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STUDIES ON THE PEREVALENCE, ANTIBIOGRAM, ASSESSMENT OF RISK FACTORS
AND PUBLIC HEALTH SIGNIFICANCE OF STAPHYLOCOCCUS AUREUS IN BEEF
AND ENVIRONMENT AT ADDIS ABABA

MSc Thesis



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DEDICATION

This thesis manuscript is dedicated to my father Adugna Kebede and my mother Ayelech Bekele who have provided me with encouragement, financial support and patience.

STATEMENT OF AUTHOUR

I, the undersigned, declare that the thesis is my original work. Sources of materials used in the thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for an advanced (MSc) degree at Addis Ababa University College of veterinary medicine and agriculture and is deposited at the university college library to be borrowed under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree or diploma.

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LIST OF ABBREVIATIONS

| | |
|-------------------------------|--|
| a_w | Water Activity |
| BAP: | Blood Agar Plates |
| CCPs | Critical Control Points |
| CDC | Centers for Disease Control and Prevention |
| CHP | Center for Health Protection |
| DNase | Deoxyribonuclease |
| ELISA | Enzyme Linked Immunosorbent Assay Kits |
| FAO | Food and Agriculture Organization |
| FBD | Food Borne Disease |
| FME | Fatty Acid Modifying Enzyme |
| GMP | Good Manufacturing Practices |
| H ₂ O ₂ | Hydrogen Peroxide |
| HACCP | Hazard Analysis Critical Control Point |
| IgG | Immunoglobulin G |
| KDa | Kilo Dalton |
| MRSA | Methicillin-Resistant <i>Staphylococcus aureus</i> |
| MSCRAMMS | Microbial Surface Components Recognizing Adhesive Matrix Molecules |
| NAP | Nutrient Agar Plates |
| NCCLS | National Committee for Clinical Laboratory Standards |

LIST OF ABBRVIATTIONS (Continued)

| | |
|--------|--|
| PAB | Purple Agar Base |
| RT-PCR | Reverse Ternscriptase PCR |
| SEs | Staphylococcal Enterotoxins |
| SOP | Standard Operation Protocols |
| SOP | Standard Operation Protocols |
| SSSS | Staphylococcal Scalded Skin Syndrome |
| TSS | Toxic Shock Syndrome |
| TSST | Toxic Shock Syndrome Toxin |
| TSST-1 | Toxic Shock Syndrome Toxin-1 |
| VISA | Vancomycin-Intermediate <i>Staphylococcus aureus</i> |
| WHO | World Health Organization |
| µg | Microgram |

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ABSTRACT

*A cross-sectional study was conducted from October 2013-April 2014 to determine the prevalence of Staphylococcus aureus from Addis Ababa abattoir and retail shops from beef Carcasses and equipment to determine antibiotic resistance profiles of the isolates found in and around Addis Ababa. A total of 888 samples comprising of 384 abattoir and 384 retail shops. carcasses swabs were in the transport medium using systematic random sampling. The swab samples were also obtained from abattoir equipment (n=60) and retail shop equipment n=(60). In addition, questionnaire survey was conducted to assess the hygienic practice during working time and possible risk factors regarding the contamination of meat. Staphylococcus aureus was isolated and identified using standard bacteriological techniques. The prevalence of Staphylococcus aureus in abattoir, retail shops and equipments was 9.3%, 19.7%, 17.5% respectively. Consequently, statistically significant association ($p= 0.00$) was observed in the prevalence of *S. aureus* between abattoir and retail shop Antimicrobial susceptibility test was also conducted on 133 isolates of *S.aureus*, using the disc diffusion susceptibility method. In this study, varying level of resistance of *S. aureus* was against bacitracin, methicillin, neomycin, and teteracyclin 95.1% was noticed. The susceptibility pattern indicated that *S. aureus* isolates exhibited varying level of resistance to two or more antimicrobial agents. Preventive measures to avoid the presence of pathogenic Staphylococcus isolates by adopting the good hygienic practices during slaughtering and handling of beef carcasses are emphasized.*

Keywords: *Abattoir, Antimicrobial, susceptibility, Beef, Carcass, Isolation, prevalence: Staphylococcus aureus.*

1. INTRODUCTION

Ethiopia has the largest cattle population (52.13 million) in Africa (CSA, 2007) and contributes 40% to the annual agricultural output, and 15% total gross domestic product. Cattle produce a total of 1.5 million tonnes of milk and 0.331 million tonnes of meat annually (FAO, 2005).

Meat, an excellent source of protein in human diet, is highly susceptible to microbial contaminations, which can cause its spoilage and foodborne infections in humans, resulting in economic and health losses (Komba *et al.*, 2012; Ahmad *et al.*, 2013). It is the most perishable of all important foods since it contains sufficient nutrient needed to support the growth of microorganisms. Foodborne 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).

Foodborne diseases occur commonly in developing countries particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory system, lack of financial resources to invest in safer equipment and lack of education for food handlers (Haileselassie *et al.*, 2013). It often follows the consumption of contaminated foodstuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria as *Salmonella species.*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Escherichia coli* O157: H7 (Nouichi and Hamdi, 2009)

Generally, animal proteins such as meats, meat products, fish and fishery products are commonly regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al.*, 2008). In fact, tissue from healthy animals are sterile, however, it has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract but that more added from knives, cloths, air, carts and equipment in general. External contamination of meat is a constant possibility from the moment of bleeding unit consumption. The possible sources of

these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves are all implicated (Goja *et al.*, 2013)..

Foodborne 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; Pal, 2013). Foodborne 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).

Foodborne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. Contaminated raw meat is one of the main sources of foodborne illnesses (Pal, 2012). The risk of the transmission of zoonotic infections is also associated with contaminated meat (Pal, 2007). International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs) (Nafisa *et al.*, 2010).

Food safety is one of the leading issues for the agricultural industry, for both livestock and producers, and bacteriological safety is the main factor which is considered as very important (Smith, 2000). 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, foodborne 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). 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 (WHO, 2002).

Staphylococcosis, an infectious bacterial zoonosis of global significance, is caused by *Staphylococcus aureus* which is a 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 cattle meat, because it can produce enterotoxins which may cause food poisoning in humans (Quinn *et al.*, 2002; Busani *et al.*, 2006). Enterotoxin-producing *S aureus* plays an important role as causative organism of food intoxications. In many countries, *S. aureus* is considered to be the second or third most common pathogen causing outbreaks of food poisoning only outnumbered by *Salmonella* species, and in competition with *Clostridium perfringen* (Aycicek *et al.*, 2001).

Symptoms like vomiting, abdominal pain and diarrhea usually occur approximately 2–6 h after the consumption of food containing enterotoxins. Although some cases have been reported, a fatal outcome is very rare. Staphylococcal enterotoxins (SEs) are resistant to proteolytic enzymes of the human intestinal tract and are heat stable proteins with a molecular weight of approximately 27–31 kDa. Thus, cases of illness might occur although no viable bacteria can be

isolated from the suspected foodstuff. So far, a family of nine major serological types of SEs have been described of which the staphylococcal enterotoxins A to D are isolated most often from outbreaks of food poisoning (Virtoria *et al.*, 2001).

In Ethiopia, the widespread habit of raw beef consumption is a potential cause for foodborne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene (Pal, 2012). Raw meat is available in open-air local retail shops without appropriate temperature control and this is purchased by households and also minced meat (Kitfo) is served at restaurants as raw, slightly-cooked or well- cooked. Meat processing at retail level is likely to contribute for the higher levels of contamination in minced beef as compared to carcasses The presence of even small numbers of pathogens in carcass meat and edible offal may lead to heavy contamination of minced meat when it is cut into pieces; as more microorganisms are added to the surfaces of exposed tissue (Haimanot *et al.*, 2010).

Moreover, antibiotic resistance levels are also elevated among foodborne pathogens such as *Salmonella* species, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni.*, and *Escherichia coli* O157: H7. Although, it is difficult to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, the presence of such bacteria in food items could play a role in the spread of antimicrobial resistance amongst foodborne pathogens. Thus, adequate information should be gathered to develop an effective strategy to reduce the outbreak of foodborne illnesses and resistance burden in the community (Lin *et al.*, 2009). Some stapilococci are methicillin-resistant (MRSA) and vacomicin resistant and infection caused by these resistant strain may be fatal because of lake of alternative antibiotics (Bhunia, 2008).

The aim of 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 *S.aures* in beef carcass, and equipments.

1.1 Objectives

This study, therefore, aimed at investigating the microbial quality of meat available in common retail shops and Addis Ababa abattoir and to determine susceptibility pattern of bacterial isolates.

1.1.1 General objectives

The general objective of this research is undertaking microbiological studies of *S. aureus* from beef carcasses, environment, and its potential implications as health hazard to consumers.

1.1.2 Specific objectives

- To determine the prevalence of *Staphylococcus aureus* in abattoir, retail shops, equipments, and establish their association with contamination of beef carcasses.
- To isolate and characterize *Staphylococcus aureus* recovered from beef carcasses, retail shops and environment.
- To conduct antimicrobial drug resistant pattern of isolates of *Staphylococcus aureus* from beef, abattoir environment and retail shops.
- To assess the risk factors and public health significance of *Staphylococcus aureus* in butchers and meat handlers.

2. LITERATURE REVIEW

2.1 Meat Production and Consumption Trend in Ethiopia

Meat is the main edible part of domestic mammals; however, recent definition includes species, as well as fish, shellfish, poultry and exotic species such as frogs and allegation (Bradeeba and Sivakumaar, 2013). Similarly, meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including the lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood. Recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savory sensation (Iroha *et, al.*, 2011).

Meat production and consumption is also important in the Ethiopian economy. The annual contribution of ruminants to meat production in Ethiopia is estimated at over 3.2 million tonnes, representing over 72% of the total meat production. The Livestock Marketing Authority (LMA 2004) estimated the annual potential for export at 72,000 t of meat with an equivalent value of USD 136 million. (NEPAD–CAADP (2005) reported that the Middle East and North African countries which are considered important for the country’s export in livestock and livestock products are Saudi Arabia, United Arab Emirates, Bahrain, Yemen, Jordan, Kuwait, Oman, Qatar, Iran, Syria and Egypt. Their annual demand is estimated at USD 1.1 billion consisting of 206,846 t of meat and 12 million heads of live animals cattle and shoats (Workneh, 2006).

