Isolation and Identification of Staphylococcus Species from Ready-To-Eat Meat Products in and Around Debre-Zeit, Ethiopia

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ABSTRACT

The study was undertaken with objective of i) to identify the factors which are responsible for food borne 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 Staphylococcus isolated from ready-to-eat meat products. In the present study, all 57 eating establishment i.e. hotels (23) restaurants (27) and cafes (7) present in and around Debre-ziet were included and only 22 managers were responded to the questionnaire survey. It was observed that out of 99 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, kitchen and food storage areas in the eating establishments were graded as good (15), satisfactory (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 had undergone monthly, 18.2% quarterly and 9.1% annual medical checkup. 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 these isolated (111) were subjected to antibiotics sensitivity and found that polymyxin-B was most sensitive, followed by norfloxacin, trimethoprim, gentamycin and neomycin; whereas ampicillin was most resistant to the present isolates. In view of above results, Staphylococcus species existed 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 implication of Staphylococcus species in ready-to-eat meat products on human health was discussed and recommendation 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.

Keywords: Coagulase, Enterotoxins, Food products, Meat, Staphylococcus

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 true incidence is difficult to evaluate, since many countries do not have an epidemiological surveillance system in place, and whereas 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 year (Acha and Szyfres, 2001)

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Food borne disease 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 food borne diseases and source of the causative microorganisms.

There are many organisms, which cause food borne diseases in human beings, among which salmonella spp, listeria monocytogenes, and clostridium perfingens 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 Bennerman, 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.
- To Isolate and identify staphylococcus species from ready-to-eat meat products.

**MATERIALS AND METHODS**

**Study Area**

The study area was in and around Debre Zeit, which is located 45Km south east of Addis Ababa. The area is located at 9°N latitude and 40°E longitude at an altitude of 1850 Mts. It has an annual rainfall of 866 mm, which occurs from June to September and dry season extends from October to February. The average temperature range from 14°C to 16°C with a relative humidity 61.3 % (CAS, 2001).

**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.

**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 Ziet town. The Sample size was determine as per the recommendation Standard of International Organization (ISO, 6888-1:2002) for the detection of *Staphylococcus* species.

**Sample Collection**

All the 57 public retail establishments of hotels, restaurants and cafes in-and-around Debre Zeit were included in the this study. A total of 384 samples of ready-to-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 from hotels and restaurants 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 March 2007. All the 384 samples were labeled 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.

STUDY METHODOLOGY

Culture 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 25 g 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 of portion of various dilution were spread on Blood Agar plates (5 % difibrinated sheep blood) and the plates were incubate aerobically at 37°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 subculture on Blood Agar plate and on nutrients slants and incubated at 37°C. The slant was preserved and maintained for characterizing the isolates.

Gram’s Staining

All the suspected culture of Staphylococcus species were staining by Gram’s stain and observed under microscope for Gram’ 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 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°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 performed as a tube coagulase test. The selected Staphylococcus was subculture into Brain Heart Infusion broth and incubated 37°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 rabbit plasma were incubated at 37°C and examined after 4 h and 24 h of incubation and observed for the clot formation. Any coagulation of plasma was regards as positive at either of the reading 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 particularly with coagulase-positive with other staphylococci. The suspected culture was incubated on PAB media plate with 1 % of maltose and incubated at 37°C for 24 h. rapid fermentation of maltose by S.aureus caused yellow discoloration of medium due to change in pH. 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 and colonies that did not produce any change on the medium were taken as S.hyicus.

Anti-Microbial Susceptibility Testing

The Staphylococcal cultures isolated from ready-to-eat-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 (µg), 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 comparable with that of 0.5 McFarland standards. Muller –Hilton Agar (MHA) plate were prepared, and a sterile cotton swab was dipped into the Tryptone Soya Broth culture and swabbed on the surfaced 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 °C under aerobic condition. The isolated were classified in accordance with the guideline of the National Committee for Clinical Laboratory Standard (NCCLS, 2002) as susceptible, intermediate or resistance for each antibiotic tested according to the manufacturer’s instructions by measuring the zone of inhibition around the antibiotic disc.

**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 proportion were applied to compute as the number of food samples positive for staphylococcus divided by the number of samples examined in each food item. The overall prevalence of Staphylococcus spp. Divided by the total number of samples examined.

**RESULTS**

In the questionnaire survey, 22 managers of 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, cleanliness 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 butcher 68.2%, municipal abattoirs 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% had not idea about this.

When enquired about the health status of workers and whether they had undergone regular medical checkup or not 68.2%informed that they had undergone monthly medical checkup; 18.2% quarterly check up ; 9.1% annual checkup 4.5% had not undergone any medical checkup. Regarding the inspection of eating establishments by the sanitary inspection, 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)

**Table1. Detail of questionnaire survey**

<table>
<thead>
<tr>
<th>Source</th>
<th>Hotels</th>
<th>Restaurants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of managers interviewed</td>
<td>10</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Food handlers</td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>36</td>
<td>49</td>
</tr>
<tr>
<td>Medical checkup</td>
<td>No</td>
<td>Monthly</td>
<td>Yearly</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Wearing of protective clothing</td>
<td>Apron only</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>
Prevalence of Staphylococcus Species in Ready-To-Eat Meat Products

In the present study, all the five ready-to-eat meat products tested were positive Staphylococcus species. Out of a total 384 ready-to-eat 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

