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ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCUS SPECIES FROM READY-TO-EAT MEAT PRODUCTS IN AND AROUND DEBRE-ZEIT.

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment for the requirements for the Degree of Master of Veterinary in Tropical Public Health

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DEBRE-ZEIT, ETHIOPIA

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LIST OF ABBREVIATIONS

a_w	Water activity
BHI	Brain Heart Infusion
BPA	Baird Parker Agar
BPW	Buffer Peptone Water
CDC	Center for Disease Control
CNS	Coagulase-Negative Staphylococcus
CPS	Coagulase-Positive Staphylococcus
CSA	Central Statistical Authority
FBD	Food Borne Diseases
FDA	Food and Drug Administration
ICMSF	International Commission on Microbiological Specification for Food
IOS	International Organization for Standardization
MHA II	Muller-Hinton Agar II
MSA	Manitol Salt Agar
NCCLS	National Committee for Clinical Laboratory Standards
NIAID	National Institute of Allergy and Infectious Diseases
OIE:	Office International des Epizooties.
PCA	Plate Count Agar
RTE	Ready-To-Eat
SEs	Staphylococcal Enterotoxins
TSST-1	Toxic Shock Syndrome Toxin-1
TVC	Total Viable Counts
USDA	United States Department of Agriculture
WHO	World Health Organization

ABSTRACT

Worldwide, millions of people suffer from communicable and non communicable diseases caused by contaminated foods. Very young, elderly and immunocompromised people do suffer more and many fatalities occur due to foodborne diseases and toxicity due to food poisoning. There are many organisms involve in food poisoning, among which *Staphylococcus* species are of importance and more frequently encountered organisms in food toxicity. The present study was undertaken i) to identify the factors which are responsible for foodborne diseases in hotels, restaurants and cafes in and around Debre-Zeit by questionnaire survey and personal observation of the eating of establishments ii) to isolate and identify *Staphylococcus* species from ready-to-eat meat products iii) to determine the antibiotic sensitivity of the Staphylococci isolates from the ready-to-eat meat products. This study was undertaken during October 2006 to March 2007 as a cross-sectional study. In the present study, all 57 eating establishments i.e. hotels (23) restaurants (27) and cafes (7) present in and around Debre-Zeit were included and only 22 managers were respondend to the questionnaire survey. It was observed that one of food handlers in these establishments 85 were female and the rest were male and only 59.1%wore apron only, 36.4% apron with hairnet and 4.5% with apron and shoes. The general cleanliness of the premises, kichen and food storage areas in the eating establishments were graded as good (15), satifactory (25) and not good (17). The meat and meat products were supplied to these places by local butchers (63.6%), municipal abattoir (13.6%), bought from local market and/or by slaughtering sheep and goats in their backyard (13.6%) and 4.5% obtained from export abattoir. Only 17 of the 22 managers interviewed informed that they are aware of knowledge of food poisoning and 5 managers had no knowledge about it. Only 68.2%workers undergone monthly, 18.2% quarterly and 9.1% annual medical check up. Regarding inspection of the premises by sanitary inspectors were done weekly (27.3%), monthly (27.3%), quarterly (36.4%) and had no inspected by them (9.1%). A total of 384 samples of ready-to-eat meat products i.e. burger (47), cooked ground meat (103), roasted meat (116), roasted chicken (19) and siga wait (99) were collected for the isolation of *Staphylococcus* species by using standard bacteriological techniques. Among these *S.aureus* (41), *S.intermedius* (33), Coagulase-Negative Staphylococcus (22) and *S.hyicus* (15) species were isolated. All the five varieties of samples tested yielded *Staphylococcus* species i.e. burger (25.5%), cooked ground meat (30.1%), roasted meat (29.9%), roasted chicken (36.8%)

and siga wet (27.3%). All there isolates (111) were subjected to antibiotic sensitivity and found that polymyxin-B was most sensitive, followed by norfloxacin, trimethoprim, gentamycin and neomycin; where as ampicillin was most resistant to the present isolates. In view of the above results, *Staphylococcus* species are present in most of the ready-to-eat meat products in eating establishments of Debre-Zeit and may pose potential sources of food toxicity to consumers. The present isolates were only sensitive to higher antibiotics. The implications of *Staphylococcus* species in read-to-eat meat products on human health was discussed and recommendations were forwarded to conduct further research on enterotoxins produced by *Staphylococcus* species and their effect on public health, proper legislation to monitor eating establishments regarding to serve and store hygienic food in hygienic environment.

Key words: Coagulase, Enterotoxins Food products, Meat, *Staphylococcus*

1. INTRODUCTION

Worldwide, millions of people suffer from communicable and non-communicable diseases caused by contaminated food. These diseases take a heavy toll in human life and suffering, particularly among infants, children, elderly and other susceptible persons. They also create an enormous social, cultural and economic burden on communities and their health system (Van der Vanter, 1999).

The Centre for Disease Control estimates that each year in the USA, 76 million people get sick, more than 300,000 are hospitalized, and 5,000 very young, elderly and the immuno-compromised Americans die as a result of foodborne illness. Recent changes in food production and distribution system, microbial adaptation, and lack of support for public health, resources and infrastructure have lead to the emergence of naive as well as traditional foodborne diseases (NIAID, 2005).

The true incidence is difficult to evaluate, since many countries do not have an epidemiological surveillance system in place, and where a system exists, mild and sporadic cases are not usually reported. In countries with a reporting system, the number of outbreaks has increased considerably in recent years (Acha and Szyfres, 2001

International partnerships are also important when investigating outbreaks involving multiple countries, which are becoming more common with the ever-increasing global movement of food and people. Improved surveillance can detect outbreaks, even if they are dispersed over a broad area (Andrea *et al*., 2004). Food illness of microbial origin that is caused by the consumption of food has been recognized as a major threat to public health. The problem is highly aggravated in the developing world (Isaacson *et al*., 1999).

Foodborne diseases are global public health problem and their implication are great for health and economy and they cause wide variety of illness and sometime even cause mortality in human beings. The survival of spores through the cooking process, germination, proliferation and production of toxins in food are responsible for human foodborne diseases and source of the causative microorganisms.

To date, around 250 different food-borne diseases have been described and bacteria are the causative agents of two-thirds of foodborne disease outbreaks. Among the predominant bacteria involved in these diseases are S*taphylococcus aureus,* which is a leading cause of gastro-enteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxin preformed in the food (Yves *et al*., 2003). Although foodborne illnesses present typically with gastro-intestinal symptoms including nausea, vomiting, abdominal pain, and diarrhea but it is important to remember that the disease can present in various forms i.e. fever, neurologic symptoms etc. (David, 2002). Staphylococcus species are widely spread in various niches such as clinical environments; meat products, milk and dairy products, and food manufacturing plants, it has become increasingly important to identify the staphylococcus at species level.

There are many organisms, which cause foodborne diseases in human beings, among which Salmonella spp, *Listeria monocytogenes,* and *Clostridium perfringens* to *staphylococcus* species are of importance. Staphylococci are found to be normal flora and mucous membrane of warm-blood animals, but they are also isolated from a wide range of foodstuffs such as meat, cheese or milk and from environments source such as soil, dust, air or natural water (Kloos and Schleifer, 1986). The large numbers of these organisms tends to be found in the nasal passages, axillae and perineal areas (Kloos and Bannerman, 1994).

Staphylococci are able to multiply readily in many foods, however in ready meat products and dairy products are probably the most implicated. Contamination of food products by Staphylococcus species may occur during the phase of manufacturing and handling of final products (Rosec *et al*.; 1997 and Letertre *et al.*; 2003). Although growth usually is constrained by the presence of competing organisms, staphylococci thrive in environments relatively free of competition from other bacteria, such as foods with high concentration of salt and sugar that impede the growth of other organisms (ICMSF, 1996).

Countries like Ethiopia, where surveillance system for foodborne diseases does not exist and is difficult to estimate the amount of the cases. There is no specific concerned body to monitor and evaluate the incidence of the foodborne diseases. There are many areas within the food production chain, from the farm to retail establishment, where foods may be contaminated and/or mishandled. It is therefore, important for all areas of food production to be monitored. Therefore, the objectives of this study are:

- ✓ To determine the prevalence of *Staphylococcus* species in different ready-to-eat meat products in the study area of in-and-around Debre- Zeit.
- ✓ Isolation and identification of *Staphylococcus* species from ready-to-eat meat products.