It has been established that composition of food depends on the level and distribution of income and the degree of urbanization in a country, among other factors. As income grows, there is a tendency to consume more livestock products. Likewise, urbanization and the consequent improvements in infrastructure encourage diversity of diets, with shifts toward livestock products and nutritious foods given recent income growth in Ethiopia and the government’s focus on developing the industrial sector, which is likely to lead to expansion of urban centers, there is

potential for growth in the demand for livestock products. Channeling this demand growth so that the potential gains will thoroughly improve the welfare of producers and consumers requires an understanding of the consumption patterns of livestock products,. Kedir (2005), Ulimwengu *et al.* (2009), and Tafere *et al.* (2010)

2.2 Microbial contamination of meat

Meat is a good source of animal protein and the expectation of all consumers is to purchase meat that is safe and wholesome (Endale and Gurm, 2013). Meat produced in an unhygienic condition could pose threat to the health of the consumers as well as impair the keeping quality of such meat. Contamination of meat can result from contaminated working surfaces, equipments and the workers hands used in the processing (Lues *et al.*, 2007). The quality of water used in meat processing at the butcher shops also play a major role in reducing or increasing meat contamination, as water is used in washing the working surfaces, carcasses, blood of meat, equipment and workers hands. Bacterial contamination of meat products is unavoidable consequence of meat processing (Jones *et al.*, 2008).

The beef meat contains 70-73% of water, 20-22% of protein, and 4.8% of lipids. This chemical composition exposes beef meat to the contamination by spoilage and pathogenic bacteria when adequate hygienic measures during the preparation, transport and marketing are not respected. In most developing countries, the absence or non respect of the existing hygienic practices in slaughtering, transportation and marketing has been found to be one of the major causes of meat contamination by pathogenic and non pathogenic microorganisms (Eugene *et al.*, 2013) Furthermore, meat are sold in the open markets on tables that are not well cleaned and disinfected. Thus exposing meat to a number of microorganisms which may be pathogenic or non pathogenic. The contamination of meat at the end consumers level, correspond to the combination of contaminations at different stages of meat preparation including the slaughtering, transportation and marketing. During the slaughtering process, the stages of skinning and the

dressing were identified to be the critical points for carcasses microbiological contamination(Gill *et al.*, 2003).

Ethiopian meat production and marketing has been plagued by lack of quality and sanitation, prevalence of disease and unqualified meat production process. Several scientists have indicated the importance of continuous assessment on meat microbial load qualities Kirton. (1989) recommended the continuous investigation and inspection of meat to provide safe and wholesome meat for human consumption. As far as microbial load level of Ethiopian meat is concerned, there is limited information on the microbial quality of Ethiopian beef that is being retailed in different outlets. In addition, the need for assuring safety cannot be underestimated in this modern world From the European point of view, the major source of carcass contamination in the meat industry is the live animal, particularly during the winter months when animals are confined and have dirty bodies Skin and hair, dirty with feces, may carry millions of bacteria to slaughtering and meat-processing environments (Minihan *et al.*, 2003; Pardi *et al.*, 2006).

2.3 Control strategies of beef meat contamination

To lower the incidence of foodborne disease, many experts and stakeholders urge the development of a science- and risk-based food safety system, in which decision makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks . Such a system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum (Okonko *et al.*, 2013). The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the keeping quality the meat. So, to increase meat quality assurance in accordance with microbial load assessment is deemed necessary (Yousuf *et al.*, 2008).

The problems involving meat consumption safety during recent decades are still a concern for consumers. Owing to the issue's serious consequences, strictness and even new relationships between cause and effect exert great impact on consumers' acceptance, on the global food trade, and especially on scientific community, to the point that they impose new paradigms for food supply, such as Safe Food Program. Coupled to meat inspection, the new model imposed a series of routinely check-ups applied to the process of cattle slaughter and meat producing. It is expected that the endorsement of these practices provides the timely implementation of corrective measures to correct potential problems before they become serious. Results should be divulged and freely shared as experiences that deepen scientific knowledge on the epidemiology of foodborne diseases, in accordance with the principles of risk analysis. Meat and meat by-products are considered to be a major source of foodborne diseases and the most important link between food producing animals and humans (Mayerhofer *et al.*, 2004).

Since bacterial contamination on the carcass surface is an inevitable consequence of the skinning and other slaughtering processes, special care before, during and after slaughter may reduce to a minimum the initial level of carcass contamination. Care must be the hallmark during the entire meat process, namely, during refrigeration, boning, processing, storage, transportation, distribution, including treatment at the consumers' home (Minihan *et al.*, 2003; Pardi *et al.*, 2006). Most food is potentially hazardous only after the principles of hygiene, cleaning and disinfection have been trespassed. On the other hand, if food is submitted to conditions that permit the entry and the multiplication of infectious or toxigenic organisms, it will surely be a vehicle for the transmission of diseases (ICMSF, 2000).

Control practices under the hazard analysis and critical control points (HACCP) system are the most effective measures in preventing microbial contamination of carcasses during slaughter (Bolton *et al.*, 2001). In fact, when applied during all the stages of meat processing against all plausible risks of carcass contamination, they reduce the risk of foodborne illness. Nevertheless, since routine monitoring of each pathogen is expensive, time consuming and difficult, the use of indirect techniques, such as appropriate indicator organisms, is more convenient and practical (Arthur *et al.*, 2004).

Meat inspecting authorities around the world are requiring meat packing plants to implement HACCP systems for the control of hazardous microbial contamination of meat. Although HACCP systems must take account of physical and chemical, as well as microbiological hazards, the latter hazards are the major concern in the production of raw meats at packing plants. The procedures recommended for implementing HACCP systems depend upon the subjective assessment of risks, with consequent uncertain identification of critical control points (CCPs) for the control of microbiological hazards. If the CCPs in a process are misidentified, the standard operating procedures (SOPs) prescribed for the control of hazards are likely to be ineffective (Jeffrey *et al.*, 2003).

There is then good reason for supposing that some of the HACCP systems that are currently operated at meat packing plants give only variable and often inadequate control over the microbiological conditions of raw meats. As subjective assessment of meat plant processes must always be uncertain, it has been suggested that HACCP systems at meat plants be based on microbiological data that allow estimation of the effects on the microbiological condition of the product of individual operations within any process. Then, operations can be modified and reevaluated, as necessary, to attain a final product that complies with stringent food safety objectives. An FSO is a statement of a maximum level of microbiological contamination considered acceptable for consumer protection. For that purpose, it is necessary to refer to the mean numbers of indicator organisms on products, as pathogenic organisms are generally too few and are present too infrequently to be useful for the routine investigation and evaluation of process performance (Brown, 2000).

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 aureus* (Baron, 2007; Randolph *et al.*, 2007).

2.4 staphylococci

2.4.1 Historical Background staphylococci

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).

Staphylococcus aureus is a Gram-positive, catalase positive commensal bacterium colonising both humans and animals. *S. aureus* is known for causing food poisoning through the production of enterotoxins. Worldwide, strains have emerged that are resistant to a wide range of antibiotics. In the Netherlands, 0.6% of all *S. aureus* strains isolated from hospitals between 1999 and 2003, were resistant to meticillin. From 2004 till 2007, this number increased to 1.1%, which is still well below the average for Europe: 23.7%, according to the European Antimicrobial Resistance Surveillance System (Shah, 2003; Todar, 2008).

The four special characteristics 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).

2.4.2 Nomenclature

Clinically, the most important genus of *Staphylococcaceae* family is *Staphylococcus* (Rho and Schaffner, 2007). Currently, 47 species and 24 subspecies are recognized in the genus *Staphylococcus* that are collectively referred to as staphylococci are described (Euzéby, 2013).

The scientific classification of *Staphylococcus* is as follows: Kingdom Bacteria, Phylum Firmicutes, Class Bacilli, Order Bacillales, Family *Staphylococcaceae*, Genus *Staphylococcus*. The pathogenic staphylococci, *S. aureus*, *S. intermedius* and *S. hyicus* (most strains) are coagulase-positive (Morrison, 2008).

2.4.3 The Organism and its Characteristics

S. aureus which is Gram positive, non-capsulated, non-motile catalase positive and non sporulated organism, 0.8-1 micrometer in diameter (Pal, 2007). The optimum temperature for growth is 35 °C to 37 °C, they can grow at temperatures ranging from 6 °C to 48 °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 (aw) 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), aw is 0.90 (range: 0.86 to 0.99) and greatest toxin production is in the presence of oxygen. (Adams and Moss, 2005; Aycicek *et al.*, 2005; Morandi *et al.*, 2007; Ash, 2008).

The bacterium multiply by simple division into two, and under suitable conditions of environment and temperature, this occurs every 15-30 minutes. Thus, one cell could become over 2 million in 7 hours and 7000 million cells after 12 hours continuous growth (Jay, 2000). It is well known that the organism produces various extracellular active substances, such as coagulase, hemolysins, nuclease, acid phosphatase, lipase, protease, fibrinolysin, enterotoxins

and toxic shock syndrome toxin. These active substances are though contribute to the pathogenicity of organism (Mohamed, 2013).

S. aureus toxin 5 distinct antigenic types labelled A, B, C, D, E. They are water-soluble, low molecular weight proteins that are heat stable resist boiling for 30 minutes. Their mode of action are exotoxins produced by chromosomal genes (Loir *et al.*, 2003).The organism Can be commonly found on the skin or in the nasal passages of most humans and animals. It has been implicated in a number of diseases in humans, ranging from minor, uncomplicated skin infections to more serious infections, such as bacteremia and pneumonia (Charlene *et al.*, 2013) Others include skin and soft tissue infections, such as cellulitis and abscesses, acute bacterial endocarditis, bacteraemia, infections associated with intravenous cannula sites, central venous access sites, osteomyelitis and post-operative wound infections (Pal, 2007; Monika , 2013).

2.4.4 Epidemiology

Staphylococci are widespread in nature and occupy a variety of niches. As a result of their ubiquity and adaptability, they are a major group of bacteria inhabiting the skin, skin gland and mucous membrane of humans, other mammals, and birds (Pal, 2001; Nagase *et al.*, 2002; Pal and Seid, 2013). They are sometimes found in the mouth, blood mammary glands, and intestinal genitourinary and upper respiratory tracts of these hosts.

