRSA may become transiently contaminated, become carriers or they may become infected and develop disease (Witte *et al.*, 2007). It has been demonstrated that MRSA is able to colonize the nasal cavity of farmers, farm attendants and their family members, veterinarians, veterinary students, laboratory personnel and meat inspectors who have been exposed to colonized or infected animals as well as to the dust from animal houses (Armand-Lefevre *et al.*, 2005).

It has been found that large numbers of staphylococci must be present in foods to cause SFP. Staphylococci may grow to large numbers in foods without causing changes in the odor, taste or physical appearance and providing no warning signal to the consumer (Bendahou *et al.*, 2008). Disease transmission by staphylococci species can occur in the following settings: gastrointestinal, skin and hair infections, systemic infections and nosocomial infections (Shah, 2003; Todar, 2008).

2.7. Pathogenesis and Clinical Features

Staphylococci are important pathogens due to the combination of toxin mediated virulence, invasiveness and antibiotic resistance (Soejima *et al.*, 2007). The major potential virulence factors of pathogenic staphylococci are surface proteins that promote colonization of host tissues, invasions that promote bacterial spread in tissues, surface factors that inhibit phagocytic engulfment, biochemical properties that enhance their survival in phagocytes, immunological disguises, membrane damaging toxins that lyse eukaryotic cell membranes, exotoxins that damage host tissues, inherent and acquired resistance to antimicrobial agents (Shah, 2003; Smith, 2007; Todar, 2008)

S. aureus produces a wide range of pathogenicity and virulence factors such as; staphylokinase, hyaluronidases, phosphatases, coagulases and haemolysins, deoxyribonuclease (DNase), catalase, lipases, proteases. Food poisoning is specifically caused by enterotoxins (Sandel and McKillip, 2004). *S. aureus* also produces lipase in infected patients. Furthermore, lipase interferes with the

phagocytosis of the infectious lipase-producing *S. aureus* cells by host granulocytes, thus indicating a direct involvement of lipase in pathogenesis (Gündoğan and Devren, 2010).

A variety of enzymes and toxins including hyaluronidase (spreading factor), deoxyribonuclease, fibrinolysin, lipase, protease, hemolysins, leukocidin, dermonecrotic toxin, hemolysins, exfoliative toxin, and enterotoxins also can contribute to a strain's pathogenicity and virulence (Andreasen, 2003; Levinson, 2008).

S. aureus produces seven protein exotoxins designated A, B, C₁, C₂, C₃, D, and E. Toxin types A and D, either singly or in combination, are most frequently implicated in outbreaks of food poisoning. Susceptibility varies between individuals but it has been estimated that in outbreaks less than 1 µg of pure toxin has been required to elicit symptoms. The toxins are small, single-chain polypeptides each containing a single disulfide loop near the molecule's centre. As a result of their compact structure they are resistant to gut proteases and heat stable, being inactivated only by prolonged boiling. Since the enterotoxins will survive heat processes that eliminate the producing organism, toxin detection in a food is a more reliable indication of hazard than viable counting procedures (Levinson, 2008).

Several toxins can kill leukocytes (leukocidins) and cause necrosis of tissues *in vivo*. Of these, one of the most important is alpha toxin, which causes marked necrosis of the skin and hemolysis. The cytotoxic effect of alpha toxin is attributed to the formation of holes in the cell membrane and the consequent loss of low-molecular-weight substances from the damaged cell (Adams and Moss, 2005).

All staphylococci produce catalase. Catalase is an important virulence factor because H₂O₂ is microbicidal and its degradation limits the ability of neutrophils to kill. *S. aureus* causes disease both by producing toxins and by inducing pyogenic inflammation. The typical lesion of *S. aureus* infection in humans is an abscess (Levinson, 2008).

Most strains of *S. aureus* are coated with a small amount of polysaccharide capsule (microcapsule) that is antiphagocytic. Protein A is the major protein in the cell wall. It is an important virulence factor because it binds to the Fc portion of IgG at the complement-binding site, thereby preventing the activation of complement. In addition, teichoic acids are polymers of

ribitol phosphate. They mediate adherence of the staphylococci to mucosal cells and play a role in the induction of septic shock. Polysaccharide capsule is also an important virulence factor. The peptidoglycan of *S. aureus* has endotoxin-like properties; i.e., it can stimulate macrophages to produce cytokines and can activate the complement and coagulation cascades. This explains the ability of *S. aureus* to cause the clinical findings of septic shock yet not possess endotoxin (Levinson, 2008).

A second important toxin, P-V leukocidin, is a pore-forming toxin that kills cells, especially white blood cells, by damaging cell membranes. The importance of P-V leukocidin as a virulence factor is indicated by the severe skin and soft tissue infection caused by methicillin-resistant *S. aureus* strains that produce this leukocidin. A severe necrotizing pneumonia is also caused by strains of *S. aureus* that produce P-V leukocidin (Levinson, 2008).

Food poisoning by *S. aureus* is characterized by a short incubation period, typically 2-4 h. The onset of symptoms in staphylococcal food poisoning is usually rapid, within hours of ingestion. Consumption of food with preformed toxin usually leads to rapid (6-12 hours) onset of predominant upper gastrointestinal symptoms. The symptoms can be very acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested and the general health of the victim (Andreasen, 2003; Ash, 2008).

The most common symptoms are prostration, nausea, vomiting and abdominal cramping. Some individuals may not always demonstrate all the symptoms associated with the illness. In severe cases, headache, dehydration, muscle cramping, transient changes in blood pressure, marked pallor and collapse may require treatment by intravenous infusion. The short incubation period is characteristic of intoxication where illness is the result of ingestion of a pre-formed toxin in the food (Forsythe, 2000; Shah, 2003).

The pathogenesis of *S. aureus* in poultry is not completely defined, but for infection to occur, a breakdown in natural defense mechanisms of the host must occur. In most cases, this would involve damage to an environmental barrier, such as a skin wound or inflamed mucous membrane, and hematogenous dissemination where a locus of infection (e.g. osteomyelitis) is established (Andreasen, 2003). It causes a variety of infectious diseases such as furuncles,

abscesses, pneumonia, osteomyelitis, and several forms of carditis and meningitis (Gündoğan and Devren, 2010).

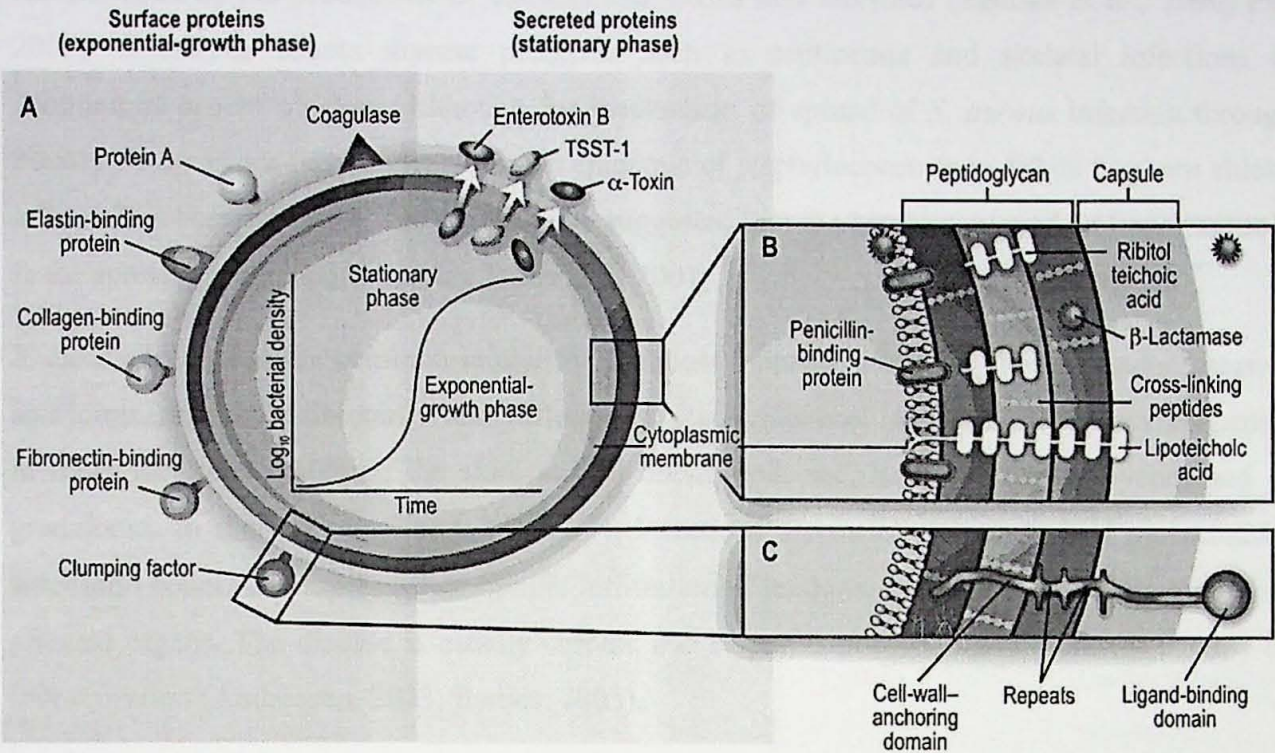


Figure 3: Pathogenic factors of *S. aureus*, with structural and secreted products both playing roles as virulence factors. A) Surface and secreted proteins. B) Cross-sections of the cell envelope. C) TSST-1, toxic shock syndrome toxin (Gordon and Lowy, 2008).

2.8. The Disease Patterns

S. aureus, the principal etiological agent of staphylococcosis, occurs as normal flora of the skin, nose, throat and mucous membrane of man and a wide variety of animals including avian. The disease staphylococcosis is a highly infectious bacterial zoonosis of global significance which causes considerable morbidity and mortality among the susceptible subjects (Pal, 2007). However, diabetic patients have more chance to attract the infection (CDC, 2004).

Staphylococcosis is reported from a wide variety of animals including; buffalo, camel, cat, cattle, dog, horse, geese, goat, monkey, pig, poultry, rabbit and sheep (Quinn *et al.*, 2002; Pal, 2007). *S.*

aureus causes severe animal diseases, such as suppurative disease, mastitis, arthritis, gastroenteritis, abortion and urinary tract infection that are associated with numerous virulence factors, such as the production of extracellular toxins and enzymes (Zadoks *et al.*, 2000, Pal, 2007). *S. aureus* causes disease problems such as septicemia and skeletal infections in commercial broiler chicken. Although the mechanism of spread of *S. aureus* infection through poultry flocks is not fully understood. An epidemic of staphylococcosis in White Leghorn chicks in India has been reported. Researchers also suggested that the hatchery played an important role in the spread of infection to rearing farms (Pal, 2001).

S. aureus infections are common in poultry; the most frequent sites being bones, tendon sheaths, and joints, especially tibiotarsal and stifle joints. Staphylococcal infections occur less frequently in other locations including the skin, sternal bursa, yolk sac, heart, vertebrae, eyelid, and as granulomas in the liver and lungs (Table 4). Infections are usually characterized by increased heterophil counts and marked heterophilic infiltration of tendons, synovial membranes, and other affected organs. The disease is usually chronic and responds poorly to antimicrobial therapy or immunization (Andreasen, 2003; Barnes, 2003).

Table 3: Staphylococcal-related infections in poultry (Andreasen, 2003).

Location	Age	Lesion	Usual outcome
Bone	Any, usually older	Osteomyelitis	Lameness
Joint	Any, usually older	Arthritis/Synovitis	Lameness
Yolk sac	Chicks, poults	Omphalitis	Death
Blood	Any	Generalized necrosis	Death
Skin	Young	Gangrenous dermatitis	Death
Feet	Mature	Bumble foot	Lameness

Humans are likely to spread MRSA to companion animals, and these can then be a reservoir for humans both in the community and in health care facilities. Horses can become colonized and/or infected with MRSA from humans or from other animal sources in their environment. There are sporadic reports of human disease, usually minor skin infections, attributable to an equine source. The primary reservoirs of MRSA in affected countries are pigs, veal calves, and broilers (EFSA, 2006).

Staphylococcus species are an important food borne human pathogens through toxin production as it can grow in improperly processed and stored food. Furthermore, they are also versatile pathogen of human and animal through invasion in which they cause a wide variety of disease ranging from slight skin infection to more severe disease such as pneumonia and septicemia (Pal, 2007; Salandra *et al.*, 2008; Bien *et al.*, 2011).

They are the major causes of SFP by releasing enterotoxins into food and toxic shock syndrome by releasing of super antigens into the blood stream. Staphylococcal food poisoning occurs with the ingestion of contaminated food in which the enterotoxigenic strains of staphylococci can multiply reaching about 10^5 CFU/g of food; this bacterial load allows the production of an amount of 20 ng to 1 µg of SE sufficient to determine symptoms in human beings (Quinn *et al.*, 2002; Salandar *et al.*, 2008). The hazard to public health by ingestion of foods contaminated with staphylococci is particularly linked to the ability of 50% of these strains to produce thermostable SEs associated with food poisoning (Kerouanton *et al.*, 2007; Bien *et al.*, 2011).