2. LITERATURE REVIEW

2.1. Meat products commonly implicated in Foodborne *Staphylococcus* species

2.1.1. Meat and poultry products

The *Staphylococcus* species are the most important foodborne pathogens found in ready- to-eat meat products that can result in debiliting illness. Toxicity due to Staphylococcal enterotoxin is generally considered as mild, self-limited illness with low mortality. Normal temperatures used in cooking will not destroy the toxins, and foods containing staphylococcal enterotoxin usually look and taste normal (Merson, 1973).

In recent years various staphylococci-contaminated food products have been implicated in food-poisoning outbreaks (Uche and Agbo, 1985, Miwa *et al*., 2001 and Loir *et al*., 2003). In the 1990's, however, contaminated ready-to-eat meat products led to several outbreaks, some of which were highly publicized and had prefound epidemiological impact (Soriano *et al*.; 2002). Staphylococcal food poisoning is caused by ingestion of enterotoxin preformed in food contaminated essentially by human manipulation.

Although coagulase-positive *staphylococcus aureus* is the main agent responsible for food intoxication (Kloos and Bannerman, 1995), by several Staphylococcal species other than *S.aureus* reportedly produce Staphylococcal Enterotoxins (Jay, 1992). For example, among the coagulase negative species *S. cohnii*, *S.epidermidis*, *S.xylosus*, and *S.haemolyticus* have been found to produce one or several, Staphylococcal Enterotoxins (Bautista *et al.*, 1988).

Many family outbreaks of staphylococcal food poisoning are caused simply by the contamination of cooked meat such as ham and similar products by the hands of food handlers in shops and homes (Fitzgerald *et al*., 2001). As a result, about 90% of staphylococcal outbreaks of foodborne diseases can be traced back to a poultry and meat products (Hagstad and Hubbert, 1986).

Bean and Griffin, (1990) reported Staphylococcal gastroenteritis outbreaks due to poultry products (96), followed by bakery products (26), beef (22), turkey (20), chicken (14) and from eggs (9) in the USA during 1973-1987.

2.1.2. Staphylococcal Enterotoxins in food products

Staphylococcal enterotoxins are exoproteins which, when produced in food that is then ingested by humans, gives rise to symptoms of acute gastroenteritis (Brun and Bes, 2000). Several types of Staphylococcus Enterotoxins have been identified on a serological basis and with SEA to SEE being the most frequently encountered (Adesiyun *et a*l., 1998, Balaban and Rasooly, 2000, Omoe *et al*., 2002, Letertre *et al*.; 2003).

These toxins usually produces by Coagulase-Positive Staphylococcus, mainly *Staphylococcus aureus* strains (Debuyser *et al*., 2001) Pyrogenic toxins include Staphylococcal Enterotoxins, Toxic Shock Syndrome Toxin, exfoliatins A and B, and Streptococcus Pyrogenic toxins. These toxins share same structure, function and sequence similarities. They have polygenetic relationships, and capable of more or less potent emetic activity (Balaban and Rasooly, 2000)

Enterotoxins are produced mainly by coagulase-positive Staphylococci, some coagulase-negative Staphylococci, involved in a variety of human and animal infection (Kloos and Bannerman, 1995). Very little is known about the growth of coagulase-negative Staphylococcus in foods. These strains have rarely been implicated in food poisoning because they do not grow rapidly in foods. Nevertheless coagulase-negative Staphylococci can contaminate foods because humans are common carriers of these microorganisms and may be related to specific human infection (Bergdoll, 1995). Coagulase-negative staphylococci are normal abundant colonizers of humans and become pathogenic only in certain situations. They are commonly isolated in clinical specimens and several species are recognized as important agents of nosocomial infections, especially in neonates (Becker *et al.*, 2001)

Toxic Shock Syndrome Toxin (TSST-1) is the causative agent in Toxic Shock Syndrome in humans, and enterotoxins (SEs), in addition to their ability to cause food poisoning (Kenney, *et al*.; 1993; Bezek and Hull, 1995). Drancourt and Raoult, 2002. reported that *S. aureus* was responsible for staphylococcal toxin-mediated food poisoning, toxic shock syndrome and for some pyrogenic infections.

2.2. Epidemiology

2.2.1. Source of infection

In developing countries, the source of infection is mainly the contaminated environment and water source where the animals are crowded together. The two most important sources to foods are nasal carries and individuals whose hands and arms with boils and carbuncles, and are permitted to handle foods by them (Acha and Szyfres, 2001).

The contamination of food products in processing plants by the *Staphylococcus* species has often been shown to result from food handlers and symptomatic carriers suffering from infected skin lesions (Desmarchelier *et al*., 1999; Mosupe and Holy, 2000; Nagase *et al,* 2002 and Nel *et al*., 2004).

Entry of the organisms into the food processing plant occurs through worker’s shoes and clothing, transporting equipment, raw foods of animal origin and healthy human carriers who handle food products (Rocourt and Cossart, 1997).

Whatever the source of food-poisoning bacteria, fault lies in preparation and storage and this lead to the multiplication of the organism in the cooked food (vehicles), which is the immediate cause of the outbreak. The organisms spread from raw to cooked foods or from persons to cooked food by their methods of food preparation and preservation (Hobbs and Gilbert, 1978).

2.2.2. Mode of transmission

Many foodborne microbes are present in healthy animals (usually in their intestines). Meat and poultry carcasses can become contaminated during slaughter by contact with small amounts of intestinal contents and similarly, fresh fruit and vegetables can be contaminated when they are washed or irrigated with water that is contaminated with animal manure or human sewage (WHO, 1984).

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. More common hazard arises through cross-contamination from raw to cooked meat or other foods (ICMSF, 1986).

Animal feeds are frequently contaminated by a variety of bacteria, which usually enter the feed mixture in the protein supplements like meat, bone, fish, and soyabean meals and they all have been shown to be frequently and heavily contaminated (Wray and Sojka, 1977).

The passage of bacteria from one food to another through the food handler is an important means of spread with the hands providing a way of transport. Contamination of food products by *Staphylococcus* species may occur during the phase of manufacturing and handling of final products (Berynestad and Granums, 2002)

2.3. Clinical Symptoms

Symptoms of foodborne illnesses are not pleasant and usually include one or more of the following: diarrhea, vomiting, headache, nausea, and dehydration. Foodborne illness is generally classified as an infection, intoxication, or a toxico-infection (FDA, 1992). The food poisoning is abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (never diarrhea alone) (Bergdoll, 1989).

was Consumption of food with preformed toxin usually leads to rapid (6-12 hours) onset of predominant upper gastro-intestinal symptoms and one of the organisms that produce such toxins are *Staphylococcus aureus* (Mead *et al*., 1999)

2.4 Factors required for *staphylococcus* species growth in food

Microorganisms, individually and as a group, grow over a very wide range of temperatures. The lowest temperature at which a microorganism has been reported to grow is at -34 ^{O}C and the highest is some where in excess of 100 ^{O}C (Novick, 2000). In general, bacteria require higher value water activity (a_w) for growth than fungi, and Gram-negative bacteria require more a_w than Gram-positive bacteria (FDA, 1992).

The *Staphylococcus* species grow in the temperature range of 7 ^{O}C to 48 ^{O}C and produce enterotoxin from 10 ^{O}C to 48 ^{O}C, with optimum enterotoxin production at 40 ^{O}C to 45 ^{O}C. Although it is usually more luxurient under aerobic than under anaerobic conditions. Increasing CO_2 tension enhances multiplication of some strains. 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 (ICMSF, 1996).

Vegetables and pasta products have a high pH in raw state, become ideal for bacterial growth when heated (Jay, 2000). In order to grow and function normally, the microorganisms in food require the following: water, source of energy, source of nitrogen, vitamins and related growth factors and minerals.