Washing the skin with soap and water usually eliminates many of the gram-negative bacteria but gram-positive cocci tend to rise to the surface of the skin from pores and can be present in even larger numbers on the surface after washing. Scrubbing disturbs the superficial layers of the skin and may further spread *Staphylococcus aureus*. The salt tolerance of the *Staphylococcus aureus* gives them a selective advantage on the skin, as the sweat has a high salt content (Quinn *et al.*, 1999)

Staphylococcus aureus is a major species of primates, though specific ecovars or biotypes can be found occasionally living on different domestic animals or birds. In human, *S aureus* has a niche preference for the anterior nares, especially in adult. where, it can exist as resident or transient member of the normal flora *S aureus* selectively adheres to nasal epithelial mucosal cell (Gotz *et al.*, 2006).

Foodborne 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). Entry of the microorganisms into the food processing plant occurs through workers shoes and clothing, transporting equipment, raw foods of animal origin and healthy human carriers (Acha and Szyfers, 2001).

2.4.5 .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, and at any one time of these, 50% carry food poisoning strains (Atanassova *et al.*, 2001),,and 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).

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).

2.5 The staphylococcus food poisoning

2.5.1. The staphylococcal enterotoxins and food poisoning

The SEs are short proteins belonging to a large family of pyrogenic toxin super antigens encoded by phage, chromosome or plasmid genes with a disulphide bridge secreted in the medium and soluble in water and saline solutions. They are rich in lysine, aspartic acid, glutamic acid, and tyrosine residues. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity (Loir *et al.*, 2003; Salandra *et al.*, 2008).

The amount of enterotoxins produced is determined by factors such as the composition of the food, competition from other microorganisms (the presence of other bacteria affects the production of enterotoxin apparently by limiting the multiplication of the staphylococci), temperature and time (Salyers and Whitt, 2002). A family of 14 different SE types has been identified, which share structure and sequence similarities, of which the antigenic types (named SE-A, B, C, D, E) are most commonly encountered in SFP (Kerouanton *et al.*, 2007). In general, SE-A is recovered from food poisoning outbreaks more often than any of the others, with SE-D being second most frequent and the fewest number of outbreaks are associated with SE-E (Jay, 2000; Shah, 2003).

Recently, additional SEs have been identified: SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, and SEU. Many of these newly discovered enterotoxins are structurally similar to the classic enterotoxins, which suggest that they also may illicit foodborne illness when consumed in large enough doses. The significance of these SEs in causing foodborne intoxication remains largely unknown and requires both future research and increased surveillance. When SEs are expressed systemically, they mediate two illnesses, TSS and SSSS. In both diseases, exotoxins are produced during an infection, The toxins act on the emetic receptors on the abdominal viscera causing stimulation of the emetic center of the brain via vagus and sympathetic nerves. The nerve stimulation ultimately results in causing diarrhoea and vomiting (Atanassova *et al.*, 2001; Walderhaug, 2007).

When SEs are expressed systemically, they mediate two illnesses, TSS and SSSS. Toxic shock syndrome toxin is produced when SEs are expressed systemically and it is the cause of TSS. It is very weakly related to enterotoxins and does not have emetic activity (Bania *et al.*, 2006; Smith, 2007). Toxic shock syndrome is an acute life-threatening illness mediated by staphylococcal superantigen exotoxins and can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST is released systemically and the host lacks appropriate neutralizing antibodies (Salyers and Whitt, 2002). Staphylococcal scalded skin syndrome, also known as Ritter's disease characterized by dermatologic abnormalities (Shah, 2003; Todar, 2008). Because of the importance of these toxins in the public health and food sectors, an efficient screening to detect the prevalence of enterotoxic strains in foods is required.

2.5.2 Pathogenesis and Clinical Features

The virulence factor of *S. aureus* is extensive, with both structural and secreted products playing a role in the pathogenesis of infection. In establishing an infection, *S. aureus* has numerous surface proteins, called “microbial surface components recognizing adhesive matrix molecules” (MSCRAMMs) that mediate adherence to host tissues. MSCRAMMs bind molecules such as

collagen, fibronectin, and fibrinogen, and different MSCRAMMs may adhere to the same host-tissue component. MSCRAMMs appear to play a key role in initiation of endovascular infections, bone and joint infections, and prosthetic device infections. Different *S. aureus* strains may have different constellations of MSCRAMMs and so may be predisposed to causing certain kinds of infections (Gordon and Lowy, 2008).

The invasion of host tissues by staphylococci apparently involves the production of a huge array of extracellular, proteins, toxins, enzymes proteins having affinity for Igs. *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 molecules 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 can 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).

Besides the above given toxins, *S. aureus* releases certain extracellular enzymes like coagulase, staphylokinase, a lipase, a deoxyribonuclease (DNase) and a fatty acid modifying enzyme (FAME). In addition, *S. aureus* expresses a number of factors that have a potential to interfere with host defense mechanisms e.g capsular polysaccharide, protein A and antibiotic resistance. *S. aureus* secretes two type toxin with superantigen activity, enterotoxins (SEs) and toxic shock syndrome toxin (TSST-1). Beside this, the exfoliatin toxin, associated with scalded skin syndrome, causes separation within the epidermis, between the living layers and superficial dead layers (Zhu and Standl, 2007).

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 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 (Pal, 2001; 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 preformed toxin in the food (Forsythe, 2000; Shah, 2003).

2.5 3 Public Health Significance of Staphylococcus aureus

Staphylococcal infections are frequent, but usually contained by immune mechanisms to the site of entry. The highest incidence of disease usually occurs in people with poor personal hygiene, overcrowding and in children. However, anyone can develop a serious staphylococcal infection including fit young people (Rho and Schaffner, 2007).

Food safety is the assurance that food will not cause any harm to the consumer when it is prepared and/or consumed according to its intended use. Food safety is a growing global concern, not only for its continuing importance to public health, but also because of its impact on international trade (Ayalew *et al.*,2013; Pal, 2013).

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 foodborne 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. Foodborne 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. Diarrheal 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). *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 (Bhunia, 2008).

The presence of small numbers of *S. aureus* on foods is not uncommon. It will occur naturally in raw meat and poultry 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

(Pal,2001). Since large numbers, typically $> 10^6$ 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. (Waters *et al.*, 2011).

The meat, available at retail outlets comes through a long chain of slaughtering, and transportation, where each step may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environment are major factors contributing in bacterial contamination of meat. Contaminations can be compounded during transportation, storage and handling of meat at butcher shops (Ahmad, 2013)

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).

2.5.4. Diagnosis of staphylococcal food poisoning

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

A food suspected of causing *S.aures* food poisoning must be assayed both for the presence of viable *S.aures* and the presence of SE (Pal and Seid, 2013) The short incubation period, severity

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; Pal and Seid,2013).

The need to identify enterotoxins in foods encompasses basically two areas (a) food that have been incriminated in food-poisoning outbreak and (b) food that are suspected of containing enterotoxin. In the former case, the identification of enterotoxin in food supports a staphylococcal food-poisoning outbreak or episode. In the later case, the presence or absence of toxin will determine the marketability of the product. The latter cannot be overemphasized because it is difficult to prevent the presence of staphylococci in some type of foods. The isolation and determination of enterotoxigenicity of staphylococcal isolates in food can serve as a signal of potential toxin formation if the food is time – temperature abused, which would allow for the proliferation of the organism. The most common approaches involve culture methods, biological serological testing and nucleic acid based assays (Bennett and Monday, 2003;Pal and Seid,2013).

Staphylococcal entererotoxin detection on culture supernatant by classic immunodiffusion, agglutination and ELISA methods is lengthy and does not always detect low toxin concentrations. Also antiserum is commercially available only for SEA, SEB, SEC .SED and SEE. Experimental testes have been developed for some of the new toxins (SEG, SHE and SEI), but they are not commercialized due to difficulties in purification and preparation of specific antibodies (Cremones *et al.*, 2005). The immunologic tests for enterotoxin detection include the immunodiffusion, radioimmunoassay immunoenzymatic (ELISA) assays (Olivera and Hirooka, 1999); and a wide varity of ELISA have been described for SE detection (Poli *et al.*, 2002).

Polymerase chain reaction (PCR), a recent technique, has proven to be a more useful and reliable tool for detecting such genes (Tamemaparau *et al.*, 2002; Cunaha *et al.*, 2006). However, the PCR is only capable of showing that a gene is present or not in the sample without indicating whether enterotoxins are produced. Nevertheless, with reverse transcriptase PCR (RT-PCR), sequences of mRNA responsible for enterotoxin production can be detected, thus, proving gene activity. (Cunaha *et al.*, 2006).

2.5.5. Treatment

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). Drugs used successfully for treatment include penicillin, streptomycin, tetracyclines, erythromycin, novobiocin, sulfonamides, lincomycin, and spectinomycin (Pal, 2007). 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). 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).

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. It is important to mention that MRSA strains are resistant to all beta lactam drugs, including both penicillins and cephalosporins (Rho and Schaffner, 2007; Levinson, 2008).

2.6 .Economic Implications

Food safety is of particular concern in a developing country not only because of the high prevalence of foodborne 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 foodborne 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). A study from the US Centers for Disease Control and Prevention (CDC) reports that FBD cause approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths and costs annually 5-6 billion USD in the United States each year (Jay, 2000). Identified pathogens account for an estimated 14 million illnesses, 60,000 hospitalizations, and 1800 deaths. *Salmonella*, *Listeria*, and SFP organisms are responsible for 1500 deaths. Unidentified pathogens account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3200 deaths. Overall, FBD appear to cause more illnesses but fewer deaths than previously estimated. Among FBD, SFP is of major concern in global public health programs (Loir *et al.*, 2003; Baron, 2007).

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 the general population to as high as 4.4% for highly sensitive persons such as immunocompromised persons, elderly persons and children (Atanassova *et al.*, 2001; Aycicek *et al.*, 2005; Kerouanton *et al.*, 2007)

Other economical loses 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).