Humans are an important source of the organism, so the presence of staphylococci in cooked or processed foods can serve to indicate poor hygiene amongst food handlers (Adams and Moss, 2005). The two most important sources to foods are nasal carriers and individuals whose hands and arms are inflicted with boils and carbuncles, who are permitted to handle foods. No viable organisms need to be ingested, although, the organism needs to grow to levels of about 10^5 CFU/g of food before the food becomes toxic. Typical symptoms are nausea and vomiting with occasional abdominal cramping and diarrhea. Death from SFP is very rare, although such cases have occurred among elderly, infants and severely debilitated persons (Rho and Schaffner, 2007; Waters *et al.*, 2011).

S. aureus is an imperative pathogen associated with activation of diverse types of infection characterized by inflammation dominated by polymorphonuclear leukocytes. This bacterium frequently causes lung infection, which is attributed to virulence factors (Bien *et al.*, 2011). The pathogen can cause a wide variety of infections, which can be divided into three types: (i) superficial lesions such as wound infection, (ii) toxinoses such as food poisoning, scalded skin syndrome and toxic shock syndrome, and (iii) systemic and life-threatening conditions such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis, and bacteremia (De Sousa and De Lencastre, 2004; Bien *et al.*, 2011).

S. aureus causes abscesses, various pyogenic infections (e.g., endocarditis, septic arthritis, and osteomyelitis), food poisoning, scalded skin syndrome, and toxic shock syndrome. It is one of the most common causes of hospital-acquired pneumonia, septicemia, and surgical-wound infections. It is an important cause of skin infections, such as folliculitis, cellulitis, boil, pustule, abscess and impetigo (Pal, 2007). *S. epidermidis* can cause endocarditis and prosthetic joint infections. *S. saprophyticus* causes urinary tract infections. Kawasaki syndrome is a disease of unknown etiology that may be caused by certain strains of *S. aureus* (Levinson, 2008; Todar, 2008).

2.9. Public Health Significance of *Staphylococcus* species

Singer and co-investigators (2007) showed that there is a strong correlation between the health of production animals and level of food-related human illnesses. In addition, the WHO report states that in industrialized countries up to 30 per cent of the population is reported to be affected by a food-borne disease each year, implying that the proportion in developing countries might be much higher (WHO, 2007).

In the industrial world, legislation and regulations have been implemented, involving both the public and the private sectors. However, in many developing countries such measures do not exist. Food borne illnesses are, therefore, still major problems in developing countries (Stamoulis *et al.*, 2004). In addition, in the less developed countries the consequences of food borne illness are more serious. Diarrhoeal disease is a major cause of morbidity and mortality in poor countries, particularly among children. It has been estimated that some 1,500 million children

under 5 suffer from diarrhea each year and that over 3 million die as a result (Adams and Moss, 2005; Randolph *et al.*, 2007).

In developing countries, the surveillance systems of FBDs hardly exist and it is therefore, difficult to estimate the real magnitude of the problem (Randolph *et al.*, 2007). Even in countries where surveillance services are very efficient, the precise incidence of food poisoning is not known, as outbreaks are often not reported to public health authorities. Hence, the incidence of FBD caused by staphylococci is thought to be much higher than reported cases (Jay, 2000; Stamoulis *et al.*, 2004).

Among FBD, SFP is of major concern in global health programmes. Staphylococcal organisms alone have found to cause hospitalization rates as high as 14%. Although not considered especially lethal, death can ensue if large amounts of SE are ingested: fatality rates range from 0.03% in general population to as high as 4.4% of highly sensitive persons such as immunocompromised persons, elderly and children (Stewart, 2003; Aycicek *et al.*, 2005; Kerounaton *et al.*, 2007).

S. aureus is among the most prevalent causes of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multidrug resistance. The enterotoxins are low molecular weight proteins which can be differentiated by serology into seven antigenic types: SEA, SEB, SEC1, SEC2, SEC, SED and SEE. These toxins are highly heat-stable and resistant to cooking and proteolytic enzymes (Jay, 2000; Kerouanton *et al.*, 2007).

Though frequently described as enterotoxins the *S. aureus* toxins are strictly neurotoxins. They elicit the emetic response by acting on receptors in the gut, which stimulate the vomiting centre in the brain via the vagus and sympathetic nerves (Adams and Moss, 2005).

Furthermore, the enterotoxins are immunosuppressive and mutagenic, and can stimulate production of interferon, interleukin-1 and -2, and tumor necrosis factor. All the enterotoxins have been shown to have superantigenic activity (Chiang *et al.*, 2008). The staphylococcal enterotoxins are more stable in many respects than most proteins. In the active state, they are resistant to proteolytic enzymes such as trypsin, chymotrypsin, rennin and pepsin. Although pepsin can digest the enterotoxins at pH values of 2.0 and below, this acidic level does not exist

in the stomach under normal conditions, particularly in the presence of food. This makes it possible for the enterotoxins to pass through the stomach to the intestinal tract, where they stimulate the emetic and diarrheal actions (Adams and Moss, 2005; Loeto *et al.*, 2007).

The presence of small numbers of *S. aureus* on foods is not uncommon. It will occur naturally in poultry and other raw meats as a frequent component of the skin microflora. Contamination by food handlers is also probably a frequent occurrence in view of the high rate of human carriage. Since large numbers, typically $> 10^6 \text{ g}^{-1}$, are required for the production of enough toxins to cause illness, contamination is necessary but is not alone sufficient for an outbreak to occur. In particular, temperature and time conditions must also be provided that allow the organism to grow. Studies in the United States and the UK have found that poultry products and cold, cooked meats are the most common vehicles (Waters *et al.*, 2011).

The growth of *S. aureus* in foods presents a public health hazard since many strains of *S. aureus* produce enterotoxins, which cause food poisoning (Pal, 2001). Some strains of *S. aureus* are capable of producing heat-stable toxins (enterotoxins) in food that act at the digestive level (Genta and Heluane, 2001). Although it is a ubiquitous organism, the largest reservoir of enterotoxin producing staphylococci is man. It is carried on the mucous membranes of the nose by a significant percentage of the human population and some strains of this species can produce a powerful toxin capable of eliciting a vomiting response. Colonization of the nose and throat with the organism will automatically imply its presence on the skin and food may also be contaminated from infected skin lesions or by coughing and sneezing (Adams and Moss, 2005; Waters *et al.*, 2011).

Although a variety of animals carry coagulase positive staphylococci, human carriers are the main reservoirs for *S. aureus* (including MRSA and MSSA) responsible for human food borne staphylococcal intoxication. Control of staphylococcal food poisoning involves temperature and time control as well as the hygienic behavior of food handlers. Food handlers with septic lesions should be excluded until treated successfully. Where hand contact is unavoidable hands should be cleaned thoroughly and disposable gloves used where practical (EFSA, 2006). Food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms (Pal, 2001). Coagulase-negative staphylococci were the

predominant bacteria species (41.7%) isolated from fingernail contents, followed by *S. aureus* (16.5%) (Andargie *et al.*, 2008).

Transmission of MRSA between humans and animals (e.g., dogs, horses, pigs) has been reported (Voss *et al.*, 2005). Indistinguishable bovine and human strains were isolated by phenotyping and genotyping methods; in which MRSA was isolated from cows with subclinical mastitis and from a person who worked with these animals (Juhász-Kaszanyitzky *et al.*, 2007).

Clear association of the organisms with food borne illness was demonstrated; in which staphylococci were able to cause poisoning by consuming milk from a cow with staphylococcal mastitis. Four biotypes of *S. aureus* are recognized but the use of biotyping is limited since nearly all of the strains isolated from human sources belong to biotype A. Phage typing schemes are used with *S. aureus*; most food poisoning strains belong to serogroup III (Adams and Moss, 2005).

With regard to some of the non- *S. aureus* species, *S. cohnii* is found on the skin of humans and occasionally in urinary tract and wound infections. Human skin is the habitat of both *S. epidermidis* and *S. haemolyticus*, and the latter is associated with human infections. Although many of the coagulase-negative species are adapted primarily to nonhuman hosts, their entry into human foods is not precluded. Once in susceptible foods, their growth may be expected to lead to the production of enterotoxins (Soejima *et al.*, 2007; Levinson, 2008).

Slaughterhouses and food-processing establishments are the next links in the chain of food safety. The post-slaughter poultry carcass is a suitable growing medium for many pathogens, including human pathogens. Hygiene procedures when handling the carcass are, therefore, crucial and should be carefully planned and monitored to avoid contamination and cross-contamination of the food products. Packaging, transport, shelf-life and storage, as well as the maintenance of the cold-chain are important considerations (Andargie *et al.*, 2008; Waters *et al.*, 2011).

2.10. Treatment of Staphylococcal infections

The objective of treatment in human patients is to replace fluids, salt and minerals that are lost by vomit or diarrhea (Sandel and McKillip, 2004). Some strains of *Staphylococcus* have acquired genes making them resistant to multiple antimicrobial agents. The organisms are uniformly resistant to penicillins and cephalosporins (Rho and Schaffner, 2007). Therefore, sensitivity tests should always be performed because antibiotic resistance is common since, often infection can be treated successfully (Andreasen, 2003).

Drugs used successfully for treatment include penicillin, streptomycin, tetracyclines, erythromycin, novobiocin, sulfonamides, lincomycin, and spectinomycin. First or second generation cephalosporins such as cephalothin, cephalexin and cefuroxime are usually safe in patients who are hypersensitive to penicillins. Erythromycin and its newer relatives are used in milder infections. Infections can also be treated with combination therapy using sulfa drugs and minocycline or rifampicin (Andreasen, 2003; Normanno *et al.*, 2005).

Approximately 20% of *S. aureus* strains are methicillin-resistant by virtue of altered penicillin-binding proteins. The drug of choice for these staphylococci is vancomycin, to which gentamicin is sometimes added. Trimethoprim-sulfamethoxazole or clindamycin can be used to treat non-life-threatening infections caused by these organisms. Note that these MRSA strains are resistant to all beta-lactam drugs, including both penicillins and cephalosporins (Rho and Schaffner, 2007; Levinson, 2008).

2.11. Prevention and Control of Staphylococcal infections

Control is both important and difficult as staphylococci can persist for months in dust, curtains and human carriage is often permanent. Reservoirs and routes of spread differ, so different measures are appropriate in different circumstances. Prevention is much concerned with the destruction of the bacteria and with the inhibition of growth (Loir *et al.*, 2003; Chiang *et al.*, 2008).

Effective methods for preventing SFP are aimed at eliminating food contamination through high standards of personal hygiene to avert contamination by food handlers. This is through public education in relation to hand washing, wearing gloves during food preparation and storing foods at proper temperature to inhibit growth or destroy the pathogen and minimize toxin production as heating food after toxin formation will not be effective (Acco *et al.*, 2003; Baron, 2007).

Even if the actual numbers of pathogenic organisms on broiler carcasses leaving processing plants are low, those pathogens in prepared (and undercooked) poultry products are amplified if held at room temperature. The handling of raw carcasses can be a source of cross contamination of other foods. Poultry products must be properly cooked, cooled and stored, to prevent food borne illness, particularly in commercial or institutional food service settings where larger quantities of food are being prepared at one time (Baraki *et al.*, 2005).

Moreover, persons with lesions containing purulent exudates should not be permitted to handle food until proper medical advice is sought. In general, measures such as serving hot meal immediately after cooking, reheating cooked foods thoroughly, rapid refrigeration of cooked foods, proper washing of hands before and after food preparation, avoiding food service worker with skin infections and using clean utensils and equipments will certainly reduce the incidence of food poisoning outbreaks due to *Staphylococcus* (Baron, 2007; Randolph *et al.*, 2007)

MRSA control programmes and the application of strict hygiene measures can help to reduce transmission (Eveillard *et al.*, 2006; Ben-David *et al.*, 2008). Any management procedure reducing damage to host defense mechanisms will help prevent staphylococcosis. Because wounds are a portal of entry for *S. aureus* into the body, anything reducing the chance of injury will help prevent infection. Maintenance of good litter quality will reduce foot pad ulceration. Particular attention should be given to hatchery management and sanitation (Andreasen, 2003; Stamoulis *et al.*, 2004).

2.12. Economic Implications of Staphylococcal Infections

Food safety is of particular concern in a developing country context not only because of the high prevalence of food-borne illness and other hazards associated with food, but also because of the

considerable economic and social costs that, in turn, reflect prevailing levels of economic development. In developing countries it is estimated that 2.1 million people died from food borne diseases in 2000. Estimated occurrence of *S. aureus* intoxication in Africa, Central and South America, South East Asia and Western Pacific regions is very frequent (WHO, 2002).

Collectively, bacterial diseases continue to be a significant cause of economic loss in the poultry industry. Staphylococcal infections are a worldwide problem in chickens and turkeys and cause economic losses due to decreased weight gain, decreased egg production, and condemnation of carcasses at slaughter (Barnes, 2003). Foods borne illness not only affect the health of individuals who become ill, but it can also have dramatic economic impact to the food establishments (NIAID, 2005).

Other economical losses could include; exports may be barred owing to non-compliance with microbiological and other standards, importation of substandard food items as well as trade rejections. Losses from export rejection not only rob countries of critical revenue but also of credibility as reliable trading partners. These disadvantages mainly arise from the country's food product processors and exporters failed to meet the importing country hygiene and processing quality standards (Smith and Robert, 2005).