Microorganisms causing food poisoning must be able to grow in the foods, which act as the vehicle of infection. Most bacteria grow at varying rates at 37 ^{O}C the temperature of the human body. Much of the food eaten by man is favorable for growth of bacteria. Staphylococci can tolerate more salt than other pathogenic bacteria (Fung *et al*., 1985).

2.5. Public Health Significance

Foodborne diseases are extremely costly. Health experts estimate that the annual cost of foodborne disease in the United States is about 5 to 6 billion dollars in direct medical expenses and loss of productivity. People infected with foodborne microbes may have no symptoms or develop symptoms ranging from mild intestinal discomfort to bloody diarrhea. Foodborne illness not only affects the health of individuals who become ill, but it can also have dramatic economic impact to the eating establishment (FDA, 1992; NIAID, 2005).

The practice of backyard slaughter of morbid animals or even dead animals for either human consumption or skin trade has contributed to the outbreaks in man and animals (Soulsby, 1982). Foodborne diseases are among the most widespread global public health problems of recent times, and their implication for health and economy is increasingly recognized (Van der Vanter, 1999). The survival of the spores through the cooking process, germination, proliferation and production of toxin in food, are responsible for human sickness. (Rocourt, 1996).

In developing countries like Ethiopia where surveillance system for foodborne diseases is not existent and it is difficult to estimate the problem. Even in countries where surveillance services are very efficient, the precise incidence of food poisoning is not known, as outbreaks are often not reported to Public Health Authorities (Jay, 2000)

2.6. Diagnosis

2.6.1 Clinical consideration

Foodborne illnesses are considered to be any illness that is related to food ingestion; gastro-intestinal 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 an outbreak (NIAID, 2005).

2.6.2. Bacteriological techniques used in the diagnosis of *staphylococcus* species in food

Currently available methods for detection and identification of bacteria in food samples are conventional bacteriological methods, which are compared and validated. These methods are usually very sensitive and they do not require sophisticated and expensive equipment. For food and environmental samples, the use of selective media and suitable growth conditions are provided and other enrichment methods are employed to limit the growth of contaminating microorganisms to reasonable numbers and multiplication of foodborne bacteria to a level that are enough for their detection (OIE, 2004).

The Staphylococcal organisms were isolated by inoculating suitably diluted food samples by spread plate method (Herbert, 1990, Nortje' *et al*., 1999); for enumeration of Total Viable Counts by using different media and incubation temperatures (Nikanenen and Aalto, 1978, Vanderzant and Splittstoesser, 1992); presumptive diagnosis of Staphylococcus organisms by inoculing on Mannitol Salt Agar (Blair *et al*, 1967), and also differentiating from Coagulase-Positive Staphylococcus from Coagulase-Negative Staphylococcus (Dugud,1989) and confirmed by using rapid latex- agglutination test (Perssnne *et al,* 1997, Van- Griethujsen *et al*, 2001).

2.7. Prevention and Control

The best way to prevent foodborne illnesses is by consuming the food as soon as it is cooked and the left overs can be preserved by refrigeration (Hobbs and Gilbert, 1978).

Prevention of foodborne outbreaks of staphylococcal intoxication include: good sanitation to prevent contamination, especially by food handlers, persons with purulent lesions containing should not be permitted to handle food until proper medical advice is sought and storing foods at proper temperature (Hagstad and Hubbert (1986)

Comprehensive educational program for the consumer and food handler, both in commercial establishments, about the origin and personal and environmental hygiene is of paramount importance (WHO, 1988).

NIAID (2005) has advised some basic ways to prevent most foodborne diseases and they are listed below:

- ✓ Wash hands carefully before preparing food.
- ✓ When handled with raw meat or poultry, wash hands, utencils, and kitchen surfaces with hot soapy water.
- ✓ Cook beef and beef products thoroughly, especially burger.
- ✓ Cook poultry meat thoroughly.
- ✓ Eat cooked food promptly and refrigerate leftovers within 2 hours after cooking.

A few simple precautions can reduce the risk of foodborne disease as indicated by CDC, (2005).

Cook: Meat, poultry and eggs thoroughly.

Separate: Do not cross-contaminate one food with another.

Chill: Refrigerate leftovers promptly.

Clean: Wash and rinse fresh fruits and vegetables in running tap water to remove visible dirt and grim.

Report: Report suspected foodborne illness to the local health department.

3. MATERIALS AND METHODS

3.1. Study area

The study area was in and around Debre-Zeit, which is located 45 km south east of Addis Ababa. The area is located at 9^O N latitude and 40^O E longitude at an altitude of 1850 Mts. It has an annual rainfall of 866 mm, which occurs from June to September and the dry season extends from October to February. The average temperature ranges from 14 ^{O}C to 26 ^{O}C with a relative humidity of 61.3 %(CSA, 2001).

3.2.1. Questionnaire survey

A questionnaire was prepared and interviewed the hotel and restaurant managers to evaluate the effect of predisposing factors that contribute to the prevalence of *Staphylococcus* species in ready-to- eat meat products in-and-around Debre-Zeit.

3.2.2. Study design and sample size

To determine the prevalence of Staphylococcus species in ready-to-eat meat products, a cross-sectional study type of investigation was conducted in hotels, restaurants and cafes in-and-around Debre-Zeit town from October 2006 to March 2007. The sample size was determined as per the recommendation of International Standard Organization (ISO, 6888-1: 2002) for the detection of *Staphylococcus* species

3.2 3. Sample collection

All the 57 public retail establishments of hotels, restaurants and cafes in-and-around Debre-Zeit were included in this study. A total of 384 samples of ready-to-eat meat products, i.e. Burger, Cooked ground meat, Roasted meat, Roasted chicken and Siga wet were collected from hotels, restaurants and cafes. These ready-to-eat meat samples in the present study were chosen to know the prevalence of *Staphylococcus* species in these foods, since these were the most popular food items sold in all the hotels and restaurants in-and-around Debre-Zeit. Minced beef was supplied by local butchers and dressed chicken by local poultry farms to the hotels and restaurants in-and-around Debre-Zeit. The samples were collected between October 2006 to Marh, 2007. The specific ingredients for these foods are detailed in Annex. 2. All the 384 samples were aseptically collected into a sterile screw capped sample bottles and kept in an icebox containing ice packs. Samples were labelled for identification, type of sample, code of eating establishments and sampling date and transported to the Microbiology laboratory of the Faculty of Veterinary Medicine, Debre-Zeit, and processed the samples following day.

3.3. Study methodology

3.3.1. Cultural procedure

Isolation and identification of *Staphylococcus* species from ready-to-eat meat products was done according the methods described by Carter, 1984 and Quinn *et al.*, 1999. A 25g of food sample was transferred to a sterile Stomacher (Lab. Seward 400) bag and 50 ml of sterile Buffer Peptone Water (BPW) (Oxoid) was added. The sample was homogenized in a stomacher for 2 min. Then 10-fold dilution of the sample was prepared with sterile BPW, and 0.1 ml portion of various dilutions were spread on Blood Agar Plates (5% difibrinated sheep blood) and the plates were incubate aerobically at 37 ^{O}C and examined after 24 h of incubation for growth. The colonies provisionally identified on the basis of staining reaction with Gram's stain, morphology and hemolytic pattern (typical and atypical). The representative colonies were subcultured on Blood Agar Plate and on nutrient slants and incubated at 37 ^{O}C. The slant was preserved and maintained for characterizing the isolates.

Gram's staining

All the suspected cultures of *Staphylococcus* species were staining by Gram's stain and observed under microscope for Gram's reaction, size, shape arrangement etc.

Catalase test

The culture to be tested for catalase test was picked up by bacteriological loop from the agar slant and mixed with a drop of 3% hydrogen peroxide on a clean slide. If the organism is positive, effervescence of oxygen is liberated within a few seconds and the catalase negative culture will not produces effervescence. Those positive cocci were considered as Staphylococci.

Manitol Salt Agar

The colonies that were confirmed by staining reaction and catalase test were streaked on MSA plate and incubated at 37 ^{O}C and examined after 24-48 h for growth. The presence of growth and change of pH in the media (red to yellow color) regarded as presumptive identification of *S.aureus* of Coagulase-positive Staphylococcus.