2.6.1 Status of Food Poisoning in Ethiopia

Epidemiologic data related to foodborne 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). Major causative agents of food borne illness outbreaks in developing countries, are *Salmonella*, (5.3%) *V. parahaemolyticus* (9.3%) *S. aureus* (10.2) Norovirus and (18.2%) Unknown (27.6) (Smith and Robert , 2005)

2.7 Prevention and Control

Ground beef should be cooked until it becomes pink. Meat from cattle, like that of other mammalian and avian species, can be contaminated by feces during slaughter and processing. Thus, all precautions should be taken to minimize this risk, and foods of animal origin should be well cooked before they are eaten. Personal hygiene, particularly hand washing is also important (Acha and Szyfres, 2001; Pal, 2007).

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; Pal, 2007).

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; Pal, 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. (Andreasen, 2003; Stamoulis *et al.*, 2004). Comprehensive education program for the consumer and food handler, both in commercial establishment, about the origin and personal and environmental hygiene is of paramount importance (Pal, 2007).

2.8 Antimicrobial Resistance

Antimicrobial resistance is the most puzzling question of public healthy in the earlier decade 21 century. Among bacteria this question seems to be more alarming due to its short generation time and efficient gene recombination mechanisms (Soares de Souza *et al.*, 2012). Drug resistance is

an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals, and specifically in the developing countries by use of medicines in humans (Threlfall *et al.*, 2000, Bogaard and Stobberingh, 2000).

A major concern is that the high levels of antibiotic resistance are a result of the use of antibiotics in food animals. A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans (White *et al.*, 2001). The epidemiological and clinical importance of *staphylococcus* species is not only because of its distribution and pathogenicity but especially due to its ability to overcome antimicrobial effects. (Soares de Souza *et al* 2012)

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).

Methicillin resistant *S aureus* (MRSA) is the major cause of nosocomal infection in human. In addition, community acquired MRSA has now become a major concern (Otter and French, 2008). New evidence also suggests that domestic animals, including food animals, are capable of serving as reservoir and shedders of MRSA, and the transmission between host species may be possible (Loo *et al.*, 2007).

3. MATERIAL AND METHODS

3.1 Study area

The study was carried out in Addis Ababa central Ethiopia. Addis Ababa is the capital city of Federal Democratic Republic of Ethiopia, and it has an area of 51,000 hectare in the central highlands with an average altitude of 2000-2560 meters above sea level. The area is characterized by bimodal rainfall with an average of 1100 mm, the highest percentage of rain falls during the long rainy season from June to September. The short rainy season is from February to April. Addis Ababa has an estimated human population of 3.15 million (CSA, 2007).

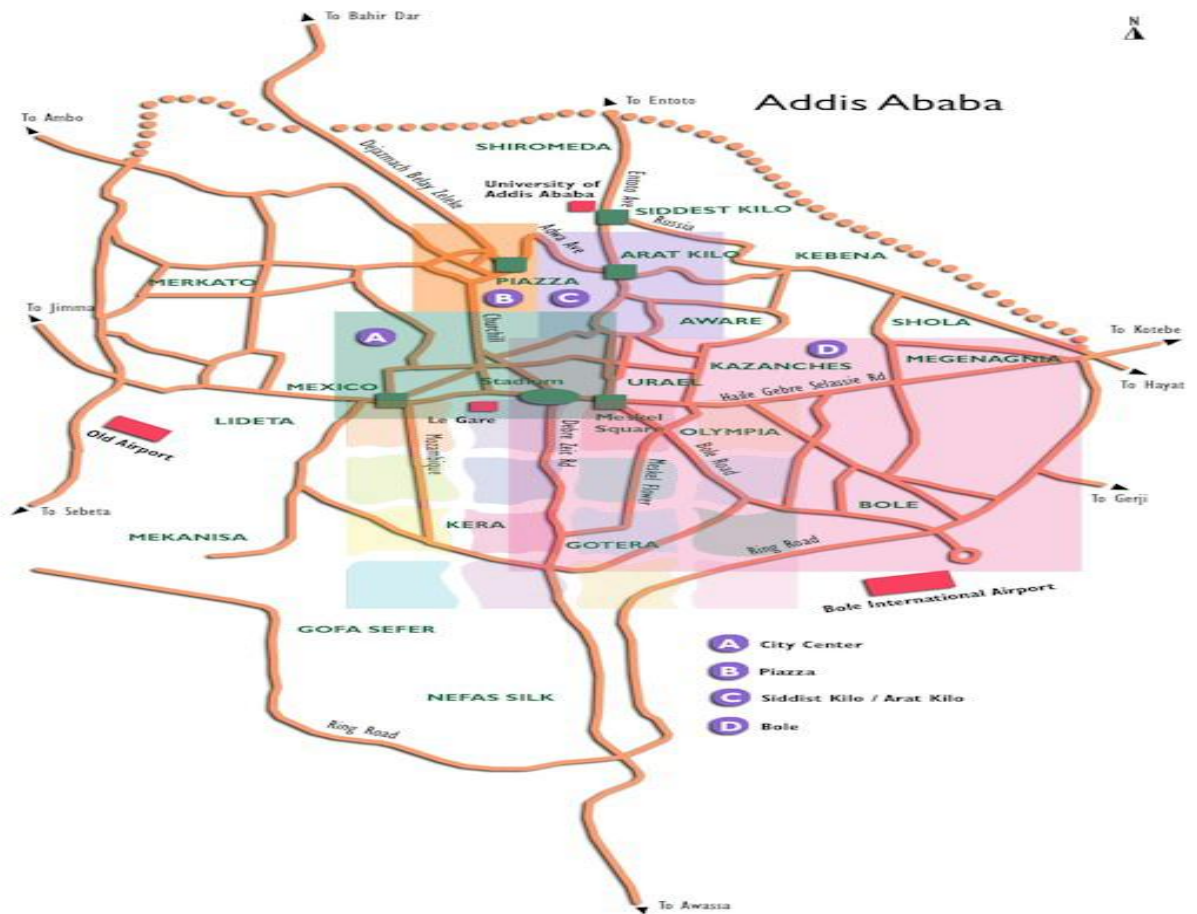


Figure1:. Map of Addis Ababa (CSA.,2005)

3.2 Study sites Abattoir and retail shops

Addis Ababa Abattoir Enterprise was established before 65 years ago and is located at the heart of Addis Ababa. The abattoir has different components such as slaughter hall, chilling room, detention meat room, condemned meat room, hide and skin room, veterinary office, water supply cold and warm, electric generator, vehicles and incinerator. The abattoir has six separate slaughter halls for bovine (three), ovine and caprine (two) and swine one.

The abattoir is a high output abattoir in the country providing 50% of the daily beef requirements of the city's residents. Most of the cattle slaughtered at the abattoir are adult males of local zebu, through lesser numbers of crossbreed males, calves as well as culled dairy cows. Species of animals slaughtered include bovine, ovine, caprine and swine.

The highest numbers of animals are slaughtered on Wednesdays and Fridays and mainly during holidays. In the abattoir, regular meat inspection is being conducted by meat inspector as well as veterinarians from Ministry of Agriculture. The abattoir has both clean and dirty areas, so that after skinning and evisceration, carcass follows the clean lines until inspection and transporting while those offal, skins etc, to dirty areas as by product preparation, like pet animal feeds, hide and skin for sale as well as, those unfit ones and condemned organs to incinerators for burn (CAS, 2005).

3.3 Study Design

A cross-sectional study design was employed to determine the prevalence of *Staphylococcus aureus* and antibiotic susceptibility test from beef carcasses at retail shops and Addis Ababa municipal abattoir from October 2013 to April 2014.

3.4 Questionnaire Survey

A descriptive survey design was used to answer questions concerning the current status of food hygiene and sanitation practiced in the abattoir, butchery shops and at home. Hygiene and sanitation were determined by the use of structured interview and through direct observations of the hygienic status and practices by abattoir and butchery shop workers. The target population constituted all the owners of retail shops in the city as well as the abattoir workers. The questions were originally written in English and translated into the Amharic language when administered. The answer were then translated to English and entered into the original form.

3.5 Study population and Sample Size Determination

There is no previous investigation about the prevalence of *S. aureus* in the study areas. Hence, the average expected prevalence rate was assumed to be 50% for the area within 95 % Confidence Intervals (CI) at ± 5 % desired accuracy was determined following the formula published in (Thrusfield, 2005).

$$n = \frac{1.96^2 \times P_{\text{exp}} \times (1 - P_{\text{exp}})}{d^2}$$

Where, n= required sample size,

d= desired absolute precision,

P_{exp} = expected prevalence (50%)

Therefore, the calculated sample size was 384 samples. A total of 888 samples comprising of 384 from Addis Ababa slaughter house and 384 from retail shops. swab samples were used for the study. In addition, 40 knives, 40 cutting tables and 40 from hooks were collected from all swab sample was collected.

Table.1: Distribution of the type and number of samples collected

| Type of sample | Number of samples |
|----------------|-------------------|
| Abattoir | 384 |
| Retail shops | 384 |
| Hook | 40 |
| Knives | 40 |
| Cutting Tables | 40 |
| Total | 888 |

3.6. Sampling techniques

Swabs were randomly sampled using systematic random sampling method from cattle population being presented for slaughter on each visit to Addis Ababa slaughter house. The target population constituted all the owners of retail shops, selected site in the city as well as the abattoir workers.

3.7 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 swab samples. 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 week from selected a retail shops and Addis Ababa municipal abattoir Selected carcasses were swabbed using the method described in ISO6888-2; (2005) by placing sterile template (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab (2X3 cm) fitted with shaft, were first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England), rubbed first horizontally and then vertically several times on the carcasses. The abdomen (flank), thorax (lateral), crutch, breast (lateral), are sites with the highest rate of contamination (ISO6888-2, 2005) were chosen for sampling. On completion of the rubbing process, the shaft were broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube.

Swab samples were also taken from knives, cutting table and hooks involved in the slaughter process. For convenience, the swabs acquired from the equipments and the working environment was considered as samples from the immediate slaughter environment. Each of the sterilized test tube were labeled by mentioning sample number, type of samples (carcass, equipment), and date. Finally, the carcass swabs were kept in the cold chain using ice box and ice packs and shipped to The food microbiology laboratory Ethiopian Institute public Health (EIPH) immediately to for analysis.

3.8 .Laboratory work

3.8.1 Isolation and identification of bacteria

Staphylococci were isolated and identified according to standard techniques (Quinn *et al.*, 2002; ISO 6888-2, 2003) Samples were processed and analyzed separately using the following procedure.