2.13. Status of Staphylococcal Food Poisoning in Ethiopia

Food borne illnesses have significant impact worldwide including developed nations. For example, the Center for Disease Control and Prevention (CDC, 2004) has made estimates that 76 million cases, more than 300,000 hospitalizations and 5000 deaths occur in a year in the US from food-borne diseases. In addition, 400 – 500 outbreaks are reported. Epidemiologic data related to food-borne diseases are inadequate in Ethiopia. But it can be evidenced that these are very common in the country because of many reasons including poverty, lack of awareness, poor water supply, poor personal hygiene and environmental sanitation, etc. (Baraki *et al.*, 2005).

Table 4: Major causative agents of food borne illness outbreaks in developing countries, 2003 (Smith and Robert 2005).

	<i>Salmonella</i>	<i>V. parahaemolyticus</i>	<i>S. aureus</i>	Norovirus	Unknown
Outbreaks	416	732	808	1,442	2,180
Rate	5.3%	9.3%	10.2%	18.2%	27.6%

2.14. Diagnosis of Staphylococcal infections

2.14.1. Clinical consideration

Food borne illnesses are considered to be any illness that is related to food ingestion; gastrointestinal tract symptoms are the most common clinical manifestation of food borne illnesses. The severity of food borne illness depends on: incubation period, duration of the resultant illness, predominant clinical symptoms and population involved in the outbreak (NIAID, 2005).

The short incubation period, brevity of illness and usual lack of fever help distinguish staphylococcal intoxication from other types of food poisoning. Diagnosis is easier when a group of cases presents the characteristic acute, predominantly upper gastrointestinal symptoms which occur shortly after consumption of a common high risk food. The clinical suspicion can be supported by laboratory tests including isolation of *S. aureus* and detection of enterotoxins from food remnant (CHP, 2011).

2.14.2. Laboratory techniques used in the detection of *Staphylococcus* species in foods

Currently available methods for detection and identification of bacteria in food samples are conventional bacteriological methods, which are compared and validated. These methods are usually very sensitive and they do not require sophisticated and expensive equipment. For food

and environmental samples, the use of selective media and suitable growth conditions are provided and other enrichment methods are employed to limit the growth of contaminating microorganisms to reasonable numbers and multiplication of food borne bacteria to a level that are enough for detection (OIE, 2004).

2.15. Antimicrobial Resistance of *Staphylococcus* species

S. aureus is a common etiological agent of food borne intoxication and is a significant marker of food quality and surface cleanliness (Normanno *et al.*, 2005). Poultry meat is a common vehicle of food borne illness, with *S. aureus* usually being one of the causes of outbreaks involving large numbers of people (Geornaras and von Holy, 2001). Antimicrobial agents used in therapy and as feed supplements to promote growth in food animals may increase the spread of drug-resistant bacteria and the transfer of drug-resistant bacteria from animals to humans (Chaslus-Dancla *et al.*, 2000; Lin *et al.*, 2009).

More than 90% of *S. aureus* strains contain plasmids that encode β -lactamase, the enzyme that degrades many, but not all, penicillins. Some strains of *S. aureus* are resistant to the β -lactamase-resistant penicillins, such as methicillin, by virtue of changes in the penicillin-binding protein in their cell membrane. These strains are commonly known as methicillin-resistant *S. aureus* (MRSA). Rare strains called vancomycin-intermediate *S. aureus* (VISA), with reduced sensitivity to vancomycin, have emerged, as having strains fully resistant to vancomycin (Levinson, 2008; Waters *et al.*, 2011).

3.2. Type and Origin of Samples

The present study was performed on the carcass of poultry and immediate slaughter environment (processing equipments and workers hand). The swab method was used to collect the samples. During the aforementioned period, four broiler farms were studied. Two of the farms (farms 1 and 2) were considered as large scale farms; in which they were considered as commercial farms, perform slaughtering of 1000-3000 broilers per day and brought their products to local market only and the other two farms (farms 3 and 4) were small scale farms with 1 or 2 houses located in living community areas and raised about 1000 to 3000 birds. The two large scale farms were selected because they were the only broiler farms which were currently supplying chicken meat in large amounts and carried out slaughter operations on an unremitting basis. The small scale farms were randomly selected.

The carcass swabs were taken at tow points in the slaughtering process after defeathering and evisceration. The personnel sampled were those who perform evisceration, cutting and washing. Sterile swabs moistened with sterile distilled water were used. The swabs were used to swab areas in between fingers and the wrist area of the hand of workers (Kaskova *et al.*, 2006; Çepoğlu *et al.*, 2010).

During the slaughtering process in the large scale farms, the birds were hanged upside down by their feet by shackles on a conveyor, which moved them to the slaughtering area. Slaughtering was performed manually using knife. The birds were bled for few minutes until they reach defeathering machine. The blood was collected in a tank located under the conveyor.

After bleeding, the birds entered to the deafeathering machine as part of scalding procedure. Following scalding and plucking activities, inedible organs, including the intestinal tract and lungs were removed. The eviscerated carcass was then rinsed internally and externally with potable water by removing the birds from the conveyor. After rinsing, the carcasses were immersed in water then depending on convenience; they could be processed at the day of slaughter or kept in refrigerator to be processed in the next day. Subsequent to processing, the carcasses were weighed individually and sorted at 4⁰C. Cleaning of the plant was performed by washing the crates, cutting tables, knives and floor by water and detergent on daily basis.

Slaughtering operation in small scale farms were performed in the following way; the chickens were slaughtered in the open air condition or in a tent like enclosure in the compound of the farm and there was no use of specialized equipments. Starting from cutting of the neck to the end packing, the process was performed on the ground.

A total of 384 samples were obtained from feather, breast, neck, wing, skins of carcasses after defeathering (CAD) and evisceration (CAE), equipments; including knives, cutting table and abattoir personnel hands have been included in the sample.

3.3. Study Design

A cross sectional study type was employed to determine the prevalence and species type of staphylococci isolated from chicken meat, equipments and workers hand. The study was performed during a period of nine months between November 2011 and April 2012. Swab samples were taken from the chicken carcass including the feather, breast, neck and wing. Workers hand swab samples were also obtained. Equipment swabs that included knives and cutting tables were collected and cultured for the presence of *Staphylococcus* spp. The isolates were further examined for their susceptibility to various antimicrobials.

Simple random sampling technique was used to take the samples from chicken carcass, workers hand and processing equipments. According to the formula recommended by Thrusfield (2007), the sample size was determined to be 384 for a 95% confidence interval, 5% desired level of precision and with assumption of 50% expected prevalence of *Staphylococcus* species in poultry meat, processing equipment and workers hand was taken since there are no earlier reference on the prevalence values available in this area.

3.4. Sample Collection, Handling and Transportation

Bacterial contamination of carcasses or cuts is generally on the surface, and this should be recognized in the collection of subsamples. Because defacing of carcasses or cuts is often economically undesirable, the surface swab technique is recommended for the sampling of

unfrozen meat. The equipment surfaces in the food industry provide the microbes growing in biofilms with liquids and excess of nutrients, for this fact samples were also taken from the equipments in the processing units (Huber *et al.*, 2011).

The samples were collected once or twice a week from selected poultry farms. Sample collection was performed by using sterile swabs for taking surface swab samples. First the sterile swabs were pre-wetted in sterile distilled water and rubbed firmly across the sampling area several times in all directions. Then swabs were introduced into sterile test tubes containing a diluent (peptone and 10% calf serum). Swabs from the carcass of the poultry were taken from feather, neck, breast and wing. Swabs of the equipments included cutting tables and knives. Swab samples were also taken from hands of workers involved in the slaughter process. For convenience, the swabs acquired from the equipments and the workers' hands were considered as samples from the immediate slaughter environment. Each of the sterilized test tubes were labeled for farm code, sample number, code of swab sites (carcass, equipment or workers hand), and date.

Subsequent to the collection samples were transported in cold chain using ice box and ice packs to the Institute of Biodiversity Conservation Microbial Laboratory for microbiological analysis. The samples were kept under refrigeration temperature of +4 °C until being processed within 24 hours of collection. Samples were analyzed microbiologically according to the standard set by the International Organization for Standardization (2003).

3.5. Cultural procedure

The techniques recommended by International Organization for Standardization, ISO (2003) was employed for the isolation and identification of *Staphylococcus* species from chicken meat, equipment surface and workers hand. The transported samples were processed within 24 hours of their arrival. The bacteriological media used for the study were prepared following the instructions of the manufacturers.

The primary culturing was made on blood agar plate (BAP) enriched with 5% heparinized sheep blood and incubated at 37 °C for 24 hours under aerobic condition. Then the plates were examined for the presence of *Staphylococcus* colonies. Isolates supposed to belong to

Staphylococcus were determined based on their morphological aspects like; opaque, white or creamy, grayish, white or yellow colonies and their hemolytic pattern were also recorded.

Presumed staphylococci colonies were then sub-cultured on nutrient agar plate (NAP) and incubated at 37 °C for 24 hours to get a pure culture. The presence of golden yellowish or sometimes opaque white, circular, entire convex colonies helps to determine and further differentiate the staphylococci species (Genta and Heluane, 2001). The pure isolates from NAP were preserved and maintained for biochemical differentiation tests and characterization of isolates.

3.6. Identification of *Staphylococcus* Species

After growth presumptive colonies were identified by using conventional bacteriological techniques on the basis of colony characteristics, pigment production and hemolysis. Identification of staphylococci organisms and species assignment were done based on Gram staining, catalase test, oxidase test, carbohydrate dissimilation (mannitol and maltose) and coagulase test.

3.6.1. Morphological Identification

All the suspected colonies of *Staphylococcus* species from nutrient agar plates were subjected to Gram's stain and were examined under light microscope for their Gram's reaction, morphology and cell arrangements. First alleged colonies of *Staphylococcus* were smeared on a glass slide by taking single and separate colony using sterile inoculating loop. Following fixation; the smears were stained using the procedure of Gram's staining and examined under light microscope using oil immersion objective. The Gram's stained smears from typical colonies that demonstrated Gram-positive cocci occurring in pairs or irregular clusters resembling 'bunches of grape' were presumptively taken as *Staphylococcus* species (Jay, 2000; Quinn *et al.*, 2002).

3.6.2. Biochemical identification

Catalase test: Colonies that demonstrate the Gram's reaction identical with the *Staphylococcus* species were further tested for the presence of catalase enzyme. Pure colonies of the isolates were

picked from the nutrient agar using a sterile loop and mixed with a drop of hydrogen peroxide (H_2O_2) on a clean microscope glass slide. Positive reaction was indicated by the liberation of bubbles of oxygen within few seconds and those with negative reaction did not produce bubbles (Adams and Moss, 2005). The catalase positive isolates were considered as staphylococci.

Oxidase test: The test was used to detect the production of cytochrome-c-oxidase by microorganisms (Roberts and Greenwood, 2003). Colonies assumed to be staphylococci were streaked firmly across a piece of filter paper moistened with 1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride with a sterile wooden stick in a Petri-dish. The development of dark purple color along the streaked line within 10 seconds was taken as positive reaction (Quinn *et al.*, 2002; Fratomico *et al.*, 2005).

Mannitol fermentation: The colonies that were identified by Gram's reaction, catalase and oxidase as *Staphylococcus* were cultured on mannitol salt agar and incubated at 37°C and examined after 24-48 hours. The presence of bacterial growth and change in the color of the media from red to yellow (change of pH) were regarded as confirmative indication of the salt tolerant staphylococci. An indicator is incorporated in the media; phenol red which detects changes in pH of the media.

In the presence of the acidic metabolic product of mannitol, the indicator turns the color of the media into yellow. Fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium. Colonies that develop weak or delayed yellow discoloration of the medium after 24 hours of incubation were considered as *S. intermedius* and colonies that failed to develop any change in the medium were considered as *S. hyicus* and coagulase negative *Staphylococcus* (Jay, 2000; Roberts and Greenwood, 2003).

Coagulase test: A tube coagulase test was performed to detect the presence of the enzyme coagulase. From the surface of each selected colony of staphylococci an inoculum was taken with sterile loop and transferred into a tube of brain-heart infusion broth and incubated at 37 °C for 24 hours. Then, 0.5 ml of each culture was added aseptically to sterile test tubes containing 0.5 ml of citrated rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37 °C along with a negative control tube containing a mixture of 0.5 ml of brain-heart infusion both and 0.5

ml of rabbit plasma. Clotting was evaluated at 30 minutes intervals for the 4 hours of the test and then after 24 hours of incubation. The reaction was considered positive, if the degree of clotting varies from a loose clot to a solid clot that is immovable when the tube is inverted (Quinn *et al.*, 2002). When there was no degree of clotting, the result was taken as a negative reaction; in such cases re-examination was made after 24 hours of incubation.

Maltose fermentation: Purple agar base (PAB) with the addition of 1 per cent maltose is useful medium to differentiate the pathogenic staphylococci (Quinn *et al.*, 2002). The suspected colony from nutrient agar was cultured on PAB with the addition of 1% maltose and incubated at 37 °C for 24-48 hours. This presumptive identification is based on the fact that *S. aureus* rapidly ferment maltose and the acidic metabolic product causes the pH indicator (bromocresol purple) to change the medium and colonies to yellow. *S. intermedius* gives a weak or delayed reaction and *S. hyicus* does not ferment maltose but attacks the peptone in the medium producing an alkaline reaction (a deeper purple) around the colonies (Fratamico *et al.*, 2005).

3.7. BioLog Identification

The BUG (BioLog Universal Growth Medium) which is a recommended medium for aerobic bacteria was employed to isolate *Staphylococcus* species. The identification of *Staphylococcus* species was performed using the OmniLog Identification System after autoclaving all media and materials at 121 °C for 15 minutes (BioLog User Guide, 2008).