Coagulase test

Coagulase test was determined by the method described by (ISO, 6888-2:2002). This test was preformed as a tube coagulase test. The selected Staphylococcus was subcultured into Brain Heart Infusion broth and incubated 37 ^{O} C 24 h. Then 0.1 ml of broth culture and 0.3 ml of sterile rabbit plasma were put into a narrow sterile tube along with a control tube containing a mixture of 0.1 ml of sterile BHI broth and 0.3 ml of rabbit plasma were incubated at 37 ^{O}C and examined after 4 h and 24 h of incubation and observed for the clot formation. Any coagulation of plasma was regarded as positive at either of the readings when compared to the control.

Purple Agar Base

This test was carried out by using commercially available Purple Agar Base (Difco) with the additional one percent maltose to differentiate the pathogenic staphylococci particularity with coagulase-positive with other Staphylococci. The suspected culture was inoculated on PAB media plate with 1% of maltose and incubated at 37 ^{O}C for 24 h. Rapid fermentation of maltose by *S. aureus* caused yellow discoloration of the medium due to change in pH (Figure 1). The acidic metabolic product of maltose was detected by bromocresol purple indicator incorporated in the medium. Colonies that develop weak or delayed yellow color after 24 hours of incubation were considered as *S. intermedius* (Figure 2) and colonies that did not produce any change on the medium were taken as *S.hyicus* (Figure3).

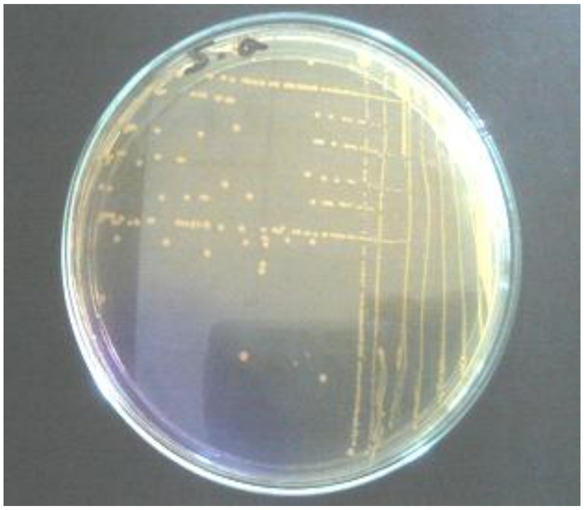

Figure 1: Maltose fermenting *S. aureus*

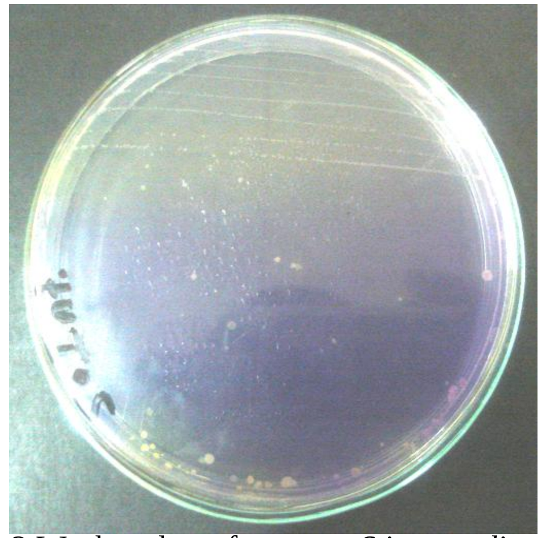

Figure 2:Weak maltose fermenter *S.intermedius*

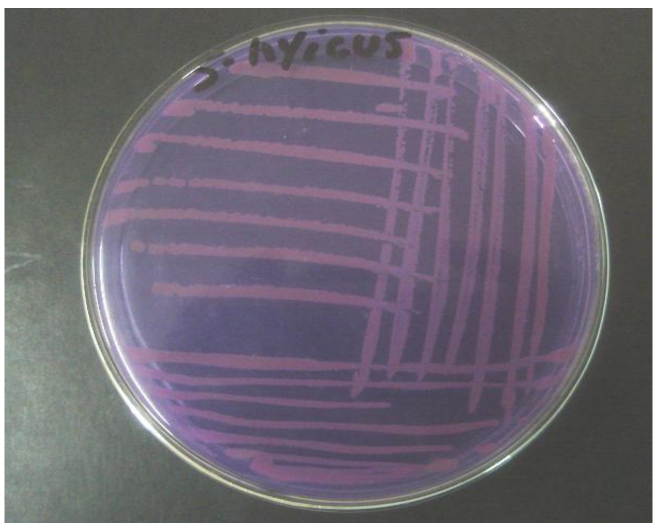

Figure 3: Non maltose fermenting ***S. hyicus***

Anti-microbial susceptibility testing

The Staphylococcal cultures isolated from ready-to-meat products in the present study were tested for anti-microbial susceptibility by disc diffusion method (Kirby-Bauer *et al.,* 1966). The following antibiotics were used for testing: ampicillin (10μg), erythromycin (15μg), streptomycin (10μg), gentamycin (10μg), tetracycline (30μg), norfloxacin (10μg), trimethoprim (25μg), chloramphenicol (30μg), polymyxin-B (300units/disc) and neomycin (30μg/disc). Antibiotic discs used were from Oxoid, Hampshire, England company. Colonies of 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 using sterile saline or adding more isolated colonies to obtain turbidity visually comprobale with that of 0.5 McFarland standards. Muller-Hilton Agar (MHA) plate was prepared, and a sterile cotton swab was dipped into the Tryptone Soya Broth culture and swabbed on the surfaces of MHA plate. Then the antibiotics discs were placed mounted on the agar plate using sterile forceps and pressed gently to ensure the complete contact with the agar surface. The plates were read 24 hours after incubation at 37^{O}C under aerobic condition. The isolates were classified in accordance with the guideline of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) as susceptible, intermediate or resistance for each antibiotic tested according to the manufacture's instructions by measuring the zone of inhibition around the antibiotic disc.

3.4. Data management and analysis

Microsoft Excel was employed for data entry, computation of descriptive statistics and drawing of graph. Descriptive Statistics such as percentages and proportions were applied to compute some of the data. Prevalence of staphylococcus species in each meat product was computed as the number of food samples positive for staphylococcus divided by the number of samples examined in each food item. The overall prevalence of *Staphylococcu*s spp. divided by the total number of samples examined.

4. RESULTS

4.1. Questionnaire survey

In the questionnaire survey, 22 managers of the 57 eating establishments i.e. hotels 23, restaurants 27 and 7 cafes were participated in the interviews. Of these 22 managers, 10 were from hotels and 12 from restaurants in-and-around Debre-Zeit. These establishments were observed for hygienic practices, cleaniness of the premises, kitchen, personal hygiene and health status of workers, their protective clothing, inspection by sanitary inspector, source of meat, knowledge of food poisoning etc.

In the present study, it was observed that 85.9% of the total food handlers (85) were female and the rest were male (14). Regarding wearing of protective clothing by the workers, 59.1% wore apron only; 36.4% (8) apron with hairnet and 4.5% (1) apron with shoes.

Regarding the general cleanliness of premises, kitchen and food storage area in the eating establishments, 15 (26.3%) were good; 25 (43.9%) satisfactory and 17(29.8%) were not good. The meat and meat products were supplied to these establishments by local butchers 68.2%; municipal abattoirs 13.6%; 13.6% bought from local market and by slaughtering sheep and goats in their backyards and 4.5% were obtained from abattoir enterprises (export abattoir). A total of 77.3% managers responded that they have the knowledge of food poisoning and its clinical signs in people and 22.7% had no idea about this.

When enquired about the health status of workers and whether they had undergone regular medical check up or not 68.2% informed that they had undergone monthly medical check up; 18.2% quarterly check up; 9.1% annual check up 4.5% had not undergone any medical check up. Regarding the inspection of eating establishment by the sanitary inspectors, 27.3% weekly, 27.3% monthly, and 36.4% quarterly inspected and 9.0 % informed their establishments were not inspected by the inspectors (Table 1).