3.8.1.1. Primary identification of staphylococcus

i Cultural procedure

The bacteriological media used for the study were prepared following the instructions of the manufacturers. The primary culturing was done by directly streaking on blood agar plate (BAP) enriched with 7% 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 characteristic such as like; opaque, white or creamy, grayish or yellow colonies. Suspected colonies were subcultured onto different blood agar plates, and further incubated at 37°C for 24 hrs. After growth representative colonies were identified by using convectional bacteriological techniques on the basis of colony characteristics, pigment production and beta hemolysis.

Presumed staphylococci colonies were then subcultured 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 helped to 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 .The final identification of the organism and species assignment were based on Gram staining ,catalase test

,oxidase test, carbohydrate dissimilation (manitol ad maltose) fermentation and coagulase test by using rabbit plasema (ISO 6888-2,2003) .

ii Gram staining

First suspected 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 (Quinn *et al.*, 2002). Representative colonies were subcultured onto different nutrient agar plate and incubated 37°C for 24 hours to get pure cultures.

iii. Catalase test

Colonies that demonstrate the Gram's reaction identical with the *Staphylococcus* species was 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 (H₂O₂) on a clean microscope glass slide. Positive reaction indicated by the liberation of bubbles of oxygen within few seconds and those with negative reaction did not produce bubbles The catalase positive cocci isolates were considered as staphylococci.

iv. Oxidase test

The test was used to detect the production of cytochrome-c-oxidase by microorganisms. 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 plastic stick in a Petri-dish. The development of dark purple color along the streaked line within 10 seconds was taken as positive reaction (Fratamico *et al.*, 2005).

v. Oxidation-Fermentation (O-F) test

The convectional O-F medium is most suitable for non-fastidious Gram negative bacteria. The modification of the O-F medium which is applied for the identification of *Staphylococcus* and *Micrococcu* was used. according to Quinn *et al.*(2002).

3.8.1.2. Secondary identification of staphylococcus

i. Coagulase test

This test was conducted to differentiate the pathogenic staphylococci species from the non-pathogenic ones. From the surface of each selected colony of staphylococci an inoculum was transferred with sterile loop in too brain heart infusion broth and incubated at 37°C for 24 hrs. After growth, 0.1 ml of each culture was added aseptically to 0.3 ml of the rabbit plasma, and incubated 37°C. Clotting was examined after 4-6 hrs of incubation by tilting the tub; if the test was negative, re-examination was made after 24 hrs of incubation. When the volume of the clot occupied more than half of the original volume of liquid, it was considered as positive (ISO, 2003).

ii Mannitol salt agar

The colonies that were identified by primary identification test and coagulase-positive isolates *Staphylococcus* were cultured on mannitol salt agar and incubated at 37⁰C 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* (Jay, 2000; Roberts and Greenwood, 2003).

iii Purple agar base

The purple agar base (PAB) with the addition of 1 per cent maltose is useful medium to differentiate the pathogenic staphylococci (Quinn *et al.*, 2002; ISO, 2003). The suspected colony from nutrient agar was cultured on PAB with the addition of 1% maltose and incubated at 37 ⁰C for 24-48 hours. This presumptive identification was based on the fact that *S. aureus* rapidly ferment maltose and the acidic metabolic product caused the pH indicator (bromocresol purple) to changed 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.

Table 2: Interpretation of result based on reactions of coagulase positive staphylococci on purple agar base with 1% maltose

| Species | Maltose fermentation | Reaction o purple agar with 1%malto |
|--------------|----------------------|---|
| S aureus | +++ | Diffuse yellow color around colonies Rapid reaction with 24 hr of incubation. |
| S itermedius | ± | Little change in the medium, slight yellow zone under colonies may have a yellowish ting. |
| S hyicus | - | Diffuse deep purple (alkaline) zone around the colonies. |

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

Source: Quinn *et al.* (2002)

iv DNase Test Agar

The suspected colony from nutrient agar was cultured on DNase test agar plates by streaking a heavy inoculum of test organism. A 1–2 cm streak approximately 5 mm wide .the plates incubated were at 37°C for 24 hours and the plates were flooded with 1 % NHCl.and Observed for clearing around the streak.. A zone of clearing around the streak indicates DNase activity.

3.9 Antimicrobial susceptibility testing

The staphylococcal organisms isolated from the beef carcasses, equipment surfaces and Kiev cutting tables in the present study was tested for their antibiotic susceptibility. The antibiotic susceptibility test was performed on 133 isolates of *S. aureus*. The isolates were tested for 13 commonly used commercially available antimicrobials using the Kirby-Bauer disk diffusion method by 0.5 McFarland standard on Muller Hinton agar plats.

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 were prepared using a sterile cotton swab dipped into tryptone soya broth culture, and then the surface of MHA plate were swabbed.

Later the antibiotic discs amoxicillin (10 µg), bacitraicin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), clindamycin (30 µg), cloxacillin (12.5 µg), erythromycin (15 µg), methicillin (5 µg), neomycin (30 µg), norfloxacin (10 µg), penicillin G (10 units), ampicin (5 µg) and vancomycin (30 µg) .were placed on the agar plate using sterile forceps, and pressed gently to ensure complete contact with the agar surface. Antibiotic discs used were from Oxoid, (Hampshire, England). The plates were incubated for 24 hours at 37⁰C under aerobic condition. Inhibition zones were measured and interpreted as susceptible, intermediate and resistant according to NCCLS guidelines (NCCLS, 2012).

The inhibition zone was reported as the diameter of the zone of surrounding the individual disk which bacterial growth was absent. Based on this, the isolates were defined as resistant, intermediate and susceptible according to the guide lines of the manufacturer manual and (NCCLS, 2012)

3 10 Questionnaire survey

Questionnaire survey was conducted on each meat shop workers and Addis Ababa abattoir slaughter house workers to assess the hygienic practices during the work and possible risk factors regarding the contamination of meat. Structured questions were prepared and pre-tested 9 peoples. The questions were originally written in English and translated into the Amharic language when administered. The answer were then translated to English and entered in to the original form.

3.11. Statistical Analysis

All coded data were entered in MS Excel and then analyzed using SPSS version 20. The overall prevalence of *S. aureus* in beef carcasses was determined using standard formula. Descriptive statistics such as means and frequencies was used to present the findings of the questionnaires. Difference among and between proportions of the groups of with certain determinant factor were determined by chi- square (χ^2) test and general linear model. A p-value <0.05 was considered indicative of a statistical significant difference.

4. RESULTS

4.1. Prevalence and Distribution of *Staphylococcus aureus*

A total of 888 samples which comprise 384 from abattoirs carcasses swab, 384 from retail shops carcasses swab and 120 swab samples from equipment were taken from Addis Ababa, and were examined microbiologically for the presence of *Staphylococcus aureus*. From total 888 samples investigated, the overall prevalence of *Staphylococcus aureus* was 136 (15%). In 752 (84.6%) samples other bacteria were grown. The frequency of isolation of *staphylococcus aureus* varied between sample sources and among different sample types. *Staphylococcus aureus* were isolated from abattoir 36 (9.3%), retail shops 76 (19.5%), and equipment 21(17.6%).The highest prevalence was recorded in retail shops followed by equipment (17.6%) and abattoir (9.3%).

Table 3: Prevalence of *S. aureus* in abattoir and butchers

| Source of sample | Number of Animals examined | Total positives | Positive proportion from its source (%) | OR | CI of OR | X ² | P-Value |
|------------------|----------------------------|-----------------|---|-----|----------|----------------|---------|
| Abattoir | 384 | 36 | 9.4 | 1 | | | |
| Butcher | 384 | 76 | 19.8 | 2.4 | 1.6-3.6 | 16.7 | 0.00 |
| Total | 768 | 112 | 14.6 | | | | |

OR= odds ratio, CI= confidence interval, X²= Chi square

The prevalence of *S. aureus* in both abattoir and butcher was found to be 14.6%. The prevalence in butcher was higher (19.8%) than that of abattoir (9.4%) which was found to be 2.4 (OR= 2.4, CI= 1.6-3.6) times higher with statistically significant (X^2 16.7, P=0.00) difference. It is also illustrated in figure 2 below.

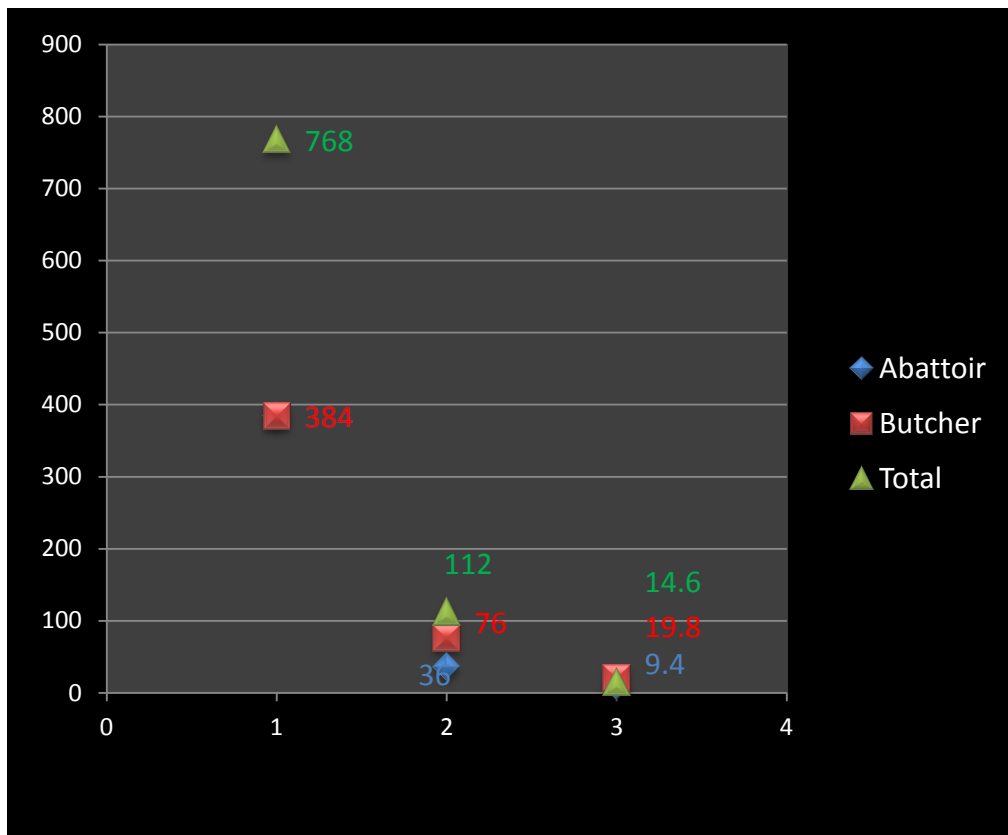


Figure 2: Prevalence of *S. aureus* identified from abattoir and butcher of the study area

Figure 2 in the above demonstrated that the red colored line (butcher) is lined above the blue lined one (abattoir) in both the total positives and positive proportion from its positives.