Pure colonies from mannitol salt agar were isolated on BUG agar and inoculated at 37°C for 24 hrs. Sub-culturing was done using the same culture media to have pure culture colonies before identification was done by Omni Log. After getting pure culture colonies, identification was carried out using Standard Operation Protocols (SOP) for aerobic, identification, having 21% turbidity in 18-20 inoculating fluid. The 21% turbidity suspension was inoculated into BioLog Gram positive (GP2) Micro-Plates. The plates were placed in OmniLog at 37°C for 22 hrs (BioLog User Gide, 2008).

Following the appropriate incubation time, OmniLog Identification System automatically read each MicroPate. The patterns formed from the wells were automatically entered to the system and used for identification (BioLog User Guide, 2008).

3.8. Antimicrobial Susceptibility Test

The staphylococcal organisms isolated from the poultry meat, equipment surfaces and workers hand in the present study was tested for their antibiotic susceptibility. The antibiotic susceptibility test was performed on 53 isolates of *S. aureus*.

Agar disk diffusion was performed by using amoxicillin (10 µg), bacitracin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), clindamycin (30 µg), cloxacillin (12.5 µg), erythromycin (15 µg), methicillin (5 µg), neomycin (30 µg), nitrofurantoin (15 µg), norfloxacin (10 µg), penicillin G (10 units), polymixin B (10 µg), rifampicin (5 µg) and vancomycin (30 µg). Antibiotic discs used were from Oxoid, (Hampshire, England). Colonies isolated from pure culture were transferred into a test tube of 5 ml Tryptone Soya Broth. The turbidity of the broth incubated was adjusted by adding sterile saline or more isolated colonies to obtain turbidity visually comparable with that of 0.5 McFarland standards.

Muller-Hinton Agar (MHA) plate was prepared; using a sterile cotton swab dipped into Tryptone Soya Broth culture, the surface of MHA plate was swabbed. Then the antibiotic discs were placed on the agar plate using sterile forceps and pressed gently to ensure complete contact with the agar surface. The plates were incubated for 24 hours at 37 °C under aerobic condition. Inhibition zones were measured and interpreted as susceptible, intermediate and resistant according to NCCLS guidelines (NCCLS, 2003).

Suspension ability to novobiocin: Coagulase-negative staphylococci are commensals of the human skin flora. However, many are also significant disease causing organisms, responsible for infections in both hospital and community environments (Martineau *et al.*, 2000). *S. saprophyticus* is innately resistant to the coumarin antibiotic novobiocin. Indeed, uniform resistance to novobiocin is a property central to the identification of *S. saprophyticus* isolates (Vickers *et al.*, 2007).

All of the CNS isolates were further tested for susceptibility to novobiocin. The objective was to classify the organisms in to two groups as; *S. saprophyticus* (novobiocin resistant) and CNS other than *S. saprophyticus* (novobiocin susceptible). A presumptive identification of *S. saprophyticus* from the rest of CNS was derived from resistance to novobiocin. This was performed by inoculation of Muller-Hinton agar with the test organism by a sterile cotton swab dipped in to soya broth culture. Then discs of novobiocin 5 µg were placed on the MHA plates by using similar procedure used in antimicrobial susceptibility test. Interpretation was made according to manufacturer's instruction.

3.9. Data Management and Analysis

The data collected through laboratory results of the collected samples were entered and managed in Microsoft Excel program. SPSS for windows version 15 (SPSS Inc., Chicago, Illinois, USA) for windows was used for data analysis. A Chi-Square test was applied to examine whether the differences between the values were significant. A p-value of less or equal to 0.05 and a chi-square value greater or equal to 3.84 was considered indicative of a statistically significant difference.

4. RESULTS

4.1. Prevalence and Distribution of Staphylococci

In this study, a total of 384 samples, 340 carcass swabs and 44 swabs acquired from immediate environment, were taken from chickens slaughtered in 4 poultry farms in Debre Zeit; and were examined microbiologically for the presence of *Staphylococcus* species.

The overall isolation rate of Staphylococci from 384 samples obtained from carcasses and immediate slaughter environment of four large and small scale poultry farms in Debre Zeit and detected by bacteriological methods was 183 (47.7%). A total of 192 samples were collected from the large scale farms (n=2) and the same number of samples were obtained from small scale farms (n=2).

Staphylococci species were isolated from 82 (47.7%) of the samples from the large scale farms. A higher isolation rate (52.6%) of *Staphylococcus* species was observed within the small scale farms. The highest incidence recorded in farm 3 (55.2%) followed by farm 4 (50.0%). Slightly lower isolation rates, 36.5% and 49.0%, were observed in the two large scale farms, farm 1 and 2, respectively. Comparison was made between the large and small scale farms and among the types of farms. There were marginal statistically significant associations between the two scales of production ($p=0.052$). Whereas, among the four poultry farms no statistically significant association ($p=0.063$) in the prevalence of staphylococci.

Table 4: Isolation rates of staphylococci in the swabs of samples obtained from 4 poultry farms in Debre Zeit (Ethiopia, 2014/15).

Sample Origin	Samples collected	Positive	Negative	χ^2	P-value
Carcass	340	154	186	0.783	0.052
Immediate Environment	44	27	17		

Table 5: Proportion of isolation of staphylococci in four large and small scale poultry farms in Debre Zeit, Ethiopia (2011-2012).

Farm Type	Farm ID	Positives	Isolation Rate (%)	Total Isolation Rate (%)	χ^2	P- value
Large Scale	Farm 1	35	36.5	42.7	3.77	0.052
	Farm 2	47	49.0			
Small Scale	Farm 3	53	55.2	52.6		
	Farm 4	48	50.0			

The 384 samples were divided in two according to the sample origin as carcass swab (n=340) and immediate slaughter environment, which include equipment surfaces and workers hand (n=44). Staphylococci were isolated in 156 (45.9%) and 27 (61.4%) of the carcass swabs and immediate slaughter environment, respectively. The result showed that there was a relatively higher rate of isolation in the samples that originated from the immediate slaughter environment and that there was a statistically marginal significant association ($p=0.053$) between the prevalence of staphylococci and two sample origin types.

Table 6: Isolation rates of staphylococci on the basis of origin of samples obtained from 4 poultry farms in Debre Zeit, Ethiopia (2011-2012).

Sample Origin	Samples collected	Positives	Percentile	χ^2	P-value
Carcass	340	156	45.9	3.743	0.053
Immediate Environment	44	27	61.4		

With regard to the swab sites, prevalence of *Staphylococcus* species was 42.9%, 60.7%, 39.3%, 40.9%, 62.5% and 60.0% in the breast, feather, neck, wing, equipment and workers hand swab, respectively. The result demonstrated that a relatively higher prevalence in the equipments (62.5%) followed by feathers (60.7%) and workers hand (60.0%). Consequently, there was a statistically significant association ($p=0.017$) among these proportions of isolates in the six swab sites (Table 8).

Table 7: Distribution of isolation rates of staphylococci on the basis of swab sites of carcass and immediate slaughter environment samples acquired from 4 poultry farms in Debre Zeit, Ethiopia (2011-2012).

Swab Site	Samples collected	Positives	%	χ^2	P-value
Breast	84	36	42.9	13.82	0.017*
Feather	84	51	60.7		
Neck	84	33	39.3		
Wing	88	36	40.9		
Equipments	24	15	62.5		
Workers' hands	20	12	60.0		
Total	384	183	47.7		

4.2. Species Identification

The isolated 183 staphylococci were further identified by biochemical tests and OmniLog for their species assignment. The species identification revealed an overall isolation rate of the following four species: coagulase negative staphylococci (CNS) 67 (17.4%), *S. aureus* 53 (13.8%), *S. intermedius* 37 (9.6%) and *S. hyicus* 26 (6.8%).

In the large scale farms (farm 1 and 2), the prevalence of *Staphylococcus* species were CNS 16.7%, *S. aureus* 11.5%, *S. intermedius* 8.3% and *S. hyicus* 6.3%. In the small scale farms (farm 3 and 4), the isolation rates of the species were 18.2% CNS, 16.1% *S. aureus*, 10.9% *S. intermedius* and 7.3% *S. hyicus*. According to the four farm types, higher isolation rate of *S. aureus*, *S. intermedius* and *S. hyicus* were observed in farm 3. While, higher rate of CNS isolation was recorded in farm 2. Though, a higher isolation rate of all the species including CNS was recorded in the small scale farms (table 10). However, these differences both in the scale of productions and farm types were not statistically significant.

Table 8: Distribution of isolation rates of *Staphylococcus* species isolated from samples acquired from 4 poultry farms on the basis of farm type and ID in Debre Zeit, Ethiopia (2011-2012).

Farm type	Farm ID	Species Isolated (%)			
		<i>S. aureus</i>	<i>S. intermedius</i>	<i>S. hyicus</i>	CNS
Large scale	1	10.4	8.3	6.2	11.5
	2	12.5	8.3	6.2	21.9
Small scale	3	17.7	12.5	8.3	16.7
	4	14.6	9.4	6.2	19.8
Total		53 (13.8)	37(9.6)	26 (6.8)	67 (17.4)

In analysis of the data, with respect to origin of samples, the result revealed that the *Staphylococcus* species identified in the carcass swabs were 17.6%, 12.4%, 9.1% and 6.5% of CNS, *S. aureus*, *S. intermedius* and *S. hyicus*, correspondingly. Species identified in the immediate slaughter environment were *S. aureus*, CNS, *S. intermedius* and *S. hyicus* with isolation rates of 25.0%, 15.9%, 13.6% and 9.1%, respectively. Relatively elevated isolation rate of all species have been observed in the samples obtained from immediate slaughter environment except CNS, which is found to be higher in carcass swab samples. Consequently, a statistically significant relation ($p= 0.022$) was recorded only in the prevalence of *S. aureus* amongst the

sample origins. However, no significant associations were observed amongst the isolation rates of the rest three species and the two origins of sample.

Table 9: Proportional distribution of isolation rates of *Staphylococcus* species contamination on the basis of sample origin from the samples acquired from 4 poultry farms in Debre Zeit, Ethiopia (2011-2012).

Sample Origin	No. examined	Species Isolated (%)			
		<i>S. aureus</i>	<i>S. intermedius</i>	<i>S. hyicus</i>	CNS
Carcass	340	12.4	9.1	6.5	17.6
Environment	44	25.0	13.6	9.1	15.9
Total	384	13.8	9.6	6.8	17.4

Out of the 183 staphylococci isolates, species were identified based on swab sites as breast, feather, neck, wing, equipment surfaces and workers' hand swabs. The isolation rate of *S. aureus* in breast, feather, neck, wing, equipment and workers hand swabs samples were 13.1%, 14.3%, 10.7%, 11.4%, 20.8% and 30.0%, correspondingly. A relatively higher isolation rate of *S. aureus* was recorded from the samples obtained from workers hand swab. *S. intermedius* were isolated in 10.7%, 9.5%, 4.8%, 11.4%, 20.8% and 5.0% of the breast, feather, neck, wing, equipment and workers hand swabs samples, respectively. A higher rate (20.8%) of *S. intermedius* isolation was documented in the slaughtering equipment surfaces.

S. hyicus prevalence in the breast, feather, neck, wing, equipment and workers hand swabs samples were 7.1%, 6.0%, 6.0%, 6.8%, 8.3% and 10.0%, respectively. Higher *S. hyicus* prevalence were observed in the swabs taken from workers hand followed by equipment surfaces. CNS prevalence according to the swab sites of breast, feather, neck, wing, equipment and workers hand were 11.9%, 29.8%, 17.9%, 11.4%, 16.7% and 15.0%, respectively. Predominance of CNS isolates has been seen in the feather samples. The comparison of *Staphylococcus* species

prevalence results in the mentioned swab sites revealed no statistically significant difference except in the isolation rates of CNS among the six different swab sites ($p=0.023$).

Table 10: Distribution of isolation rates of *Staphylococcus* species isolated from samples acquired from 4 poultry farms on the basis of swab sites in Debre Zeit, Ethiopia (2011-2012).

Swab sites	Species Isolated			
	<i>S. aureus</i>	<i>S. intermedius</i>	<i>S. hyicus</i>	CNS
Breast	11 (13.1%)	9 (10.7%)	6 (7.1%)	10 (11.9%)
Feather	12 (14.3%)	8 (9.5%)	5 (6.0%)	25 (29.8%)
Neck	9 (10.7%)	4 (4.8%)	5 (6.0%)	15 (17.9%)
Wing	10 (11.4%)	10 (11.4%)	6 (6.8%)	10 (11.4%)
Equipment	5 (20.8%)	5 (20.8%)	2 (8.3%)	4 (16.7%)
Workers' hand	6 (30.0%)	1 (5.0%)	2 (10.0%)	3 (15.0%)
Total	53 (13.8%)	37 (9.6%)	26 (6.8%)	67 (17.4%)

The 67 CNS were additionally investigated for their susceptibility to novobiocin in order to presumptively identified *S. saprophyticus* from other coagulase negative staphylococci (e.g. *S. epidermidis* and *S. haemolyticus*). The coagulase negative species identification result demonstrated the predominance of *S. saprophyticus* 68.6%. The other coagulase negative *Staphylococcus* species accounted for the 31.4%. The qualitative OmniLog reading revealed the presence of the species *Staphylococcus aureus* sub species *aureus* and *Staphylococcus pasteurii*.