Table 1. Details of questionnaire survey

Source		Hotels	Restaurants	Total
Number of managers interviewed		10	12	22
Food handlers	Male	11	3	14
	Female	36	49	85
Medical check up of workers	No	1	-	1
	Monthly	7	8	15
	Yearly	1	1	2
	Quarterly	1	3	4
Wearing of Protective clothing	Apron only	6	7	13
	Apron with hairnet	4	4	8
	Shoes with apron	-	1	1
Source of meat and meat Products	Local butcher	4	11	15
	Export abattoir	1	-	1
	Municipal Abattoir	2	1	3
	Local market	3	-	3
Knowledge of food poisoning	Yes	8	9	17
	No	2	3	5
Score for hygiene in the eating establishments	Good	7	8	15
	Satisfactory	9	16	25
	Not good	7	10	17
Visit of sanitary inspector	Weekly	2	4	6
	Monthly	4	2	6
	Quarterly	3	5	8
	No	1	1	2

4.2. Prevalence of *Staphylococcus* species in ready-to-eat meat products

In the present study, all the five ready-to-meat products tested were positive for *Staphylococcus* species. Out of a total 384 ready-to-meat meat products tested, 12 of the 47(25.5%) burger; 31 of the 103(30.1%) cooked ground meat samples; 34 of the 116(29.3%) roasted meat; 7 of the 19(36.8%) roasted chicken samples and 27 of the 99(27.3%) siga wet samples yielded *Staphylococcus* species. (Table. 2)

Table 2. Distribution of *Staphylococcus* species in different ready-to-eat meat Products from eating establishments in-and-around Debre-Zeit

Meat products	Number of samples tested	Number of samples positive for *Staphylococcus* species
Burger	47	12(25.5%)*
Cooked ground meat	103	31(30.1%)
Roasted meat	116	34(29.3%)
Roasted chicken	19	7(36.8%)
Siga wet	99	27(27.3%)
Total	384	111(28.9%)

*Number in parenthesis indicate percentage positive

4.3. Distributions of *Staphylococcus* species in ready-to-eat meat products.

A total of 111 *Staphylococcus* species were identified and *Staphylococcus aureus* was found to be the most predominantly isolated species 41 (36.9%) followed by *S. intermeduis* 33 (29.7%), Coagulase-Negative Staphylococcus 22 (19.8%) and *S.hyicus* 15 (13.5%). Among these species 89(80.2%) were Coagulase-Positive *Staphylococcus* species and 22(19.8%) for Coagulase-Negative *Staphylococcus* species isolated from meat products and species isolates for each ready-to-eat meat products. (Table. 3).

Table 3. Distribution of *Staphylococcus* species in different meat products

Meat products	*S aurues*	*S.intermedius*	Coagulase-Negative Staphylococcus	*S. hyicus*
Burger	6(50%)	3(25%)	3(25%)	0
Cooked ground meat	13(41.9%)	11(35.4%)	5(16.1%)	2(6.5%)
Roasted meat	11(32.4%)	12(35.3%)	8(23.8%)	3(8.8%)
Roasted chicken	1(14.3%)	0	0	6(85.7%)
Siga wet	10(37.0%)	7(25.9%)	6(22.2%)	4(14.8%)
Total number of species isolates	41	33	22	15

Distribution of *Staphylococcus* species in ready-to-eat meat products in present study is given Figure 4. It was observed that all the four types of Staphylococci were present in cooked ground meat, roasted meat and Siga wet were as in burger except *S.hyicus* all other there types were present. In case of roasted chicken, only *S. aureus* and *S.hyicus* were isolated. Cooked ground meat yielded more Staphylococci than other products tested. The *S.hyicus* was more in roasted chicken when compared to other products.

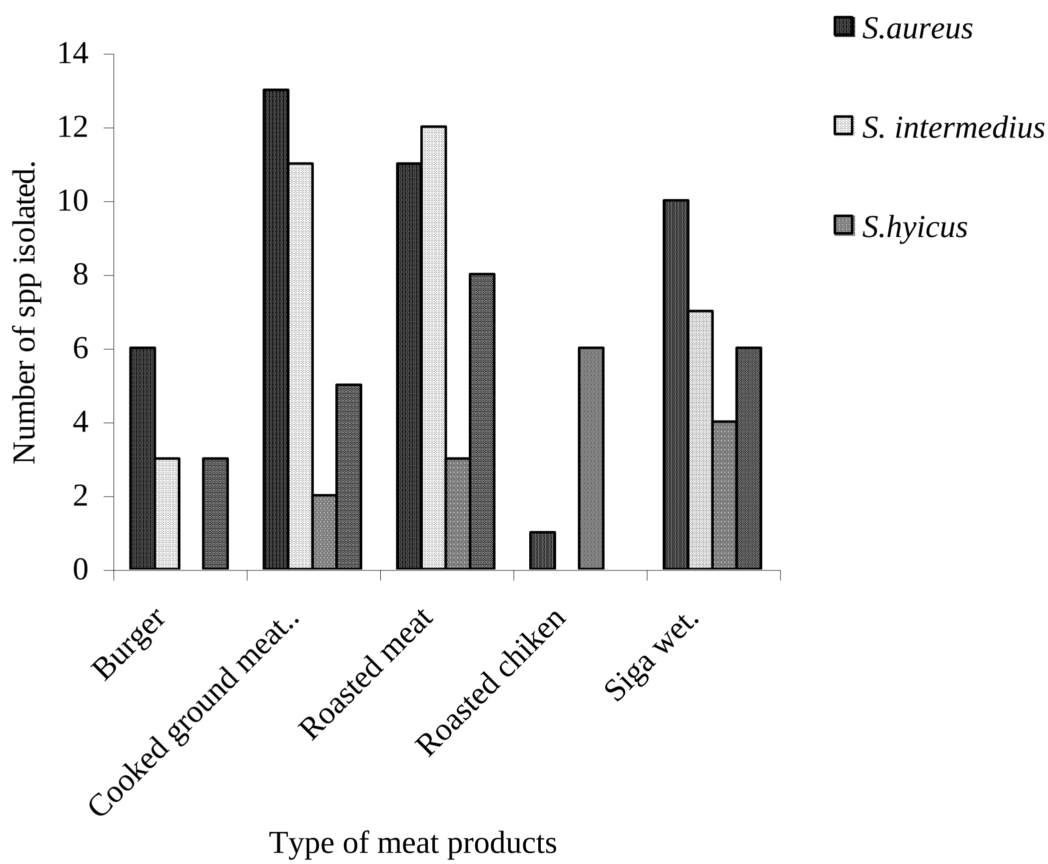

Figure 4:Distribution of different *Staphylococcus* species isolated from ready-to-eat meat products.

4.4. Anti-microbial susceptibility

In the present study, all the 111 isolates of Staphylococcus species comprising *S.aureus* (41), *S.intermedius* (33), *S.hyicus* (15) and Coagulase-Negative Staphylococci (22) were tested for their susceptibility to 10 antibiotics. It was observed that polymyxin-B was most sensitive (96.4%), followed by norfloxacin (95.5%), neomycine (91.0%), gentamycin (89.2%) etc. and others ranged from 80.2% to 84.7% in their sensitivity. The least sensitive antibiotic was ampcillin (26.1%) (Table. 4).

Table 4. Overall antibiotic susceptibility pattern of 111 isolates of *Staphylococcus* species from ready-to-eat meat products

.

Antibiotics tested	Overall sensitivity	% Overall sensitivity
Ampicillin	29	26.1
Chloramphenicol	89	80.2
Erythromycin	94	84.2
Gentamycin	99	89.2
Neomycine	101	91.0
Streptomycin	93	83.8
Trimethoprim	100	90.1
Norfloxacin	92	82.9
Tetracycline	92	82.9

As for as the different species concerned in their susceptibility, S.aureus was most sensitive to polymyxin-B followed by erythromycin, norfloxacin, trimethoprim, gentamycin etc. and least sensitive to ampcillin.