Table 4: Prevalence of *S. aureus* from different surface materials

| Source of sample | Number of materials examined | Total positives | Positive proportion from its source (%) | OR | CI of OR | X² | P-Value |
|-------------------------|-------------------------------------|------------------------|--|-----------|-----------------|----------------------|----------------|
| Cutting table | 40 | 6 | 15.0 | 1 | | | |
| Hook | 40 | 6 | 15.0 | 1 | 0.3-3.4 | 1.0 | 0.56 |
| Knife | 40 | 9 | 22.5 | 1.6 | 0.5-5.2 | | |
| Total | 120 | 21 | 17.5 | | | | |

Table 4 in the above indicates the prevalence of *S. aureus* in meat surface contact materials. Although there was no statistically significant difference (P= 0.56), knife was found relatively higher source of *S. aureus* which was around one and half (OR= 1.6, CI= 0.5-5.2) times that of both the others.

4.2. Antimicrobial Susceptibility

A total of 133 *S. aureus* isolates were tested to various antimicrobial agents by disc diffusion technique the resistant pattern varied among thirteen drugs. In general, all strains of *S. aureus* were susceptible to chloramphenicol, clindamycin and ampicillin. On the other hand, all isolates strains were found to be resistant to bacitracin, neomycin, methicillin, and 95% to tetracycline. It was noticed that 49.5%, 45.5%, 45% and 13% of the strains of *S.aureus* were resistant to pencilin G ,vancomycin, cloxacillin and norfloxacin respectively while 86.5%, 73%,72%,54% and 50% of the strains were susceptible to amoxicillin, norfloxacin, erythromycin, cloxacilin and pencilin G respectively. Intermediate susceptibility was observed in vancomycin (54%) and erythromycin (27%). amoxicillin and norfloxacin showed equal intermediate susceptibility (13%) and small intermediate susceptibility was demonstrated in tetracycline (5%).

Table 5: Antimicrobial susceptibilities amongst 133 isolates of *S. aureus*

| Antimicrobials | Susceptible | Intermediate | Resistant |
|-----------------|--------------------|--------------------|--------------------|
| | N _Q (%) | N _Q (%) | N _Q (%) |
| Chloramphenicol | 133 (100%) | 0 | 0 |
| Cloxacillin | 73 (54.8%) | 0 | 60 (45.2%) |
| Clindamycin | 133 (100%) | 0 | 0 |
| Amoxicillin | 115 (86.5%) | 18 (13.5%) | 0 |
| Bacitracin | 0 | 0 | 133 (100%) |
| Penicillin G | 67 (50%) | 0 | 66 (49.5%) |
| Norfloxacin | 97 (73%) | 18 (13.5%) | 18 (13.5%) |
| Vancomycin | 0 | 72 (54.5%) | 61 (45.5%) |
| Ampicilin | 133 (100%) | 0 | 0 |
| Neomycin | 0 | 0 | 133 (100%) |
| Erythromycin | 97(72.9%) | 36(27%) | 0 |
| Teteracycline | 0 | 6(4.5%) | 127 (95%) |
| Methicillin | 0 | 0 | 133 (100%) |

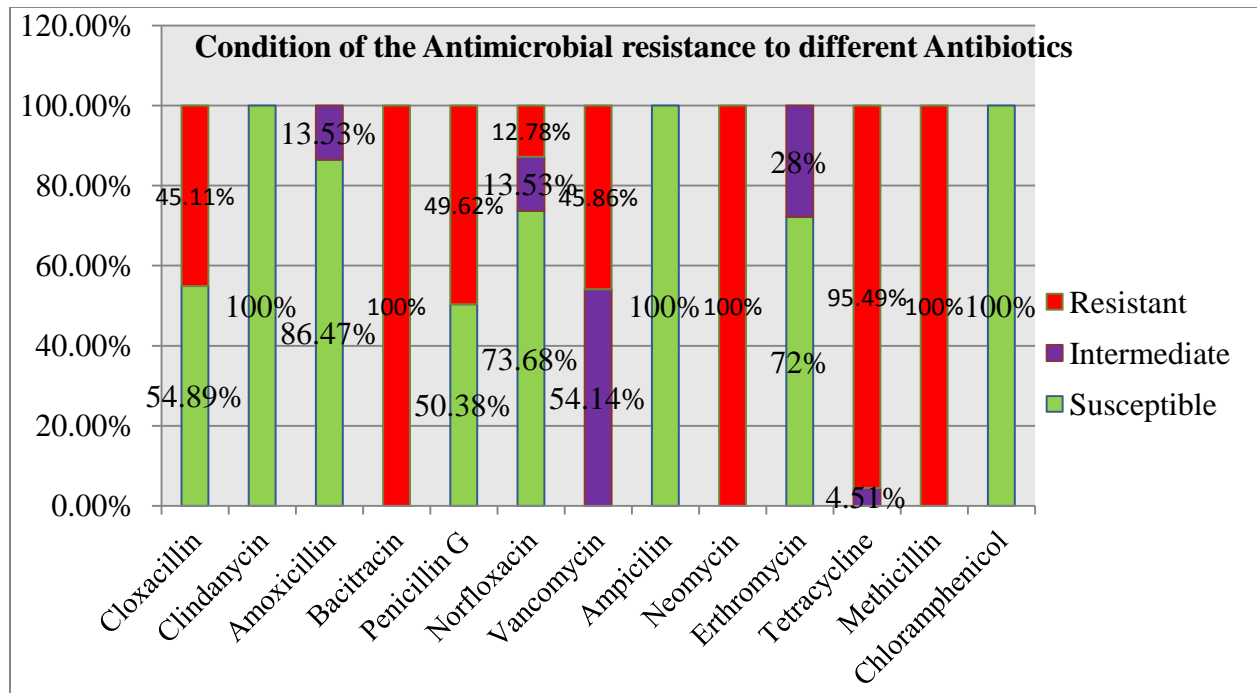


Figure 3: Drug resistance in *staphylococcus aueres* for selected antimicrobial agents

4.3. Summary of hygienic practice at retail shop workers

In all, a total of 24 retail shop workers were interviewed. Among the workers, 58.5% were illiterate, and 58.3% of the respondents had not any training on meat and butcher area hygiene. The study showed that 75 % of the workers at meat shops did not wear aprons, and 58.3% did not cover their hair and 65% of the butchers did not have cashier and serving food. It was also observed that 41.1 % of butcher shop used only water for washing purpose as shown in Table 7.

Table 6: Knowledge and skill of retail shop workers on hygienic practices

| Observation type | Values | Frequency | Percent |
|--------------------|-----------------------|-----------|---------|
| Educational status | Grade 1-8 | 8 | 33.3 |
| | Grade 8-10 | 6 | 25.0 |
| | Illiterate | 10 | 41.7 |
| Training | Yes | 14 | 58.3 |
| | No | 10 | 41.7 |
| Money | Cashier money handler | 6 | 35 |
| | Butcher money handler | 18 | 65 |
| Washing | Water only | 10 | 41.5 |
| | Water and soap | 14 | 58.5 |
| Hair cover | Not covered | 14 | 58.5 |
| | Covered | 10 | 41.5 |
| Apron | Not used apron | 18 | 75 |
| | used apron | 6 | 25 |

4.4. Knowledge of abattoirs workers on hygienic practices

Out of the total of 24 abattoir workers interviewed, 58.3% of them were educated, and 100% of the respondents had taken training regarding meat and personal hygiene. The study showed that 83.4% of the abattoir workers used aprons, and 8.3% did not cover their hair and 83.3% of the abattoir workers protective cloth was dirty. It was also noticed that 100 % of workers used water and soap for washing purpose. Furthermore, 33.3% used disinfected knives between works. It was also observed that 58.3% workers were doing their work with minor skin wounds (Table.8).

Table7: Knowledge of abattoir workers on hygienic practices

| Observation types | Values | Frequency | Percent |
|----------------------------|----------------|-----------|---------|
| Educational status | Grade 1-8 | 6 | 25 |
| | Grade 8-10 | 4 | 16.6 |
| | Illiterate | 14 | 58.3 |
| Training | Yes | 24 | 100 |
| | No | 0 | 0 |
| Ill Health | Work | 14 | 58.33 |
| | Not work | 10 | 41.6 |
| Washing | Water only | 0 | 0.0 |
| | Water and soap | 24 | 100 |
| Hair cover | Not covered | 2 | 8.3 |
| | Covered | 22 | 91.6 |
| Apron | Not used apron | 4 | 16.6 |
| | used apron | 20 | 83.4 |
| Disinfection knife | Between works | 8 | 33.3 |
| | After work | 16 | 66.6 |
| Wash overall | Daily | 6 | 25 |
| | Twice a week | 18 | 75 |
| Protective clothing status | Neat | 4 | 16.6 |
| | Dirty | 20 | 83.4 |

5. DISCUSSION

5.1. Prevalence and Distribution of staphylococcus aureus in raw meat

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

In the present study, out of 133(15%) *Staphylococcus aureus* isolated, 19.7% of the samples from retail shops Caracas swabs were contaminated by *S. aurues* and 9% of isolates from abattoir and 17.5% isolated from equipment. In study carried out in Italy, Normanno *et al.*, (2007) reported the isolation rate of *S. aureus* from sample of beef carcass at 10%. This result was found to be lower than the current study which is 15%. Schroeder *et al.*, (2005) isolated *S aureues* from abattoir sample of beef carcasses at the rate of 41% which was found in disagreement with the current study. Such dissimilarities could arise from the difference in swab sites and isolation procedure employed. In our study, of factors (income, education and knowledge of food poisoning) supposed to be associated with raw meat consumption this indicate that retail shops and abattoir at a great risk of acquiring *staphylococcus aureus* food poisoning

On other hand, according to the study by Bunnoeng *et al.*, (2014) from Thailand could isolate *S aureus* (68%) from 34 meat samples from retail shops which is higher than the finding of this study. The finding from Egypt showed that 21.8% of minced beef carcasses consist of *S. aureu* (Marwa *et al*, 2013) which is consistent with the current study. In a study from Nigeria abattoir by Iroha *et al.*,(2011) revealed 1.3% of *S aureus* from beef meat which was lower than present finding that is 9% of *S. aureus* from abattoir. Agwa *et al.*, (2014) from Nigeria also showed 14%

isolation of *S. aureus* which was similar with the present findings moreover, and Bore and co workers (2009) also reported similar results from abattoir and retail shops meat.