4.3. Antimicrobial Susceptibility

A total of 53 *S. aureus* isolates were exposed to antimicrobial agents, resistant pattern varied among fifteen drugs. In general all strains were susceptible to chloramphenicol and rifampicin. On the other hand, all strains were resistant to bacitracin, methicillin, neomycin and polymixin B. 90.6%, 90.6%, 60.4%, 81.2%, 60.4%, 69.8%, 81.2% and 50.9% of the strains were susceptible to amoxicillin, cephalothin, cloxacillin, clindamycin, erythromycin, nitrofurantoin, norfloxacin and penicillin G, respectively. While 39.6%, 9.4%, 9.4%, 9.4%, 49.1%, 39.6% of the isolates were resistant to cloxacillin, erythromycin, nitrofurantoin, norfloxacin, penicillin G and vancomycin correspondingly. Intermediate susceptibility was observed in 9.4%, 9.4%, 18.8%, 30.2%, 20.8%, 9.4% and 60.4% of the isolates for the drugs, amoxicillin, cephalothin, clindamycin, erythromycin, nitrofurantoin, norfloxacin and vancomycin, respectively.

Table 11: Antimicrobial susceptibilities amongst 53 isolates of *S. aureus* from four poultry farms in Debre Zeit, Ethiopia (2011-2012).

Antimicrobials	Resistant	Intermediate	Susceptible
Amoxicillin	0	5	48
Bacitracin	53	0	0
Cephalothin	0	5	48
Cloxacillin	21	0	32
Chloramphenicol	0	0	53
Clindamycin	0	10	43
Erythromycin	5	16	32
Methicillin	53	0	0
Neomycin	53	0	0
Nitrofurantoin	5	11	37
Norfloxacin	5	5	43
Penicillin G	26	0	27
Polymixin	53	0	0
Rifampicin	0	0	53
Vancomycin	21	32	0

5. DISCUSSION

5.1. Prevalence and Distribution

Food-borne diseases are of major concern worldwide. *Staphylococcus* species are prevalent important food-borne bacterial pathogens that cause food poisoning in humans when ingested in contaminated foods, including poultry meat (Salandra *et al.*, 2008). *Staphylococcus* species can be indeed be easily eliminated from foods by heat or competition with other flora, whereas SFs resist most of the treatments used during food processing. Hence, these pathogens have relevant public health implications (Sousa, 2008).

Data on the incidence of food-borne illness for developing countries are particularly weak, although it is recognized that a wide variety of bacterial infections and intoxication including *staphylococcus* are wide spread. Further, where incidence data are available, these are frequently outdated (WHO, 2002). In Ethiopia, the problem attains great proportions due to many reasons, basic among which are poverty and lack of public health awareness. Besides, epidemiological data related to food-borne diseases are inadequate. But it can be evidenced that they are very common in the country because of poor water supply, poor personal hygiene and environmental sanitation (Baraki *et al.*, 2005).

Despite these findings, studies that have investigated the sources of staphylococcal food poisoning have focused almost exclusively on the carriage of enterotoxigenic *S. aureus*, and rarely on enterotoxin-producing CNS, in food handlers. Therefore, enterotoxigenic CNS strains may contribute to staphylococcal food poisoning, as any enterotoxigenic staphylococcal strain can be involved in food poisoning (Udo *et al.*, 2000).

Several previous studies have established *S. aureus* as the most frequently isolated species. Huys and co-workers (2005) were able to isolate 38% of *S. aureus* from a PPP located in Sunda, South Africa. Isolates were obtained from neck skins and from rubber fingers. Fifty five per cent *S. aureus* was reported by Guven and co-workers (2010) from Turkey poultry meat samples in which 63.6% were enterotoxigenic strains. A relatively lower isolation 10.4% of *S. aureus* was

recorded by Cohen and co-investigators (2007) from poultry samples in traditional shops and supermarkets of Casablanca, Morocco. In addition, during 2003 to 2005, *S. aureus* was recovered from 0.3%, 0.4%, and 7.8% of rinse fluids from chicken carcasses, respectively, collected at 17 meat processing plants; a report by Lin and co-workers (2009). In a study conducted by Adesiji and co-workers (2011) *S. aureus* (80%) was the most frequently isolated organism in fresh raw chicken from various abattoirs and markets around Osogbo city Nigeria. In the present study, *S. aureus* was isolated in 42 (12.4%) of carcass samples and 11 (25.0%) of immediate slaughter environment.

In the present study, out of 183 (47.7%) staphylococci isolated, 13.8% of the samples (12.4% of carcass swabs) were contaminated by *S. aureus* and more than 73.3% of the isolates were resistant to several antibiotics. Results were also reported by Alvarez-Astorga *et al.* (2002) in chicken parts and processed chicken products from Spain. *S. aureus* contaminated 10% of samples, in a study performed in US from poultry meat samples; were ninety-six percent of the isolates were resistant to at least 1 antimicrobial tested (Waters *et al.*, 2011). Consequently, this result was found to be consistent with the present report.

In an investigation carried out in Croatia, Kozacinski and co-investigators (2006) reported the isolation rate of *S. aureus* from samples of chicken breasts at a percentage of 46.15%. Tiemersma and co-workers (2003) isolated *S. aureus* from samples of chicken meat by a rate of 23.3%. CNS were detected in 48.2% of samples chicken carcasses and 49.3% of human samples. In this same study, among the CNS 17.4% were *S. haemolyticus* and 14.5% *S. epidermidis*. In the present-day study, 31.4% of CNS other than *S. saprophyticus* were investigated and found to be comparable with the finding of Çepoğlu and co-workers (2010) from Turkey, that reported from a total of 125 staphylococcal isolates 72.8% of isolates from hand swabs were identified as CNS and 27.2% were coagulase positive staphylococci.

However, Saikia and Joshi (2010) could isolate *S. aureus* in 20% chicken samples from retail market in North-India. Of the 90 poultry meat samples examined from Turkey, *S. aureus* were reported to be 53.3 % by Gündoğan and Devren (2010). Kumar and Saravanan (2011) have been able to isolate 6.67 % of *S. aureus* from 210 chicken meat samples taken from retail poultry

markets of Namakkal and Tamilnadu, India. This was found to be comparable with the present study result.

High frequency of carrier status among food handlers has been identified by several investigators. Pereira and co-workers (2000) examined 55 healthy food handlers in a large industrial kitchen in Belo Horizonte (Brazil) and found that 32 (58.2%) were carriers of *S. aureus* and 17 (30.9%) carried enterotoxigenic strains in their nasal cavity and under fingernails. Also study conducted by Udo and co-investigators (2000), revealed that 81.61% of isolates from hands of healthy food handlers in different restaurants in Kuwait City were CNS and 18.4% were *S. aureus*. In the present study, *S. aureus* and CNS was isolated from 30.0% and 15.0% of workers hand, respectively; which is found to be inconsistent with the results reported by Pereira and co-workers (2000).

In a study attempted to isolate *S. aureus* from fingernail contents of both the hands among food-handlers in Gondar Town, Northwest Ethiopia, the predominant bacteria were coagulase-negative staphylococci (41.7%) followed by *S. aureus* (16.5%) (Andargie *et al.*, 2008). Though, variation is noticed with current study result in the isolation of *S. aureus* (30.0%) and CNS (15.0%) from workers hand. Such dissimilarities could arise from the difference in swab sites and isolation procedure employed.

Marino and co-investigators (2010) from Italy, have been able to isolate the species *S. aureus*, *S. epidermidis*, *S. saprophyticus* and *S. pasteurii* from food and food environments in 26.8%, 24.3%, 22.1% and 11.2%, respectively. The strains that readily formed biofilms belonged to the species *S. aureus*, *S. epidermidis* and *S. pasteurii*. In this same study *S. aureus* were isolated in 46.3% from workers hand and 21.0% from equipment surfaces. Accordingly, in the present study *S. aureus* (13.8%), *S. epidermidis* (31.4%) together with other CNS other than *S. saprophyticus*, *S. saprophyticus* (68.6%) and *S. pasteurii* were isolated. Moreover, *S. aureus* was isolated at a rate of 30.0% from workers' hand and 20.8% from equipments. Thus, the results obtained in the isolation rate of *staphylococcus* species from workers' hand and equipment surfaces were found to be consistent with the present study.

Becker and co-workers (2001) showed on both molecular and immunological levels that a substantial number of *S. intermedius* isolates harbor the potential for enterotoxin production

(Khambaty *et al.*, 2000). Based on the above information, out of the 183 *Staphylococcus* species isolated and identified in the present study, some isolates could be enterotoxigenic and chickens meat might be a potential source of food poisonings and hazard to the public health. The different rates of species isolation mentioned earlier in the reports could be explained by the different techniques used in these studies, diversification in the origin of the isolates or by geographical differences.

The high isolation rate of *S. aureus* in this study indicates poor hygiene and working practices of the meat handlers during the processing stage as well as lack of sterilization of utensils and working surfaces (EFSA, 2004). Enterotoxin-producing *S. aureus* is the most common cause of food-borne human illness throughout the world in which red meat and poultry products have been implicated (Normanno *et al.*, 2005).

This study investigated the prevalence and antimicrobial susceptibility of *Staphylococcus* species isolates in chicken carcass and immediate slaughter environment. It was observed that there was lack of measures in slaughtering, preparation, handling and storage of the chicken products. The higher contamination rate with *Staphylococcus* may be attributed to excessive handling of chicken meat and its products by workers during processing, bad personal hygiene, cross contamination and slicing machines or cutting knives.

5.2. Antimicrobial Susceptibility Profiles

Food contamination with antibiotic-resistant bacteria can also be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades (Van *et al.*, 2007).

In the present study, result of antibiogram showed that *S. aureus* isolates demonstrated higher resistance for the drugs; bacitracin, methicillin, neomycin and polymyxin B, followed by vancomycin, penicillin G and cloxacillin. Minimum resistance (9.4%) demonstrated in some of the isolates for erythromycin, nitrofurantoin and norfloxacin. Higher susceptibility was noted for

the drugs, chloramphenicol and rifampicin in all isolates; followed by a relatively lower susceptibility (50.9% to 90.6%) for amoxicillin, cephalothin, cloxacillin, clindamycin, erythromycin, nitrofurantoin, norfloxacin and penicillin G have been demonstrated. Intermediates susceptibility was confirmed for 46.6% of the antibiotics used in this study.

In a study performed by Losito and co-workers (2005) all strains of *S. aureus* were susceptible to cephalothin, vancomycin, bacitracin and rifampicin; which were found to be similar in the susceptibility of rifampicin with the present study. Some (15.2%) of the strains were resistant to penicillin G, 6.8% were resistant to chloramphenicol, 8.5% to clindamycin and 16.9% to erythromycin.

The current data demonstrated that poultry meat and slaughtering environment are frequently contaminated with multidrug-resistant *S. aureus*, in which resistance was demonstrated for more than five antimicrobial drugs. Very similar resistance pattern was reported by Waters and co-workers (2011) from US poultry meat samples. Further more, description by Tassew and co-investigators (2010) revealed that out of the 20 *S. aureus* isolates, 65% showed resistance to erythromycin, 60% to amoxicillin, and 20% to vancomycin and all isolates were sensitive to rifampicin (100%) from Jimma town, Ethiopia. In addition, 90% of the isolates were methicillin resistant. This finding was consistent with the present study, in which 100% methicillin resistance were recorded in all isolates.

While Çepoğlu and co-workers (2010) discovered that 4.70% of *S. aureus* isolates were resistant to methicillin, 1.19% to vancomycin, 33.33% to erythromycin and 29.11% to tetracycline, 3.53% isolates showed medium resistance to methicillin, 2.38% to vancomycin. Adesiji and co-workers (2011) from Nigeria reported antimicrobial susceptibility in most *S. aureus* isolates which were susceptible to erythromycin and vancomycin. This was found similar with the present study report, in which 60% susceptibility to erythromycin and intermediate susceptibility 40% for vancomycin was observed.

6. CONCLUSION AND RECOMMENDATIONS

Staphylococcal food poisoning is of major concern in public health programs worldwide. Predictive models for *Staphylococcus* species growth and SE production would be powerful tools for microbial risk assessment in poultry industries. The overall prevalence of *Staphylococcus* species in the present study from broiler carcass and immediate slaughter environment in large and small scale farms in Debre Zeit was 47.7%. *S. aureus* was isolated in 13.8% of the samples in which 25.0% were sourced from immediate slaughter environment (equipment surfaces and workers hand) and 12.4% were from carcass swabs.

The current data confirmed the presence of high level of *S. aureus* from hands of workers followed by the equipment surfaces. *S. aureus* isolated from the equipment surfaces and workers' hand could be source of contamination for the carcass and the *S. aureus* isolates were found to be multidrug resistant. Food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms. *S. aureus* isolates which are methicillin resistant and multidrug resistant that emphasizes close follow up in the utilization of antibiotics and the importance of making periodic monitoring of food establishments.

In conclusion, the results of this study clearly show that staphylococci contamination of poultry meat and its processing environment is a concern that should be continually monitored. This study offers base-line information that could serve as a foundation for further studies on the impact of food-borne pathogens on human health in the study area. The lack of corresponding culture data from humans to support the hypothesis that food supply is a major source of antimicrobial resistant *Staphylococcus* spp. present limitations in interpretation of our data.