The most sensitive antibiotics to *S.intermedius* was neomycin followed by polymyxin-B, norfloxacin etc and least sensitive to ampicillin (21.2%). All the 15 isolates *S. hyicus* were completely sensitive to polymyxin-B, chloramphenicol and norfloxacin followed by tetracycline gentamicin, neomycin, trimethoprim, streptomycin and erythromycin. This species also showed only 13% susceptibility to ampicillin.

Among the 22 Coagulase-Negative Staphylococcus species, there was 100% sensitivity to trimethoprim and norfloxacin, followed by polymyxin-B, streptomycin (95%), neomycin (91%), gentamycin and erythromycin (86%), chloramphenicol and tetracycline (82%) and ampicillin (73%). (Table 5).

Table 5. Species wise antibiotic sensitivity pattern

Antibiotics tested	*S.aureus*	*S.intermedius*	*S.hyicus*	Coagulase-Negative Staphylococcus
Ampicillin	4/41(9.8)	7/33*(21.2) **	2/15(13)	16/22(73)
Chloramphenicol	35/41(85)	21/33(64)	15/15(100)	18/22(82)
Erythromycin	38/41(93)	27/33(82)	10/15(67)	19/22(86)
Gentamycin	37/41(90)	29/33(88)	14/15(67)	19/22(86)
Neomycin	36/41(88)	32/33(97)	13/15(87)	20/22(91)
Polymyxin-B	40/41(98)	31/33(94)	15/15(100)	21/22(95)
Streptomycin	36/41(88)	25/33(76)	11/15(73)	21/22(95)
Trimethoprim	37/41(90)	28/33(85)	13/15(87)	22/22(100)
Norfloxicin	38/41(93)	31/33(94)	15/15(100)	22/22(100)
Tetracycline	33/41(80)	27/33(82)	14/15(93)	18/22(82)

* Number of isolates susceptible /number tested against that particular antibiotic.

** Percent sensitive to the antibiotic tested.

5. DISCUSSION

The surveillance of food for microbial contamination is vital for the protection of public health and consumer interests. The production of safe food also has important economic implications in an increasingly competitive global market. The general hygiene of the establishments health status of workers and their personal hygiene appear to be satisfactory in the hotels/ restaurants examined in the present study. However, source of meat for the meat products sold in these establishments may be responsible for the cross contamination of *Staphylococcus* species in the ready-to-eat meat products. This aspect was emphasized by ICMSF (1989), in which is stated that common hazard arises through cross-contamination from raw to cooked meat or other foods.

Generally Staphylococci may be expected to exist, at least in low number, in any food product that are of animal origin or in those that are handled directly, unless heat processing steps applied to effect their destruction (Nagase *et al*.; 2002; Euzeby, 2003). Many different food items can be good growth medium for Staphylococci, and have been implicated in Staphylococcal food poisoning (Bergdoll, 1989). The organisms that were isolated in the present study was important foodborne pathogens in humans, and causes diseases in domestic animals. The overall prevalence of *Staphylococcus* species in the ready-to-eat meat products procured from eating establishment was 28.9%. This result was lower than the *Staphylococcus* species recovery rate 58.6% from hotels, restaurants and cafes by Berynestad and Granums (2002), 49% in read-to-eat meat products by Payne and Wood (1974) and the 39% in meat products (Adesiyun, 1984) in Nigeria but higher than the 11.9% recorded by ICMSF (1996). Of the 384 meat products examined, a total of 111 *Staphylococcus* species were identified. Coagulase-Positive Staphylococcus species were the most frequently isolated species from the food items analyzed and accounted for 80.2% of the total isolates and the remaining 19.8% were identified as coagulase negative Staphylococci. Milne *et al*., (2002) reported 90% prevalence of Coagulase-Positive Staphylococcus species that is relatively higher than the present findings. The *Staphylococcus aureus* was found to be the most predominant of the Positive-Coagulase Staphylococcus species (36.9%) isolated followed by *S. intermeduis* (29.7%) and *S.hyicus* (13.5%), but the lower prevalence of *S. aureus* was reported in ready-to-eat meat products i.e. 3% by Valle *et al*., (1990) and 9.4% Soriano *et al*.,

(2002) which compared to the present finding. This made be done to cross contamination of different ready-to-eat products or raw meat with cooked products as suggested by ICSMF (1986) or must have originated from the throat, hands and nail of food handling persons (Hatakka *et al*., 2000; Mossel and van Net-ten, 1991). It is also to be noted that *Staphylococcus aureus* is the most resistant non-sporalating bacteria to most of the physical and chemical agents and common inhabitant of the throat, dust etc in the houses (Duguid, 1989) and this may be the reason that all the products to yield this organism in the present study.

The 29.7% frequency of *S.intermedius* in the current study was higher than the findings recorded in other countries. The *S.intermedius* species was isolated from cooked meat products at the rate of 2.6% by Brun and Bes, (2002) 9.4% by Euzeby, (2003) and 13.3% By Becker *et al*.;(2001) but nearer to present prevalence rate was the reported by Adesiyun *et al*., (1998) from cooked meat products. Khambaty *et al*., (1994) in the present study also 30% of the isolates of *S. intermedius* were from all the products except roasted chicken and may play a role in causing outbreaks of food poising. The coagulase-positive *S.intermedius* was the predominant non-*S.aureus* species isolated from foods, which was the only species has clearly involved in staphylococcal food poisoning outbreaks.

In this study *S.hyicus* was isolated and identified from different meat products at the prevalence rate of 13.5% except burger Kiss *et al.*, (1997) and Lilenbaum *et al*., (1987) have reported that *S.hyicus* is found most predominantly in milk and poultry products. Some authors have described its low distribution of in food products 0.2% (Hoover, 1983) and 2% (Balaban and Rasooly, 2000).

Coagulase- Negative Staphylococcus was the third most frequently isolated species in the current study. The present result was higher than the 3.4% as reported by Udo *et a*l., (1999). The coagulase-negative Staphylococcus inhabits the skin and mucous membranes of human beings and these microorganisms can contaminate foods if not handled properly **(**Bergdoll, 1995). This may be due to frequently handling of food items by the workers and exposure of the products to outside environments in the present study.

In the present study *Staphylococcus* species was isolated from roasted chicken samples taken from the eating establishments at the rate of 36.8% which is higher than the 22% prevalence reported in the United Kingdom Wieneke *et al*., (1993) and in France the 9.5% by Haeghebaert *et al*., (2002) very low prevalence (3.5%) was reported in Croatia, from roasted chicken Bibely, *et al*., (1994). Differently from other food items, *S.hyicus* was found to be the most common contaminant of roasted chicken followed by *S.aureus,* may be due to frequent handling, improper roasting and exposure outside and not properly covering the cooked products.

In this study, 31(30.1%) of cooked ground meat samples were found contaminated with *Staphylococcus* species. In the study conducted in the United States on cooked ground beef sample the recovery rate of *Staphylococcus* species was found to be 12.4% (USDA, 1996). Gienigeorgis, (1989) reported the prevalence of 22% in cooked meat and 22.8% isolation rate from minced beef was reported in Croatia by Bibely, *et al.,* (1994). The higher prevalence rate of *Staphylococcus* species in cooked ground meat in this study may be due to improper cooking, poor personal, knives and cutting board hygiene. USDA (1996) reported that *S. aureus* was the most predominant species recovered at the rate of 30% from cooked ground meat. Adesiyun *et al*., (1998) reported 23.1% *S.intermedius* from cooked meat products, the present finding also indicated the similar prevalence rate.

In the current study, out of 116 sample of roasted meat 29.3% were contaminated with *Staphylococcus* species. The result of the present study was higher than the prevalence of 3.3% (USDA, 1996) and 3% (Ramesh *et al*., 2002) recorded in the United States. The higher contamination rate of roasted meat in the present study compared to the other findings in other countries may be associated with the use of same cutting boards in processing of raw meat that to be roasted and spices such as green pepper and onion that to be incorporated to the roasted meat without heat treatment.

Burger was found relatively less frequently contaminated food item (25.5%) as compared to other food items examined. This result was higher than the 16% prevalence reported by Cooney, *et al*., (1980).