Goja and co investigator (2013) attempted to isolate *S aureus* from beef meat in Sudan and found that 12% prevalence rate which was somewhat lower than the current study (15%). The study conducted in Jimma on *S. aureus* isolated from minced meat found to be in agreement with the present study (Himanot *et al*, 2010).The finding from USA showed that *S aureus* was the most frequently isolated organism (20%) in fresh raw meat from various retail shops around USA (Bhargana *et al*, 2011). In the present study, *S aurues* was isolated in retail shops 76(19.7%) of carcass sample and 21(17.6%) equipment which are similar with the observation of Bhargana and others (2011). Ahmed *et al.*, r(2013) from Egypt have been able to isolate *S aureus* positive which were significantly higher for beef outlet as compare to beef abattoirs 70%v 55% , this result was found to be consistent with the current report but higher in proportion. The different rates of 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.

Marino *et al.*, (2010) from Italy, have been able to isolate the species *S. aureus* from food and food environments in 26 % The strains that readily formed biofilms belonged to the species *S. aureus*. In this same study *S. aureus* were isolated 21.0% from equipment surfaces. Accordingly, in the present study *S. aureus* 17.7% were isolated. Thus, the results obtained in the isolation rate of *S aureus* species from equipment surfaces were found to be consistent with the present study.

Charlene *et al.*, (2013) also reported from Athens Georgia that 65% of beef product from retail meat was contaminated by *S aureus* which is in disagreement result with the result of present study. The probable reason for differences in prevalence rate could be explained by the different techniques used differences in sample type or by geographical differences.

The high isolation rate of *S. aureus* in this study indicate poor hygiene and working practices of the meat handlers during the processing stage as well as lack of sterilization of equipment and working surfaces. Retail shops are one of the food industries that contribute to the problem of possible foodborne diseases and potential health hazards associated with food unless the principles of food hygiene are implemented. This fact is also supported by the result of the present finding where there is a gap in awareness of the abattoir and retail shop workers on handling of meat and maintaining hygienic status in their working area.

5.2. Antimicrobial Susceptibility Profiles

Antibiotic resistant bacteria pose a growing problem of concern, worldwide since the bacteria can be easily circulated in the environment. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous. A relatively high number of strains are resistant to the antimicrobial commonly used in the therapeutic protocol of many humans and animal infections (Normanno *et al.*, 2007)

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). Infection due to methicillin-resistant *S. aureus* (MRSA) have increased world-wide during the past twenty years (Deresinski, 2005; Ippolite *et al.*, 2010).

In the present study, result of antibiogram showed that *S. aureus* isolates demonstrated higher susceptibility to chloramphenicol, ampicillin, clindomycin followed by relatively lower susceptibility to amoxicillin (86.5%), norfloxacin (73%) erythromycin (72%), cloxaciline

(54%) and pencilin G (50%). The intermediate susceptibility was recognized in tetracycline (5%), norefloxacin and amoxicillin (13%), vancomaycen (54%). On the other hand, *S. aureus* was found completely resistant to methicilin, neomicen bacteracen.

The results of present study are almost comparable with the work of Iroha and co-workers (2011), which the strains of *S. aureus* were susceptible to clindomaycin, ampicilin and relatively lower susceptibility to erythromycin (80%) and amoxiline (60%). Çepoglu *et al.*, (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, and 3.53% isolates showed medium resistance to methicillin, 2.38% to vancomycin. This is in disagreement with this study. thus the resistance figures from different countries can considerably vary, from very low to very high probably reflecting the ues of antimicrobials in those countries.

In Nigeria, Adesiji *et al.*, (2011) reported that most of isolates of *S. aureus* were susceptible to erythromycin and vancomycin which is consistent with the present study in which 72% susceptibility to erythromycin and intermediate susceptibility for vancomycin(54%). The current data also demonstrated that beef meat and equipments were frequently contaminated with multidrug-resistant *S. aureus*, in which resistance was demonstrated for more than four antimicrobial drugs. Multidrug-resistant pathogens are not only a problem for clinical treatment of infection and illness, but also be a concern in the combination with other virulence properties *S.aureus* isolates had a significantly higher rate of biofilm formation.

Furthermore, the finding from Jimma described that, a total of 20 *S. aureus* isolates were tested for seven antibacterial agents which showed 90% were resistant to oxacillin, 85% to ampicillin, 65% to erythromycin, 60% to amoxicillin, 35% to streptomycin, and 20% to vancomycin but all (100%) of the isolates were sensitive to Co-trimoxazole. Eighteen of the 20 (90%) *S. aureus* isolates were methicillin resistant *staphylococcus aureus* (MRSA). This finding was consistent

with the present study, in which 100% methicillin resistance was recorded in all isolates of *S. aureus* (Himanot *et al.*, 2010)

The results of this study indicated that the resistance of *S. aureus* to tetracycline was higher than the findings reported previously in Ethiopia (32.3%) by Spohia (2011). In addition, lower degree of resistance to tetracycline was observed as compared with the present study in Italy (58%) (Moroni *et al.*, 2006), North Palastine, 45% (Ghaleb, 2006), South India 11.84 % (Mubarack *et al.* 2012) and USA, 23%, (Makovec and Ruegg, 2003) This could be attributed to the repeated over use of the drug for long time and in appropriate doses in our country.

This study presented the contamination status of retail meat and Addis Ababa abattoir, its surrounding environment as well as demonstrated the role of raw meat as a reservoir of antibiotic resistance bacteria that can be transferred to humans, thereby constituting a health problem. The application of hygiene practices along the food chain and prudent use of antibiotics in animal husbandry are, therefore, essential to control further emergence of antibiotic resistance

5.3. Finding of the Questionnaire survey

Foodborne diseases occur commonly in developing countries particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food-handlers (WHO, 2004) Without proper hygienic control, the environment in butchers area can act as important sources of bacterial contamination (Gill *et al.*, 1998).

A total of 48 peoples (24 from retail shop workers and 24 from abattoir) were interviewed about their knowledge on meat hygiene. From retail shops, 41.7% of them were illiterate and 58.3% of the respondents did not take any training regarding meat and retail shop area hygiene. Those who

received training were not appreciating the effectiveness of the training. About 58.5% of the workers did not use hair cover, at the same time 75% were not wear apron and 65% retail shops did not have cashier which only focused on the management of their hands and equipments. At abattoir, literate workers were 58.4%, trained 100% but ill health workers 58.33%, 8.3% of them did not cover their hair. Most of them were using apron 83%, 25% of them wash over all daily. With regard to protective cloth, 85% were dirty.

The investigation carried out in Mekelle city showed that 48% did not have cashier, 78% of the respondents did not take training regarding meat and butcher area hygiene. Those who received training were not appreciating the effectiveness of the training which only focused on the management of their hands and equipments. Though, variation was noticed when compared with the result of current study, the educational status is almost similar with the present finding in which 58% of the workers were illiterate (Mekonnen *et al*, 2013).

Another study from Mekelle which was performed by Endale *et al.*,(2013) demonstrated that, 41.7% of subjects were illiterate and 58.3% of the respondents did not take training regarding meat and butcher area hygiene. The study showed that 41.7% of the butcher workers did not wear aprons and 58.3% did not cover their hair their observation which was consistent with the finding of the present study. Endale and co investigator also reported that 91.7% of the butchers handled money which differ the current study where 75% have cashier. Furthermore 58.3 % of butcher shop used only tap water for cleaning purpose which is inconsistent with the present study. The difference in the current study could be explained by the different knowledge level and lack of regular training techniques used in city. This is well supported by who advocated training of food handlers regarding the basic concepts and requirements of personal hygiene (Adem, 2009).

In both cases the purpose of wearing apron and hair cover is to protect both the food product. and the meat handler from cross contamination, overalls should be suitable to wear over other clothing. However, in this study 75 % of the butcher workers did not wear aprons and 58.% did not cover their hair. The findings disagree with the reports of (Nel, 2004) from South Africa in which 85% butchers worn apron that indicates difference in knowledge of meat handlers in different countries. Paper currency is widely exchanged for goods and services in countries worldwide. It is used for every type of commerce. All this trade is in hard currency, with lower denomination notes receiving the most handling because they are exchanged many times, this makes it lasts less than a few years in circulation and provides a large surface area as a breeding ground for pathogens in the current study 25% butchers handled money while serving food. However, Muinde and co workers (2005) from Kenya showed 91.7% of the butchers handled money while serving food. These indicate that meat handlers are probable sources of contamination. For microorganisms it is important that, all possible measures should be taken to reduce or eliminate such contamination.

6. CONCLUSION AND RECOMMENDATIONS

Staphylococcal food poisoning is one of major concern in public health programs worldwide. *Staphylococcus* organisms are widely spread in many foods and low contamination levels that favor growth and multiplication could induce SFP. The present study confirmed the presence of high level of *Staphylococcus aureus* from retail shops (19.7%) followed by the equipment (17.5%) and abattoir (9.3%). *S. aureus* isolated from the equipment could be the source of contamination for the carcass. The presence of *S. aureus* poses a health hazard and rise concerns about the safety of these food products. This can determine the degree of public health concern as *S. aureus* are known for the enterotoxigenic potential. It is also third level pathogen that causing food poisoning in the world. *S. aureus* isolates were found to be multidrug resistant that emphasizes close follow up in the utilization of antibiotic. Food-handlers with poor personal hygiene working in food service establishments could be potential sources of infection due to *S. aureus*. To the best of our knowledge, this is the first report on the occurrence of *S. aureus* in beef carcasses from the study area. The results warrant further investigations to elucidate the public health significance, and the enterotoxigenicity of the isolates in beef meat.