Therefore, it is recommended that:

- Poultry meat handlers should be educated on the adverse effect of lack of proper personal, environmental hygiene and sanitation. In addition, consumers should be made aware of the risk of consuming inadequately cooked poultry meat.

- The long-standing practice of examining foods for coagulase-positive staphylococci as the strains of importance has undoubtedly led to underestimations of the prevalence of CNS which are enterotoxin producers. Therefore, further studies on the involvement of enterotoxigenic CNS strains which may contribute to staphylococcal food poisoning should be performed.
- The differences observed in the efficacy of the antibiotics tested against staphylococci showed that the antibiotic susceptibility tests should be performed together with the identification of the bacterial agents.
- Since all the isolates of *S. aureus* investigated in this study are methicillin resistant the occurrence of methicillin-resistant staphylococci should be continuously evaluated in surveillance programs within the scope of veterinary public health aspects.
- Implementing a Hazard Analysis and Critical Control Points system, and supporting certification of food service providers.
- Further studies should be conducted in order to develop predictive tools including modern and rapid methods of detection, since many factors affect *Staphylococcus* growth and SE production in foodstuffs.

7. REFERENCES

- Acco, M., Ferreira, F., Henriques, J. and Tondo, E. 2003. Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handlers. *Food Microbiol.*, **20**: 489-493.
- Acha, P. N. and Szyfers, B. 2001. Zoonoses and Communicable Diseases Common to Man and Animals. 3rd edi. Pan American Health Organization, Washington D. C. Vol, 1. Pp. 233-246.
- Adams, M. R. and Moss, M. O. 2005. Food Microbiology. The Royal Society of Chemistry 2nd ed., Cambridge. UK. Pp. 253-259.
- Adesiji, Y. O., Alli, O. T., Adekanle, M. A. and Jolayemi, J. B. 2011. Prevalence of *Arcobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone J. Biomed. Res.*, **3**: 8-12.
- Adwan, G., Shanab, B. A. and Adwan, K. 2005. Enterotoxigenic *Staphylococcus aureus* in raw milk in the North of Palestine, *Turk. J. Biol.*, **29**: 229-232.
- Alişarlı, M., Sancak, Y. C., Akaya, L. and Elibol, C. 2003. Investigation of *Staphylococcus aureus* isolation and thermonuclease activity and enterotoxin formation in some dairy desserts. *Turk J. Vet. Anim. Sci.*, **27**: 1457-1462.
- Allen, V. M., Corry, J. E. L., Burton, C. H., Whyte, R. T. and Mead, G. C. 2000. Hygiene aspects of modern poultry chilling. *Inter. J. Food. Microbiol.*, **58**: 3948.
- Alvarez-Astorga, M., Capita, R., Alonso-Calleja, C., Moreno, B. and Garci-Fernandez, M.C. 2002. Microbiological quality of retail chicken byproducts in Spain. *Meat Sci.*, **62**: 45-50.
- Andargie, G., Kassu A., Moges, F., Tiruneh, M. and Huruy, K. 2008. Prevalence of bacteria and intestinal parasites among food-handlers in Gondar Town, Northwest Ethiopia. *J. Heal. Popul. Nutr.*, **26**: 451-455.

- Andreasen, C. B. 2003. Staphylococcosis. In: Saif, Y. M., Barnes, H. J., Fadly, A. M., Glisson, J. R., McDougald, L. R. and Swayne, D. E. (eds.). Diseases of Poultry. 11th ed., A Blackwell Publishing Company. Iowa State Press. Pp.798-804.
- Antunes, P., Machado, J. and Peixe, L. 2006. Illegal use of nitrofurans in food animals: contribution to human salmonellosis? *Clin. Microbiol. Infect.*, **12**: 1047–1049.
- Armand-Lefevre, L., Ruimy, R. and Andremont, A. 2005. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg. Infect. Dis.*, **11**: 711-714.
- Arnold, J. W. and Silvers, S. 2000. Comparison of poultry processing equipment surfaces for susceptibility to bacterial attachment and biofilm formation. *Poul. Sci.*, **79**: 1215–1221.
- Ash, M. 2008. *Staphylococcus aureus* and Staphylococcal Enterotoxins. Food borne microorganisms of public health importance, 5th edition, AIFST (NSW Branch), Sydney, Astralia, Pp. 313-332.
- Asoh, N., Masaki, H., Watanabe, H., Watanabe, K., Mitsusima, H., Matsumoto, K., Oishi, K. and Nagatake, T. 2005. Molecular characterization of the transmission between the colonization of methicillin-resistant *Staphylococcus aureus* to human and environmental contamination in geriatric long-term care wards. *Intern. Med.*, **44**: 41-45.
- Atanassova, V., Meindl, A. and Ring, C. 2001. Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham- a comparison of classical culturing detection and RFLP-PCR. *Intern. J. Food Microbiol.*, **68**: 105-113.
- Aycicek, H., Cakiroglu, S. and Stevenson, T. 2005. Incidence of *Staphylococcus aureus* in ready to eat meals from military cafeterias in Ankara, Turkey. *Food Cont.*, **16**: 531-534.
- Bania, J., Dabrowska, A., Bystron, J., Korzekwa, K., Chrzanowska, J. and Molenda, J. 2006. Distribution of newly described enterotoxin-like genes in *Staphylococcus aureus* from food. *Inter. J. Food Microbiol.*, **108**: 36-41.

- Baraki, N., Wodajo, A., Abera, M., Oljira, L., Mitiku, H., Mengistu, S. and Ketema, F. 2005. Food-borne Diseases. Ethiopia Public Health Training Initiative. Haramaya University, Ethiopia.
- Barbier, F., Ruppe, E., Hernandez, D., Lebeaux, D., Francois, P., Felix, B., Desprez, A., Maiga A., Woerther, P. L., Gaillard, K., *et al.*, 2010. Methicillin-resistant coagulase negative staphylococci in the community: high homology of SCC mec IVa between *Staphylococcus epidermidis* and major clones of methicillin resistant *Staphylococcus aureus*. *J. Infect. Dis.*, **202**:270-281.
- Barnes, H. J. 2003. Staphylococcosis. In: Saif, Y. M., Barnes, H. J., Fadly, A. M., Glisson, J. R., McDougald, L. R. and Swayne, D. E. (eds.) Diseases of Poultry. 11th ed., A Blackwell Publishing Company. Iowa State Press. Pp.797-798.
- Baron, F. 2007. Review on *Staphylococcus aureus* and food poisoning. *Gen. Molec. Res.*, **2**: 163-176.
- Becker, K., Keller, B., von Eiff, C., Brück, M., Lubritz, G., Etienne, J. and Peters, G. 2001. Enterotoxigenic potential of *Staphylococcus intermedius*. *Appl. Environ. Microbiol.*, **67**: 5551- 5557.
- Bendahou, A., Lebbadi, M., Ennane, L., Essadqui, F. and Abid, M. 2008. Characterization of *Staphylococcus* species isolated from raw milk and milk products in North Morocco. *J. Infec. Dis. Devp. Coun.*, **2**: 218-225.
- Ben-David, D., Mermel, L. A. and Parenteau, S. 2008. Methicillin-resistant *Staphylococcus aureus* transmission: the possible importance of unrecognized health care worker carriage. *Am. J. Infect. Control.*, **36**: 93-97.
- Bhandare, S. G., Sherikar, A. T., Paturkar, A. M., Waskar, V. S. and Zende, R. J. 2007. A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food. Contr.*, **18**: 854-868.

- Bien, J., Sokolova, O. and Bozko, P. 2011. Characterization of virulence factors of *Staphylococcus aureus*: novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response. *J. Pathog.*, **10**: 1-13.
- Biolog® Data Collection Software Identification System User Guide, 2008.
- Breynestad, S. and Granum, P. E. 2002. *Clostridium perfringens* and food borne infection. *Int. J. Food Microbiol.*, **74**: 195-202.
- Busani, L., Scavia, G., Luzzi, I. and Caprioli, A. 2006. Laboratory surveillance for prevention and control of food-borne zoonoses. *Ann. Ist. Super Sanita.*, **42**: 401-404.
- Capita, R., Alonso-Calleja, C., Garcia-Fernandez, M. C. and Moreno, B. 2002. Characterization of *Staphylococcus aureus* isolated from poultry meat in Spain. *Poult. Sci. Asso.*, **81**: 414-421.
- CDC, 2004. Methicillin resistant *Staphylococcus aureus* Fact sheet.htm: 1-5.
- Çepoğlu, H., Vatansever, L. and Oral, N. B. 2010. Isolation of staphylococci from food handlers and investigation of their enterotoxigenicity and susceptibility to some antibiotics. *Kafkas Univ. Vet. Fak. Derg.*, **16**: 27-35.
- CFS, 2008. Microbiological quality of poached chicken for sale in retail outlets. Food and Environmental Hygiene Department, HKSAR. Risk assessment study. Report No.32.
- Chaslus-Dancla, E., Lafont, J. P. and Martel, J. L. 2000. Spread of resistance from food animals to man: the French experience. *Acta Vet. Scand.*, **93**: 53-60.
- Chiang, Y., Liao, W. Fan, C., Pia, W., Chiou, C. and Tsen, H. 2008. PCR detection of staphylococcal enterotoxins (SE) and survey of SE types in *Staphylococcus aureus* isolated from food poisoning cases in Taiwan. *Intern. J. Food Microbiol.*, **121**: 66-73.
- CHP, 2011. Scientific Committee on Enteric Infections and Food borne Diseases. Review of Staphylococcal Food Poisoning in Hong Kong. Department of Health, Risk assessment study. Report No 28.

- Cohen, N., Ennaji, H., Bouchrif, B., Hassar, M. and Karib, H. 2007. Comparative study of microbiological quality of raw poultry meat at various seasons and for different slaughtering processes in Casablanca (Morocco). *Poult. Sci. Assoc.*, **16**:502–508.
- CSA, 2005. Population census. [http://en.wikipedia.org/wiki/Central_Statistical_Agency_\(Ethiopia\)](http://en.wikipedia.org/wiki/Central_Statistical_Agency_(Ethiopia)). Accessed on 20 February 2012.
- Das, S. C., Chowdhury, S. D., Khatun, M. A., Nishibori, M., Isobe, N. and Yoshimura, Y. 2008. Poultry production profile and expected future projection in Bangladesh. *World's Poultry Sci. J.*, **64**: 99–118.
- De Sousa, M. A. and De Lencastre, H. 2004. "Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones," *FEMS Imm. Medi. Microbiol.*, **40**: 101–111.
- EFSA, 2004. Trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the European Union in 2004. *EFSA J.* **310**:1-278
- EFSA, 2006. Scientific opinion of the panel on biological hazards on a request from the European Commission on assessment of the public health significance of methicillin resistant *Staphylococcus aureus* (MRSA) in animals and foods. *EFSA J.*, **993**: 1-73.
- El-Jakee, J., Nagwa, A. S., Bakry, M., Zouelfakar, S. A., Elgabry, E. and Gad El-Said, W. A. 2008. Characteristics of *Staphylococcus aureus* strains isolated from human and animal sources. *Eurasian J. Agric. Environ. Sci.*, **4**: 221-229.
- Eveillard, M., Lancien, E., de Lassence, A., Branger, C., Barnaud, G., Benlolo, J. A. and Joly-Guillou, M. L. 2006. Impact of the reinforcement of a methicillin-resistant *Staphylococcus aureus* control programme: a 3-year evaluation by several indicators in a French university hospital. *Eur. J. Epidemiol.*, **21**: 551-558.
- FAO. 2003. *World agriculture towards 2015/2030 – an FAO perspective*. Rome. (available at <http://www.fao.org/docrep/005/y4252e/y4252e00.htm>)

- FAO. 2004. Antibiotic growth promoters in food animals, by Hughes, P. and Heritage, J. In Assessing quality and safety of animal feeds. Animal Production and Health. Rome. Paper No. 160, pp. 129–152.
- FAO/WHO 2002. Global Forum of Food Safety Regulators. Improving Efficiency and Transparency in Food Safety Systems Sharing Experiences, Marrakesh, Morocco: www.fao.org/docrep/meeting/004/Y3680E/Y3680E02.htm
- Forsythe, S. J. 2000. The Microbiology of Safe Food. Blackwell Science. Oxford. USA. Pp. 159-160.
- Fratamico, P. M., Bhunia, A. K. and Smith, J. L. 2005. Food borne pathogens. Microbiology and Molecular Biology, Caister Academic press, Wymondham Norfolk, UK. Pp. 270-275.
- Genta, M. L. and Heluane, H. 2001. Biochemical Identification of most frequently encountered bacteria that cause food spoilage. In: Spencer, J. T. and Ragout de Spencer, A. L. (eds). Food Microbiology Protocols. Humana Press Inc. Totowa, New Jersey. Vol. 14. Pp.3-11.
- Geornaras, I. and von Holy, A. 2001. Antimicrobial susceptibilities of isolates of *Staphylococcus aureus*, *Listeria* species and *Salmonella* serotypes associated with poultry processing. *Int. J. Food Microbiol.*, **70**: 29–35.
- Gordon, R. J. and Lowy, F. D. 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* Infection. *Clin. Infec. Dis.*, **46**: 350-359.
- Gündoğan, N. and Devren, A. 2010. Protease and lipase activity of *Staphylococcus aureus* obtained from meat, chicken and meatball samples. *GU. J. Sci.* **23**:381-384.
- Güven, K., Mutlu, B. M., Gulbandilar, A. and Cakir, P. 2010. Occurrence and characterization of *Staphylococcus aureus* isolated from meat and dairy products consumed in turkey. *J. Food Safety*, **30**: 196–212.
- Heath, S. E. 2006. Challenges and options for animal and public health services in the next two decades. *Rev. Sci. Tech. Off. Int. Epiz.*, **25**: 403–419.