Of the 99 "Siga wet" samples examined in the current study 27.3% were found contaminated with *Staphylococcus* species. This result is lower than the 84% prevalence rate in meats with spices recorded by Kneifel and Berger, (1994).

The difference in isolation of *Staphylococcus* species from the same food item in this study may be due to the difference in microbiological and sampling techniques employed, and the duration of study.

The most frequently found bacteria as the normal skin flora of dogs is *S.intermedius* and not common in other domestic animals. The high recovery rate of this species from ready-to- eat meat product in the current study may be associated with the cross transfer of the organism from dogs to meat producing animals, as farmers most commonly keep their domestic animals in close contact with dogs.

Antibiotic resistant bacterial strains are increasingly emerging worldwide because of in-descriminate use of antimicrobial drugs that result in significant public health problems (Hart and Kariuri, 1998). In the present study it was observed that polymyxin B was the most sensitive (96.4%) when compared to the other antibiotics tested. The similar result was reported by Devries (1990). This may be due to many of the broad spectrum antibiotics tested in the study are frequently used for treatment in animals and man.

The importance of this result to isolation of coagulase-negative *Staphylococcus* species from ready-to-eat meat products lies not only in the scientific aspects considering the scarcity of available data in this respect, and most of the data is available from the isolation of *Staphylococcus* species from bovine mastitis case and their susceptibility to antibiotics.

It is desirable to do more work on ready-to-eat products with respect to other organisms, in other locations to know the exact picture of microbial contamination, its possible impact on public health with respect to food poisoning, avoid outbreaks of food poisoning in people.

6 CONCLUSIONS AND RECOMMENDATIONS

The result of the present study indicated that

- ✓ All the five ready-to-eat meat products yield *Staphylococcus* specie.
- ✓ The most prevalence species are *S.aureus*, followed by *S.intermedius*, Coagulase-Negative Staphylococcus and *S.hyicus*.
- ✓ The Staphylococcal isolates were sensitive to most of the antibiotics tested and resistant to ampicillin.

Based on the above conclusions the following recommendation are forwarded:

- ✓ Owners, managers and food handlers and servers of eating establishments need awareness and training with regard to the preparation, storing, serving and in order to curb undesirable contamination.

- ✓ Identification and appropriate management of human carriers and workers of known foodborne pathogens who could transmit the foodborne diseases and toxicity in eating establishments.

- ✓ Food handlers should wear clean protective clothing and wash hands with soap and water before and after handling of food.

- ✓ All the ready-to-eat meat products from the eating establishments should be checked for other foodborne microorganisms and their toxins.
- ✓
- ✓ Hazard Analysis Critical Control Point System (HACCP) should be implemented to enhance food safety in the establishments.

- ✓ The government should have proper legislation to monitor the eating establishments regularly by the concerned public health authorities regarding methods of cooking, serving, and storing and their hygienic environment.

- ✓ There should be detailed epidemiological surveillance and reporting system to find out the situation of foodborne diseases in Ethiopia.

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8. ANNEXS

Annex 1 Questionnaire used for interviewing Hotel and Restaurant Managers

Date________

Code_________

Name of the manager________________________

Name of the Hotel/Restaurant_____________

Address__________________

FOOD HANDLERS

1. How many food handlers are in your Hotel or Restaurant?

Female______

Male________

2. Do they wear protective clothing?

Yes_______

No________

2.1. If yes, what type of protective clothing do they wear?

a) Apron only____________

b) Apron with hairnet______

c) Shoes with apron________

3. Would food handlers go to local health center for medical check up regularly?

a) Yes_______

b) No________

3.1. How many times do they go ?

a) Weekly_______

b) Monthly______

c) Quarterly ________

d) Yearly ______

SOURCE OF MEAT AND MEAT PRODUCTS

4. Supplied by

a) Local butcher______

b) Export abattoir______

c) Municipal abattoir_____

d) Slaughter of sheep and goats in backyard _____

INFORMATION ABOUT FOOD POISONING

5. Do you know any information on food poisoning in people ?

a) Yes____

b) No______

5.1. If yes, please tell any signs and symptoms of food poisoning

6. Does sanitary inspector visits your enterprise regularly?

6.1. If yes, what frequency do they visit?

a) Weekly_____

b) Monthly_____

c) Quarterly______

d) Yearly_________

GENERAL OBSERVATION ON CLEANLINESS OF THE PREMISES, KICHEN, FOOD STORAGE AREA ETC.

a) Good______

b) Satisfactory_____

c) Not satisfactory______

Annex 2 Ingredients for the preparation of ready-to-eat meat products collected for isolation of *Staphylococcus* species.

Meat samples	Ingredients
Burger	Minced beef, flour, wheat flour, egg, spices, salt, green pepper and vegetable oil
Cooked ground meat	Minced beef, butter, table salt, flour and pepper
Roasted chicken	Half part of chicken, chopped onions and vegetable oil
Siga wet	Chopped meat (sheep or goat), chopped onions, berbere, salt, oil and butter

Annex 3 Sample collection sheet for bacteriological analysis

		Type and number of samples collected						
No.	Sources	B	CGM	RM	RCH	SW	Total	Remark

B =burger CGM=cooked ground meat RM= roasted meat RCH=roasted chicken SW=Siga wet

Annex 4. Location of sampling hotels, restaurants and cafes, type and number of samples

Code	Source	Name	Type of sample	TNS	Location
1	Hotel	Bekel Mola	B=GM=RM=RCH	9	Debre-zeit
2	Hotel	Novel	B=CGM=RM=SW	11	Debre-zeit
3	Hotel	Bishoftu Plaza	B=CGM=RCH=SW	14	Debre-zeit
4	Hotel	Woll Gemtu	GM=RM=SW	7	Debre-zeit
5	Hotel	Dire	GM=RM=SW	5	Debre-zeit
6	Hotel	Zikala	GM=RM=SW	5	Debre-zeit
7	Hotel	K.B.	GM=RM=SW	6	Debre-zeit
8	Hotel	Temesgen	GM=RM=SW	6	Debre-zeit
9	Hotel	Seven-E	B=RM=GM=SW	14	Debre-zeit
0	Hotel	Genet	GM=RM=SW	6	Debre-zeit
11	Hotel	Beza	GM=RM=SW	5	Debre-zeit
22	Hotel	Wajra	GM=RM=SW	3	Debre-zeit
33	Hotel	Zoma	GM=RM=SW	5	Debre-zeit
44	Hotel	3G	GM=RM=SW	4	Debre-zeit
222	Hotel	ShuferochD/Z	GM=RM=SW	7	Debre-zeit
333	Hotel	Piramid	B=GM=RM=RCH	8	Debre-zeit
444	Hotel	BishoftuAffaf	B=GM=RM=RCH	8	Debre-zeit
55	Hotel	Ampollo	GM=RM=SW	3	Dukem
66	Hotel	Yerterara	GM=RM=SW	5	Dukem
77	Hotel	Misrakber	GM=RM=SW	5	Dukem
88	Hotel	Tinsha	GM=RM=SW	6	Dukem
99	Hotel	Werku Bikila	GM=RM=SW	6	Dukem
111	Hotel	Wendmamachoch	GM=RM=SW	6	Dukem

B=burger CGM=Cooked ground meat RM=roasted meat RCH=roasted chicken SW=Siga wet
TNS= Total Number of Sample