Therefore, based on the above conclusive remarks, the following recommendations are forwarded:

Cutting tables and utensils must be kept clean and workers in connection with meat production and distribution must be routinely medical examined.

The slaughter houses should be routinely investigated for hygiene. Meat handlers and sellers should be educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation. In addition, consumers should be made aware of the risk of consuming raw and inadequately cooked beef meat.

Meat must be transported by special refrigerator cars and stored at low temperature to prevent growth of *S. aureus* because food poisoning is caused by ingestion of improperly stored food in which *S. aureus* grew. Also meat must be stored in refrigerator at selling point, so as to avoid contamination.

In order to detect early changes in bacterial susceptibilities before a high prevalence of resistance is developed, regular monitoring of antimicrobial resistance both among pathogenic bacteria and normal flora of animals should be practiced. The genetic mechanisms, which mediate antimicrobial resistance in these bacteria, would also need further studies.

Implementing a hazard analysis and critical control points system and Good Manufacturing Practices, Good Hygienic Practices at abattoir and supporting certification of food service providers is highly imperative.

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.

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Appendix I: Questionnaire

Questionnaire survey format and observed hygiene practice assessment in slaughter house.

General information

Date of collection_____

Questionnaire Code No 01

Name: _____ sub city: _____

Educational status: Grade 1-8_____

Grade 8-10 _____

Illiterate _____

Employment status: Temporary ____ Permanent ____

1. Did you get any documented food safety training or training on personal hygiene practice and meat handling? Yes__ No __

2. What are the main strategies used to wash equipment and surfaces in the slaughterhouse?

A. With detergent and hot running water? yes_____ No_____

B. With detergent and fresh water ? yes_____ No_____

3. How often do you clean and disinfect knives and hooks in your duty?

A. Only after breaks

B. Only during shifts

4. How often you wash overalls?

A Daily

B. Twice a week

5. Do you have any legal procedure of reporting illness? Yes_____ No_____

6. Do you use Hair cover and apron? Yes ____ No ____

Always ____ Sometimes ____

7. Protective clothing statues?

A neat

B dirty

- Knowledge of butchers on hygienic practices and observational assessment in butchery shop.

Date of collection _____

Questionnaire Code No 02

Name: _____ sub city: _____

1. Educational status: Grade 1-8 _____

Grade 8-10 _____

Illiterate _____

2.. Have you ever received any lessons in personal hygiene Yes _____ - NO _____

3. Handling money? Cashier _____ Butcher _____

4 How often do you wash your hands?

A Water only

B Water and soap

5. Hair

Covered _____

Not covered _____ -

6. Aprons Used _____ Not used _____

7. Protective clothing statues?

A neat

B dirty

Appendix III Table 2 Record sheet for laboratory isolation and identification of *Staphylococcus*

| S N | Sampl e code | Type hemolise s | Primary identification testes | | | | | General |
|--------|-----------------|-----------------------|-------------------------------|--------------------------|------------------|------------------|-----|---------|
| | | | Gram stain | Shape and arrangement | Catalase test | Oxidase teste | o-f | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

| Sample number | Sample code | Secondary identification tests | | | | Remark |
|------------------|----------------|--------------------------------|-----|-----|-------|--------|
| | | Coagulase | MSA | PAB | DNase | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
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| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

Appendix IV Composition and preparation of media used for the study

❖ Nutrient agar (Oxoid, England)

Typical formula (g/l):

| | |
|-----------------------|------|
| Lab-Lemco powder..... | 1.0 |
| Yeast extract..... | 2.0 |
| Peptone..... | 5.0 |
| Sodium Chloride | 5.0 |
| Agar..... | 15.0 |

Final pH 7.4±0.2at 25°c

Instruction for uses:

Suspend 28g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 120°C for15 minutes.

❖ Blood agar (Oxioid,England)

Typical formula (g/l):

| | |
|-----------------------|------|
| Lab-Lemco powder..... | 10.0 |
|-----------------------|------|

| | |
|----------------------|------|
| Peptone..... | 10.0 |
| Sodium Chloride..... | 5.0 |
| Agar..... | 15.0 |

Final pH 7.3±0.2at 25°c

Instruction for uses:

Suspended 40g in 1 liter of distilled water bring to the boil to dissolve completely. Sterilized by autoclaving at 121°C for 15 minutes Cool to 45-50°C and add 7% sterile sheep defibrinated blood.

❖ **Mannitol salt agar (OXoid,Engeland)** Typical formula (g/l):

| | |
|-----------------------|-------|
| Lab-Lemco powder..... | 1.0 |
| Peptone..... | 10.0 |
| Mannitol..... | 10.0 |
| Sodium Chloride..... | 75.0 |
| Phenol Red..... | 0.025 |
| Agar..... | 15.0 |

Final pH 7.4±0.2at 25°c

Instruction for uses:

Suspend 111g in 1 liter distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Mix well before pouring in to sterile petri dishes.

❖ **Purple agar base (Difco,France)**

Typical formula (g/l)

| | |
|-------------------------|------|
| Proteose Peptone..... | 10.0 |
| Beef Extract..... | 1.0 |
| Sodium Chloride..... | 5.0 |
| Bromocresol Purple..... | 0.02 |
| Agar..... | 15.0 |

Final pH 6.8±0.2at 25° c

Instruction for use:

Suspend 31g of the powder in one liter of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. When preparing 0.5-1% carbohydrate fermentation, agar dissolve 5-10g of the desired in the basal medium prior to sterilization by autoclaving.

❖ **Brain heart infusion 500g** (HIMEDILA Laboratories Pvt. Ltd.23, VadhaniInd.Est.,LBS Marg. Mumbai-400 086,India, M210-500G)

Typical formula (g/l)

| | |
|---------------------------------|------|
| Calf brain infusion solids..... | 12.5 |
| Beef heart infusion | 5.0 |
| Peptose pepetone..... | 10.0 |

Sodium Chloride.....5.0

Di-Sodium phosphate.....2.5

Glucose.....2.0

Final pH 6.8±0.2at 25°c

Instruction for use:

Dissolve 37g in 1 liter of distilled water and distribute in to final containers. Sterilize by autoclaving 121°C for 15 minutes.

❖ **Muller Hinton Agar** (Deben Diagnostics Ltd 500g pack)

A susceptibility test medium recommended by NCCLS.(2012)

Beef infusion solids2.0 g

Acid Hydrolysate of casein.....17.5g

Starch.....1.5g

Agar.....17g

Final pH: 7.4 ± 0.2 at 25°C

Instruction for use:

Suspend 38g of the powder in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil to dissolve the powder completely. Autoclave at 121°C for 15 mints.

DNase test agar (Oxoid, England)

Typical Formula (g/l)

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

Final pH: 7.3 ± 0.2 at 25°C

Instruction for use

Suspend 42 g of the medium in one liter of distilled water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes.

Appendix V: O-F basal media test

The formula of O-F basal media (HIMEDIA Laboratoris Pvt.Ltd.23, Vadhani Ind.Est.,

LBS Marg.Mumbi-400 086, M 395-500G)

Typical formula (g/l)

| | |
|---------------------------------|------|
| Casein enzymic hydrolysate..... | 2g |
| Sodium Chloride..... | 5.0g |
| Agar..... | 2.0 |
| Dipotassium Sulphate..... | 0.30 |
| Bromothymol blue..... | 0.08 |

Instruction for use

Dissolve 9.4g in 1 liter distilled water and distribute into final containers. Sterilize by autoclaving at 121c for 15 minutes.

Appendix VI: procedure for coagulase test (ES ISO 6888-2, 2002)

1. Using a sterile pipette, add 0.3 ml of the rehydrate plasma to a 12×75mm test tube.
2. Using a sterile serological pipette add 0.1ml of the overnight broth culture of the test organisms to the tube of plasma or using bacteriological loop, thoroughly emulsify 2-4 colonies (loopful) from anon inhibitory agar plat in the tube of plasma.
3. Mix gently and Incubate at 37°C.
4. Examine periodically for coagulation by gently tipping the tube after the first hour and once every hour thereafter until four hours elapsed. If no clot is observed at the end of this period, at 24 Hours avoid shaking or agitating the tube during reading. Doubtful or false-negative results may occur due to breakdown of the clot.
5. Record results: Positive- any degree of clotting-from a loose clot suspended in plasma to a solid clot that is immovable when the tube is inverted. –Negative – no degree of clotting

Appendix VII: procedures for oxidase test

1. Prepare a solution of 1% tetramethyl-p- phenylenedihydrochloride,
2. Piece of filter paper is moistened in a Petri dish with fresh reagent
3. The test bacterium is streaked firmly across the filter paper with a glass rod
4. A dark purple color along the steak line with in 10 seconds indicates a positive reaction. *Pseudomonas aeruginosa* can be used as a positive control organism.

Appendix VIII: procedures for catalas test

1. Place a drop of 3% H₂O₂ on a glass slide.
2. Touch a sterile loop to a culture of the organism to be tested and pick up a visible mass of cells(colony).
3. Mix the organism in the drop of hydrogen peroxide .
4. Observe for immediate and vigorous bubbling.

Interpretation: Bubbling indicates a positive test and no bubbling indicates a negative test.

Appendix VIII: procedure of Gram's staining

1. Prepare the smear and heat fix.
2. Stain with crystal violet for 60 seconds and rinse with tap water and drain
3. Flood the slides with iodine and allow to remain 60 second and rinse with tap water and drain
4. Decolorize with 95% ethanol until 15 second and rinse with tap water and drain
5. Counter stain with safranin for 60 seconds and rinse with tap water and drain
6. Examine microscopically under oil immersion

Appendix IX: Buffered peptone water (Oxoid, England)

Composition (g/l)

Peptone10.0

Sodium chloride5.0

Final pH 7.5 ± 0.2 (at 25 0C)

Preparation

15 g of the powder was dissolved in 1 liter of distilled water. Stirred and dissolved completely. Then, sterilized by autoclaving at 121 0C for 15 minutes after dispensing into the test tubes.