- Hein, I., Jorgensen, H., Loncarevic, S. and Wanger, M. 2005. Quantification of *Staphylococcus aureus* in unpasteurized bovine and caprine milk by real-time PCR. *Res. Microbiol.*, **156**: 554-563.
- Huber, H., Ziegler, D., Pflüger, V., Vogel, G., Zweifel, C. and Stephan, R. 2011. Prevalence and characteristics of methicillin resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. *BMC Vet. Res.*, **7**: 1-6.
- Huys, G., D'Haene, K., Van Eldere, J., von Holy, A. and Swings, J. 2005. Molecular diversity and characterization of tetracycline-resistant *Staphylococcus aureus* isolates from a poultry processing plant. *Appl. Environ. Microbiol.*, **71**: 574–579.
- Iroha, I. R., Ugbo, E. C., Ilang, D. C., Oji, A. E. and Ayogu T. E. 2011. Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. *J. Publ. Heal. Epidem.*, **3**: 49-53.
- ISO/TS 6888-3: 2003. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Staphylococcus* spp. Part 1: Detection method. International Organisation for Standardisation (ISO), ISO Central Secretariat, 1 rue de Varembé, Case Postale 56, CH - 1211, Geneva 20, Switzerland.
- Jay, J. 2000. Modern Food Microbiology. 6th ed, Aspen Publishers Inc., Gaithersburg, Maryland. Pp. 441-456.
- Jones, T. F., Kellum, M. E., Porter, S. S., Bell, M. and Schaffner, W. 2002. An outbreak of community-acquired food borne illness caused by methicillin-resistant *Staphylococcus aureus*. *Emerg. Infect. Dis.*, **8**: 82-84.
- Juhász-Kaszanyitzky, É., Jánosi, S., Somogyi, P., Dán, Á., van der Graaf-van Bloois, L., van Duijkeren, E. and Wagenaar, J. A. 2007. MRSA transmission between cows and humans. *Emer. Infect. Dis.*, **13**: 630-632.
- Kaskova, A., Ondrasovicova, O., Sokol, J., Vargova, M., Smirjakova, S., Ondrasovic, M. and Chovanec, M. 2006. Efficiency of sanitary treatment in poultry breeding and poultry Meat processing plant. *Acta. Vet. Brno.*, **75**: 611–617.

- Kerouanton, A., Hennekinne, J., Letertre, C., Petit, L., Chesneau, O., Brisabois, A. and De Buyser, M. 2007. Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. *Inter. J. Food Microbiol.*, **115**: 369-375.
- Khambaty, F. M., Bennett, R. W. and Shah, D. B. 2000. Application of pulsed-field gel electrophoresis to the epidemiological characterization of *Staphylococcus intermedius* implicated in a food-related outbreak. *Epidemiol. Infect.*, **113**:75–81.
- Khan, S. A., Nawaz, M. S., Khan, A. A. and Cerniglia, C. E. 2000. Transfer of erythromycin resistance from poultry to human clinical strains of *Staphylococcus aureus*. *J. Clin. Microbiol.*, **38**:1832–1838.
- Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T., *et al.* 2005. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *J. Vet. Med. Sci.*, **67**:107– 110.
- Kozacinski, L., Mirza H. and Nevijo Z. 2006. Microbiological quality of poultry meat on the Croatian market. *Vet. Arhiv.*, **76**: 305-313.
- Kumar, T. A. and Saravanan, S. 2011. Assessment of contamination in chicken meat by food borne *Staphylococcus aureus*. *Inter. J. Res. Pure Appl. Microbiol.*, **1**: 59-60.
- Lee, J. H., 2003. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl. Environ. Microbiol.*, **69**: 6489-6494.
- Levinson, W. 2008. Medical Microbiology and Immunology. 10th ed., McGraw-Hill Companies, Inc. San Francisco, California.
- Lin, J., Yeh, K. S., Liu, H. T. and Lin, J. H. 2009. *Staphylococcus aureus* isolated from pork and chicken carcasses in Taiwan: prevalence and antimicrobial susceptibility. *J. Food Protec.*, **72**: 608-611.

- Loeto, D., Matsheka, M. I. and Gashe, B. A. 2007. Enterotoxigenic and antibiotic resistance determination of *Staphylococcus aureus* strains isolated from food handlers in Gaborone, Botswana. *J. Food Prot.*, **70**: 2764-2768.
- Loir, Y., Baron, F. and Gautier, M. 2003. *Staphylococcus aureus* and food poisoning. *Genet. Molec. Res.*, **2**: 63-76.
- Longauerova, A. 2006. Coagulase negative staphylococci and their participation in pathogenesis of human infections. *Bratisl Lek Listy.*, **107**: 448-452.
- Losito, P., Vergara, A., Muscariello, T. and Ianieri, A. 2005. Antimicrobial susceptibility of environmental *Staphylococcus aureus* strains isolated from a pigeon slaughterhouse in Italy. *Poult. Sci.*, **84**:1802–1807.
- Lourdes, M., Cunha, R., Sinzato, Y. and Silveria, L. 2004. The medical significance and identification of coagulase negative staphylococci. *J. Clin. Microbiol.*, **99**: 855-860.
- Marino, M., Frigo, F., Bartolomeoli, I. and Maifreni, M. 2010. Safety-related properties of staphylococci isolated from food and food environments. *J. Appl. Microbiol.*, **110**:550–561.
- Martineau, F., Picard, F. J., Menard, C., Roy, P. H., Ouellette, M. and Bergeron, M. G. 2000. Development of a rapid PCR assay specific for *Staphylococcus saprophyticus* and application to direct detection from urine samples. *J. Clin. Microbiol.*, **38**:3280–3284.
- Mead, G. C. 2000. Fresh and further-processed poultry. In: Lund, B. M., Baird Parker, T. C. and Gould G. W. (eds.). *The Microbiological Safety and Quality of Food*. Vol 1, Aspen Publ. Gaithersburg, Maryland, Pp. 445-471.s
- Morandi, S., Brasca, M., Lodi, R., Crenonesi, P. and Castiglioni, B. 2007. Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcus aureus* from milk and dairy products. *Vet. Bacteriol.*, **25**: 432-435.
- Morrison, R. 2008. The coagulase test in the identification of pathogenic staphylococci. *J. Appl. Bacterio.*, **25**: 432-435.

- Mosupe, F. M. and Holy, A. 2000. Microbiological hazard identification and exposure assessment of street food vending in Johannesburg. South Africa. *Int. J. Food Microbiol.*, **61**: 137-145.
- Mulders, M. N., Haenen, A. P., Geenen, P. L., Vesseur, P. C., Poldervaart, E. S., Bosch, T., Huijsdens, X. W., Hengeveld, P. D., Dam-Deisz, W. D., Graat, E. A., Mevius, D., Voss, A. and Van De Giessen, A. W. 2010. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in the Netherlands. *Epidemiol Infect.*, **138**: 743-55.
- NCCLS, 2003. Performance standards for antimicrobial susceptibility testing. Thirteenth informational supplement. Approved standard M100-S13. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Nel, S., Lues, J. R., Buys, E. M. and Venter, P. 2004. Bacterial population associated with meat from deboning room of throughput red meat abattoir. *Meat Sci.*, **66**: 667-674.
- NIAID 2005. [http://www.nibid.nih.gov/fact sheets/food borne diseases. htm](http://www.nibid.nih.gov/fact%20sheets/food%20borne%20diseases.htm)
- Normanno, G., Firinu, A., Virgilio, S., Mula, G., Dambrosio, A., Poggiu, A., Decastelli, L., Mioni, R., Scuota, S., Bolzoni, G., Di Giannatale, E., Salinetti, A. P., La Salandra, G., Bartoli, M., Zuccon, F., Pirino, T., Sias, S., Parisi, A., Quaglia, N. C. and Celano, G. V. 2005. Coagulase-positive staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *Int. J. Food Microbiol.*, **98**: 73-79.
- OIE. 2004. *Listeria monocytogenes*, In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 5th edi. Office International des Epizooties, Paris. Pp. 52-56.
- Pal, M. 2001. Epidemiology of staphylococcal food poisoning. *Bev. Food Wor.*, **28**: 11-13.
- Pal, M. 2007. Zoonoses. 2nd ed., Satyam Publishers, Jaipur, India, Pp. 138-139.
- Peacock, S. J., de Silva, I. and Lowy, F. D. 2001. What determines nasal carriage of *Staphylococcus aureus*? *Trends Microbiol.*, **9**: 605-610.

- Pereira, M. L., Carmo, L. S., Lara, M. A., Silva, S. O., Dias, R. S. and Bergdoll, M. S. 2000. Enterotoxigenic staphylococci from food handlers working in an industrial kitchen in Belo Horizonte-MG (Brazil). *Rev. Microbiol.*, **25**: 161-165.
- Persoons, D., Van Hoorebeke, S., Hermans, K., Butaye, P., De Kruif, A. and Haesebrouck, F. 2009. Methicillin-resistant *Staphylococcus aureus* in poultry. *Emerg. Infect. Dis.*, **15**: 452-453.
- Quinn, P. J., Cater, M. E., Markey, B. and Carter, G. R. 2002. Clinical Veterinary Microbiology. Mosby International Limited, Spain, Pp. 118-126.
- Randolph, T. F., Schelling, E., Grace, D., Nicholson, C. F., Leroy, J. L., Cole, D. C., Demment, M. W., Omore, A., Zinssstag, J. and Ruel, M. 2007. Role of livestock in human nutrition and health for poverty reduction in developing countries. *J. Animal Sci.*, **85**: 2788-2800.
- Rao, V. A., Thulasi, G. and Ruban S. W. 2009. Meat quality characteristics of non-descript buffaloes as affected by age and sex. *Worl. App. Sci. J.*, **15**: 1058-1065.
- Rho, M. and Schaffner, D. 2007. Microbial risk assessment of staphylococcal food poisoning. *Inte. J. Food Microbiol.*, **116**: 332-338.
- Roberts, D. and Greenwood, M. 2003. Practical Food Microbiology. Confirmatory biochemical tests. 3rd edi. Blackwell Publishing. USA. Pp. 254-255.
- Rosenstein, R. and Götz, R. 2000. Staphylococcal lipases: Biochemical and molecular characterization. *Biochemie.*, **82**: 1005-1014.
- Saikia, P. and Joshi, S. R. 2010. Retail market poultry Meats of North-East India-A microbiological survey for pathogenic contaminants. *Res. J. Microbiol.*, **5**: 36-43.
- Salandra, G., Goffredo, E., Pedarra, C., Nardella, M., Parisi, A., Dambrosio, A., Quaglia, N., Celano, G. and Normanno, G. 2008. Occurrence, characterization and antimicrobial resistance pattern of *Staphylococcus* species isolated from dairy products in Southern Italy. *Inter. J. Food Microbiol.*, **9**: 327-360.

- Sandel, M. K. and McKillip. 2004. Virulence and recovery of *Staphylococcus aureus* relevant to the food industry using improvements on traditional approaches. *Food Cont.*, **15**: 5-10.
- Schmid, D., Gschiel, E., Mann, M., Huhulescu, S., Ruppitsch, W., Bohm, G., Pichler, J., Lederer, I., Hoger, G., Heuberger, S. and Allerberger, F. 2007. Outbreak of acute gastroenteritis in an Austrian boarding school. *Euro. Surveill.*, **12**: 51-3.
- Sendi, P. and Proctor, R. A. 2008. *Staphylococcus aureus* as an intracellular pathogen: the role of small colony variants. *Trends in Microbiol.*, **11**: 1-5.
- Shah, M. 2003. Molecular pathogenesis of *Staphylococcus aureus* and other staphylococci. *J. Appl. Bacteriol.*, **59**: 207-221.
- Shiomori, T., Miyamoto, H. and Makishima, K. 2001. Significance of airborne transmission of methicillin-resistant *Staphylococcus aureus* in an otolaryngology-head and neck surgery unit. *Arch. Otolaryngol. Head Neck Surg.*, **127**: 644-648.
- Silva, W., destroy, M., Landgaf, M. and Franco, B. 2000. Biochemical characteristics of typical and atypical *Staphylococcus aureus* in mastitic milk and environmental samples of Brazilian dairy farms. *Brazilian J. Microbiol.*, **31**: 103-106.
- Singer, R. S., Cox, L. A, Dickson, J. S., Hurd, H. S., Phillips, I. and Miller, G. Y. 2007. Modeling the relationship between food animal health and food borne illnesses. *Preven. Vet. Med.*, **79**: 186-203.
- Smith, C. W. and Robert, N. 2005. Global and Local Food Safety Around the World. Center for Science in the Public Interest (CSPI), Washington. Pp. 23-29.
- Smith, K. 2007. Food borne pathogenic microorganisms and natural toxins. *Food and Drug Admin. Center for Food Safety Appl. Nutr.*, **10**: 119-150.
- Soejima, T., Nagoa, E., Yano, Y., Yamagata, H., Kagi, H. and Shinagawa, K. 2007. Risk evaluation of staphylococcal food poisoning in processed milk produced with skim milk powder. *Intern. J. Food Microbiol.*, **115**: 29-34.