Annex. 4. Cont'd

Code	Source	Name	Type of sample	TNS	Location
12	Restaurant	Farmer'sHouse	B=GM=RM=SW	11	Debre-Zeit
13	Restaurant	Tommy-1	B=GM=RM=RCH	13	Debre-Zeit
14	Restaurant	Herna	GM=RM=SW	9	Debre-Zeit
15	Restaurant	Tommy-2	B=GM=RM=SW	12	Debre-Zeit
16	Restaurant	Farmy	B=GM=RM=SW=RCH	16	Debre-Zeit
17	Restaurant	Elson	B=GM=RM=SW	11	Debre-Zeit
18	Restaurant	Say	GM=RM=SW	8	Debre-Zeit
19	Restaurant	Tabbor	GM=RM=SW	7	Debre-Zeit
20	Restaurant	Tigist D/Z	GM=RM=SW	8	Debre-Zeit
21	Restaurant	Hany-Get	GM=RM=SW	7	Debre-Zeit
22	Restaurant	Abb	GM=RM=SW	8	Debre-Zeit
23	Restaurant	Shewa Cottage	GM=RM=SW	3	Debre-Zeit
24	Restaurant	A and Z	GM=RM=SW	10	Debre-Zeit
25	Restaurant	City	GM=RM=SW	5	Debre-Zeit
26	Restaurant	G.T.S.	GM=RM=SW	9	Debre-Zeit
27	Restaurant	Facil	GM=RM=SW	9	Debre-Zeit
28	Restaurant	Shuferoch	GM=RM=SW	6	Dukem
29	Restaurant	Debretsige	GM=RM=SW	6	Dukem
30	Restaurant	Liben	GM=RM=SW	6	Dukem
31	Restaurant	Haylu	GM=RM=SW	4	Dukem
32	Restaurant	Harermeda	GM=RM=SW	5	Dukem
33	Restaurant	Kelema	GM=RM=SW	6	Dukem
34	Restaurant	Tigist D.	GM=RM=SW	6	Dukem
35	Restaurant	Burk	GM=RM=SW	6	Dukem
36	Restaurant	Adea	GM=RM=SW	3	Dukem
37	Restaurant	Chuchu	GM=RM=SW	3	Dukem
38	Restaurant	Tenkir and Bet.	GM=RM=SW	6	Dukem

B=burger CGM=Cooked ground meat RM=roasted meat RCH=roasted chicken SW=Siga wet
TNS= Total Number of Sample

Annex. 4. Cont'd

Code	Source	Name	Type of sample	TNS	Location
39	Cafe	Rose	B=RM	5	Debre-Zeit
40	Cafe	Eyerusalem	B=RM	4	Debre-Zeit
41	Cafe	Eshetu	B=RM	3	Debre-Zeit
42	Cafe	Forest	B=RM	4	Debre-Zeit
43	Cafe	Abyssinia	B=RM	4	Debre-Zeit
44	Cafe	Genu	B=RM	3	Debre-Zeit
45	Cafe	Blen	B=RM	4	Debre-Zeit

B=burger CGM=Cooked ground meat RM=roasted meat RCH=roasted chicken SW=Siga wet
TNS= Total Number of Sample

Annex 5 Composition and preparation of media used the study

Blood Agar Base (Mark Germany)

Composition:

Nutrient substrate (heart extract and peptone) 20g

Sodium chloride 5g

Agar-Agar 15.0g

Preparation

40g were suspended in 1 liter of demineralized water by heating in a boiling water bath and autoclaved at 121 ^{O}C for 15 minutes. Cooled to 45-50 ^{O}C and 5-7% sterile defibrinated blood sheep was added and mixed taking care to avoid bubble formation poured to plates. pH 6.8±0.2 at 25 ^{O}C.

Buffered Peptone Water

Composision:

Peptone from casein 10.0g

Sodium chloride 5.0g

Di-sodium hydrogen phosphate 3g

Potassium dihydrogen phosphate 1.5g

Direction

Dissolve 20g in 1 liter distilled water, and sterilize by autoclaving at 121^{O}C for 15 minutes.

Nutrient Agar (Oxoid, England)

Composition:

Lab-Lemo powder 1.0g

Yeast extract 2.0g

Sodium chloride	5.0g
Agar	15.0g

Direction

20g of media were suspended in 1 liter of distilled water brought to boil to dissolve completely. Sterilize by autoclaving at 121^{O}Cfor 15 minutes.pH 7.4±0.2

Manitol Salt Agar

Ingredients

Protease peptone No. 3	10.0g
Bacto- Beef Extract	1g
D-manitol	10.0g
Sodium chloride	75g
Phenol Red	0.025g

Direction

To rehydrate, suspend111g in 1 liter distilled or deionized water and heat to boiling to dissolve completely. Sterilize in the autoclave for 15 minutes at 121^{O}C. Cool to 45-50 ^{O}C and dispense into sterile petri dishes. Final PH 7.4± 0.2 at 25 ^{O}C.

Brain Heart Infusion

Ingredients

Pancreatic digest casein	14.5g
Agar	5g
Brain Heart Solids from infusion	8g
Peptic digest of Animal Tissue	5g
Sodium chloride	5g
Dextrose	2g

Sodium Phosphate Dibasic	2.5g
pH	7.4±0.2 at 25 ^{O}C
Distilled water	1liter

Instruction

Dissolve 52g in 1000 ml distilled water stir and dissolve completely and sterilized by autoclaving for 15 minutes at 121^{O}C .Cool to room temperature before use.

Purple Agar Base

Ingredients

Proteose Peptone No. 3, Difoco	10g
Bacto-Beef Extract	1g
Sodium chloride	5g
Bacto Bromo-Cresol- Purple	0.02g

Directions

To rehydrate the medium, dissolve 16g in 1 liter of distilled or deionized water .Sterilize in the autoclave for 15 minutes at 121C^{O}.When preparing 0.5-1% carbohydrate fermentation, dissolve 5-10g of the desired carbohydrate in the basal medium prior to sterilization ,or dissolve 16g Bacto Purple Agar Base in 900ml distilled or deionized water and aseptically add 100 ml of a sterile 5-10% carbohydrate solution (W/V) after sterilizing and cooling the basal medium .

Muleller Hinton II Agar

Formula

Beef Extract	2g
Acid Hydrolysate of casein	17.5g
Strach	1.5g
Agar	17.0g

Direction

Suspended 38g of the powder in 1 liter of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1min. to completely dissolve the powder. Autoclave at 121^{O}C for 15 min. Do not overheat.

Tryptic Soy Agar (DIFCO.TM, 500g)

Composition

Pancreatic digest of casein 15g

Enzymatic digest of Soybean meal 05.0g

Sodium chloride 5.0g

Agar 15.0g

Instruction

40g of the powder was suspended in 1 liter of purified water and mixed thoroughly. It was heated with frequent agitation and boil for 1 minute to completed dissolve the powder and autoclaved at 121 ^{O}C for 15 minutes and then dispended to sterile petry dish after reaching at 50 ^{O}C.

Annex 6 Recorded Sheet for Laboratory analysis of *Staphylococcus* species

Date	Sample no.	Sour -ces	Type of sample	Blood agar	Subculture	Gram's staining	Catalase test	MSA

MSA=Manitol Salt Agar

Annex 7 record sheets for confirmation of *Staphylococcus* species

Sample no.	Sources	Type of sample	CPS	CNS

CPS=Coagulase-Positive Staphylococcus, CNS=Coagulase-Negative Staphylococcus

Annex 8 record sheets for identification of Coagulase-Positive Staphylococcus on Purple Agar Base

Sample no.	Type of sample	*S.aureus*	*S.intermedius*	*S.hyicus*

9. CURRICULUM VITE

A. Personal data:

Full Name: Senait Getachew Zewde

Place of birth: Addis Ababa

Date of birth: 1962 E.C

Martial status: Marriage

Religion: Christian

Nationality: Ethiopian

Present address: South Omo Zone Bako Gazar Wereda, Ethiopia

Occupation: Head office, Bako Gazar Wereda agricultural and Natural Resource Development.

B. Education:

2. DVM from Higher Institute of Agricultural Science of Havana, Republic of Cuba
From 1989- 1993.

3. Junior and secondary School: 21 de Sene (Cuba), 1976 E.C

4. Junior elementary school: Karamara 1972 E.C

C. Work experience:

From 1986-1993. Animal health team leader and member of the managemen committee of the woreda agriculture and rural development office

From 1994-1996 E.C. Head office. at Wereda level.

D. Research papers

DVM: Determination of Inhibitors in milk from cow treated with acriflavine and tetracycline intrauterine.

Language

Amaharic, Spanish and English.

Others

I have certificate in training on PME (Participating in Monitoring and Evaluation).

Diploma in computer training

10. SIGNED DECLARATION SHEET

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

Name Senait Getachew

Signiture ___________

Date of submission June 25/2007

This Thesis has been submitted for examination with I approval as University advisor.

Prof. A. R. S. Moorthy____________