- Soriano, M. J., Font, G., Molto, C. and Manes, J. 2002. Enterotoxigenic staphylococci and their toxins in restaurant foods. *Trends Food Sci. Technol.*, **13**: 60-67.
- Sousa, C. P. 2008. Impact of food manufacturing practices on food-borne diseases. *Braz. J. Arch, Biol. Techolol.*, **51**: 815-823.
- Stamoulis, G. S., Pingali, P. and Shetty, P. 2004. Emerging challenges for food and nutrition policy in developing countries. *Elect. J. Agri. Dev. Econom.*, **1**: 1542–167.
- Stewart, C. 2003. *Staphylococcus aureus* and staphylococcal enterotoxins. In: Hocking, A. D. (eds.) Foodborne Microorganisms of Public Health Significance. Australian Institute of Food Science and Technology, Waterloo. Pp. 359-379.
- Sung, J. M., Lloyd, D. H. and Lindsay, J. A. 2008. *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. *Microbiology*, **154**: 1949-1959.
- Tamarapu, S., McKillip, J. L. and Drake, M. 2001. Development of a multiplex polymerase chain reaction assay for detection and differentiation of *Staphylococcus aureus* in dairy products. *J. Food Prot.*, **64**: 664-668.
- Tassew, H., Abdissa, A., Beyene, G. and Gebre-Selassie, S. 2010. Microbial flora and food-borne pathogens on minced meat and their susceptibility to antimicrobial agents. *Ethiop. J. Hea. Sci.*, **20**: 137- 143.
- Thorberg, B. M. 2008. Coagulase-negative staphylococci in bovine sub-clinical mastitis. Licentiate Thesis, Swedish University of Agricultural Science, Department of Biomedical Sciences and Veterinary Public Health, Uppsala.
- Thrusfield, M. 2007. Veterinary Epidemiology, 3rd Edition. Blackwell Science, UK. Pp. 332.
- Tiemersma, E. W., Bronzwaer, S. L., Lyytikainen, O., Degener, J. E., Schrijnemakers, P., Bruinsma, N., Monen, J., Witte, W., Altay, G., Keskin, O. and Akan, M. 2003. Tavuklardan izole edilen stafilokok suslarının identifikasyonu ve bazı antibiyotiklere duyarlılıklarının belirlenmesi. *J. Vet. Anim. Sci.*, **27**:595-600.

- Todar, K. 2008. Todar's online Textbook of Bacteriology. Available online at <http://www.Textbookofbacteriology.net>
- Turutoglu, H., Tasic, F. and Ercelik, S. 2005. Detection of *Staphylococcus aureus* in milk by tube coagulase test. *J. Appl. Bacterio.*, **49**: 419-422.
- Udo, E. E., Al-Bustan, M. A., Jacob, L. E. and Chugh, T. D. 2000. Enterotoxin production by coagulase-negative staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisoning. *J. Med. Microbiol.*, **48**: 819-823.
- Van de Venter, T. 2000. Emerging food-borne diseases: a global responsibility. *Food Nutr. Agr.*, **26**: 4-13.
- Van den Broek, I. V., Van Cleef, B. A., Haenen, A., Broens, E. M., Van der Wolf, P. J., Van den Broek, M. J., Huijsdens, X. W., Kluytmans, J. A., Van De Giessen, A. W. and Tiemersma, E. W. 2009. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. *Epidemiol Infect.*, **137**: 700-708.
- Van Looveren, M., G. Daube, L. De Zutter, J. M. Dumont, C. Lammens, P. Wijdooghe, M. Vandamme, M. Jouret, M. Cornelis, and H. Goossens. 2001. Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *J. Antimicrob. Chemother.*, **48**: 235-240.
- Van, T. H., Moutafis, G., Tran, L. T. and Coloe, P. J. 2007. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. *Appl. Environ. Microbiol.*, **73**: 7906-7911.
- Vickers, A. A., Chopra, I. and O'Neill, A. J. 2007. Intrinsic novobiocin resistance in *Staphylococcus saprophyticus*. *Antimicrob. Agents Chemoth.*, **51**: 4484-4485.
- Voss, A., Loeffen, F., Bakker, J., Klaassen, C. and Wulf, M. 2005. Methicillin resistant *Staphylococcus aureus* in pig farming. *Emerg. Infect. Dis.*, **11**: 1965-19656.
- Walderhaug, M. 2007. Food borne pathogenic microorganisms. *Center Food Saf. App. Nut.*, **28**: 48-65.

- Waters, A. E., Contente-Cuomo, T., Buchhagen, J., Liu, C. M., Watson, L., Pearce, K., Foster, J. B., Driebe, E. M., Paul, S. K. and Price, L. B. 2011. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clin. Infect. Dis.*, **52**: 1-4.
- WHO 2002. Food Safety and Food-Borne Illness. World Health Organization, Geneva.
- WHO 2007. Fact sheet No. 237: Food safety and food borne illnesses. Geneva. (available at <http://www.who.int/mediacentre/factsheets/fs237/en/print.html>).
- Whyte, R. T. 2002. Occupational exposure of poultry stockmen in current barn systems for egg production in the United Kingdom. *Br. Poult. Sci.*, **43**: 364–373.
- Witte, W., Strommenger, B., Stanek, C. and Cuny, C. 2007. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. *Emerg. Infect. Dis.*, **13**: 255-258.
- Wubete, A. 2004. Bacteriological Quality of Bovine Milk in Small Holder Dairy Farms in Debre-Zeit, Ethiopia. Faculty of Veterinary Medicine, Addis Ababa University, MSc Thesis.
- Yashoda, K. P., Sachindra, N. M., Sakhare, P. Z. and Rao, D. N. 2001. Microbiological quality of broiler chicken carcasses processed hygienically in a small scale poultry processing unit. *J. Food Quality*, **24**: 249–259.
- Zadoks, R., van Leeuwen, W., Barkema, H., Sampimon, O., Verbrugh, H., Schukken, Y. H. and van Belkum, A. 2000. Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. *J. Clin. Microbiol.*, **38**:1931–1939.
- Zhao, C., Ge, B., Villena, J. D., Sudler, R. and Yeh, E. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork and beef from the Greater Washington D.C., Area. *Appl. Environ. Microbiol.*, **67**: 5431-5436.

8. ANNEXES

Annex I: Sampling collection sheet for bacteriological analysis

Serial no	Sample code	Collection date	Type of sample	Source	No of samples

Annex II: Record sheet for laboratory isolation and identification of *Staphylococcus* species

Serial no	Type of sample	Sample code	Colony characteristics on BAP	Hemolysis	Growth on NAP	Gram's reaction (cellular morphology, arrangement, shape and Gram + or -)	Catalase test	Coagulase test	Growth on MSA	Mannitol fermentation	Maltose fermentation

Annex III: Record sheet for identification of CPS and CNS

Serial No	Date of collection	Type of sample	Source	Sample code	CPS	CNS

Annex IV: The various tests used for identification of *Staphylococcus* species

Serial No	<i>Staphylococcus</i> Spp	Hemolysis	Pigment production	Coagulase test	Carbohydrate fermentation	
					Mannitol	Maltose
1.	<i>S. aureus</i>	+	+	+	+	+
2.	<i>S. intermedius</i>	+	-	+	±	±
3.	<i>S. hyicus</i>	-	-	+	-	-
4.	CNS	-	-	-	-	-

+ = 90% or more strains are positive, ± = 90% or more strains are weak positive, - = 90% or more strains are negative.

Annex V: Media and reagents used for the isolation and identification of *Staphylococcus* spp.

1. Blood agar base (infusion agar) 500 g (HiMedia Laboratories Pvt. Ltd. 23, Vadhani Ind. Est, LBS Marg, Mumbai-400.086, India. MO73). Used for isolation and cultivation of many fastidious pathogenic microorganisms after addition of blood.

Composition (g/l)

- Lab-lemco powder 10.0
- Peptone 10.0
- Sodium chloride 5.0
- Agar 15.0
- Final pH 7.3 ± 0.2 (at 25°C)

Preparation

Suspended 40.0 g in 1 liter of demineralized (distilled) and boiled to dissolve completely. Sterilized by autoclaving at 121°C for 15 minutes. Cool to $45-50^{\circ}\text{C}$ and added 7% sterile defibrinated blood.

2. Nutrient agar 500g (Oxoid, Basingstoke, Hampshire, England). Composition (g/l)

- Lab-lemco powder 10.0
- Yeast extract 2.0
- Peptone 10.0
- Sodium chloride 5.0
- Agar 15.0
- Final pH 7.3 ± 0.2 (at 25°C)

Preparation

Suspended 40.0 g in 1 liter of demineralized (distilled) water, and boiled to dissolve completely. Then sterilized by autoclaving at 121°C for 15 minutes.

3. Purple agar base (Difco, France).

Composition (g/l)

- Proteose peptone 10.0
- Beef extract 1.0
- Sodium chloride 5.0
- Agar 15.0
- Bromocresol Purple 0.02
- Final pH 6.8 ± 0.2 (at 25 °C)

Preparation

31 g of the powder was suspended in 1 liter of purified water, and mix thoroughly. Heated with frequent agitation and boiled for 1 minute to completely dissolve the powder. Then autoclaved at 121 °C for 15 minutes. When preparing 0.5-1% carbohydrate fermentation, 5-10 g of the desired carbohydrate was desolved in the basal medium prior to sterilization by autoclaving.

4. Mannitol salt agar (Oxoid, England)

It is recommended for the selective isolation, cultivation and enumeration of *Staphylococcus* from clinical and non-clinical specimens.

Composition (g/l)

- Lab-lemco powder 10.0
- Sodium chloride 75.0
- D-mannitol 10.0
- Peptone 10.0
- Phenol Red 0.025
- Final pH 7.5 ± 0.2 (at 25 °C)

Preparation

111g of powder was suspended in 1 liter of distilled water and boiled to dissolve completely. Then sterilized by autoclaving at 121 °C for 15 minutes. Mixed well before pouring into sterile Petri-dishes.

5. Buffered peptone water (Oxoid, England)

Composition (g/l)

- Peptone 10.0
- Sodium chloride 5.0
- Final pH 7.5 ± 0.2 (at 25 °C)

Preparation

15 g of the powder was dissolved in 1 liter of distilled water. Stirred and dissolved completely. Then, sterilized by autoclaving at 121 °C for 15 minutes after dispensing into the test tubes.

Annex VI: Flow chart of ISO 6888-3 protocol

Isolation and identification of *Staphylococcus*



Primarily culture on sterile blood agar plates and incubation at 37 °C for 24-48 hours under aerobic culture conditions



Colonies are 0.5 to 1.5µm in diameter, with grey or grey white to golden yellow coloration



During Gram's staining, the organisms are spherical bacteria occurring in microscopic clusters resembling grapes

Biochemical test for confirmation



Catalase test (positive reaction)



Inoculate colonies on MSA and incubate at 37 °C for 24-48 hours, growth and change in the pH of the medium is confirmative for *Staphylococcus* and classified as highly fermentative (*S. aureus*), weak fermentative (*S. intermedius*) and non fermentative (*S. hyicus* and CNS)



Coagulase test identifies the most pathogenic CPS (*S. aureus*, *S. intermedius*, *S. hyicus*) from CNS



Inoculated CPS isolates on PBA plate with 1% maltose were incubated at 37 °C for 24-48 hours to differentiate pathogenic staphylococci, particularly the coagulase-positive isolates. The identification was based on the fact that *S. aureus* rapidly fermented maltose to change the medium and colonies to yellow. *S. intermedius* gave a weak or delayed reaction and *S. hyicus* did not ferment maltose instead consumed the peptone in the medium and produced a deeper purple color.

Annex VII: OmniLog sample preparation process for Gram positives

Initial culture medium	BUG + B	
Gram stain and observe cell morphology		
Gram stain results	Gram Positives	
Microbe type	GP-COCCUS-ROD, GP-COCCUS, GP-ROD	GP-ROD SB (spore-forming bacillus)
Culture medium	BUG + B	BUG + M + T (8drops in 3ml H2O) “+”streaking
Temperature	Typically 35-37° C	Typically 30° C
Atmosphere	Air or 6.5% CO2 (if required)	Air
Inoculating fluid	GN/GP-IF + T (3 drops)	GN/GP-IF (NO THIO)
Inoculum turbidity/ Turbidity standard	20% T GP-COC & GP-ROD & GN-FAS	28% T GP-ROD SB
MicroPlate type/ µl per well	GP2 150	GP2 150
Incubation time (hours)	4-6, 16-24	4-6, 16-24

BUG + B = Biolog Universal Growth Medium + blood; BUG + M = Biolog Universal Growth Medium + maltose; T = thioglycolate; %T = percent transmittance; GP-COCCUS & GP-COC = gram-positive cocci; GP-ROD = gram-positive rod; GP-ROD SB = gram-positive rod, spore-forming bacillus; IF = inoculating fluid

9. SIGNED DECLARATION SHEET

This is my original work, has not been presented for a degree in any other university and that all the sources of material used for the thesis have been duly acknowledged.

Dr. Sihin Tesfaye

This thesis has been submitted for examination with my approval as a University advisor

Prof. Mahendra Pal

Dr. Genene Tefera

Date of submission

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