<table>
<thead>
<tr>
<th>Meat production</th>
<th>number of samples tested</th>
<th>Number of samples positive for</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Burger</td>
<td>47</td>
<td>12 (25.5%)*</td>
<td></td>
</tr>
<tr>
<td>Cooked ground meat</td>
<td>103</td>
<td>31 (30.1%)</td>
<td></td>
</tr>
<tr>
<td>Roasted meat</td>
<td>116</td>
<td>34 (29.3%)</td>
<td></td>
</tr>
<tr>
<td>Roasted chicken</td>
<td>19</td>
<td>7 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>“Siga wet”</td>
<td>99</td>
<td>27 (27.3%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>111 (28.9%)</td>
<td></td>
</tr>
</tbody>
</table>

*Number in parenthesis indicate percentage positive

Distribution 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(36.5%) followed by S.intermedius 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 from each ready-to-eat meat products. (Table.3).

Table 3. Distribution of staphylococcus species in different meat products

<table>
<thead>
<tr>
<th>Coagulase-Negative</th>
<th>Meat products</th>
<th>S.aureus</th>
<th>S.intermedius</th>
<th>Staphylococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burger</td>
<td>6(30%)</td>
<td>3(25%)</td>
<td>3(25%)</td>
<td>0</td>
</tr>
<tr>
<td>Cooked ground meat</td>
<td>13(41.9%)</td>
<td>11(35.4%)</td>
<td>5(16.1%)</td>
<td>2(6.5%)</td>
</tr>
<tr>
<td>Roasted meat</td>
<td>11(32.4%)</td>
<td>12(35.3%)</td>
<td>8(23.8%)</td>
<td>3(8.8%)</td>
</tr>
<tr>
<td>Roasted chicken</td>
<td>1(14.3%)</td>
<td>0</td>
<td>0</td>
<td>6(85.7%)</td>
</tr>
<tr>
<td>“Siga wet”</td>
<td>10(37.0%)</td>
<td>7(25.9%)</td>
<td>6(22.2%)</td>
<td>4(14.8%)</td>
</tr>
<tr>
<td>Total number of</td>
<td>41</td>
<td>33</td>
<td>22</td>
<td>15</td>
</tr>
</tbody>
</table>

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 presented 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.
“Isolation and Identification of Staphylococcus Species from Ready-To-Eat Meat Products in and Around Debre-Zeit, Ethiopia”

**Anti-Microbial Susceptibility**

In the present study, all the 111 isolated 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%), neomycin (91.0%), gentamycin (89.2%) etc. and others ranged from 80.2% to 84.7% in their sensitivity. The least sensitive antibiotic was ampicillin (26.1%) (Table 4).

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

<table>
<thead>
<tr>
<th>Antibiotics tested</th>
<th>Overall sensitivity</th>
<th>% Overall sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>29</td>
<td>26.1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>89</td>
<td>84.2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>94</td>
<td>84.2</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>99</td>
<td>89.2</td>
</tr>
<tr>
<td>Neomycin</td>
<td>101</td>
<td>91.1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>93</td>
<td>83.8</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>100</td>
<td>90.1</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>92</td>
<td>82.9</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>92</td>
<td>82.9</td>
</tr>
</tbody>
</table>

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 ampicillin.

The most sensitive antibiotic to *S.intermedius* was neomycin followed by polymyxin-B, norfloxacin etc. and least sensitive to ampicillin (21.2%). All isolated *S.hyicus* were completely sensitive to polymyxin-B, chloramphenicol and norfloxin followed by tetracycline, gentamycin, 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 trimethoprin 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 antibiotics sensitivity pattern**

<table>
<thead>
<tr>
<th>Antibiotic tested</th>
<th><em>S.aureus</em></th>
<th><em>S.intermedius</em></th>
<th><em>S.hyicus</em></th>
<th>Coagulase-Negative Staphylococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>4/41(9.8)</td>
<td>7/33(21.2)**</td>
<td>2/15(13)</td>
<td>16/22(73)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>34/41(85)</td>
<td>21/33(64)</td>
<td>15/15(100)</td>
<td>18/22(82)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>38/41(93)</td>
<td>27/33(82)</td>
<td>10/15(67)</td>
<td>19/22(86)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>37/41(90)</td>
<td>29/33(88)</td>
<td>14/15(67)</td>
<td>19/22(86)</td>
</tr>
<tr>
<td>Neomycin</td>
<td>36/41(88)</td>
<td>32/33(97)</td>
<td>13/15(87)</td>
<td>20/22(91)</td>
</tr>
</tbody>
</table>
**DISCUSSION**

The surveillance of food for microbial contamination is vital for protection of public health and consumer interest. 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 presence 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 (1996), in which it is stated that common hazard arises through cross contamination from row 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 those that are handled directly, unless heat processing steps applied to effect their destruction (Nagaseet *et al.*, 2002; Euzebzy, 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 were important food borne pathogens in humans, and cause diseases 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 ready-to-meat products by Payne and Wood (1974) and the 39 % in meat products (Adesiyun, 1984) in Nigeria but higher than 11.9% recorded by ICMSF (1996). Of the 384 meat products examined, a total 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-Positive 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.intermidius* (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 resistance 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% frequently of *S. intermedius* in the current study was higher than the findings recorded in the 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 Euzeyb, (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 outbreak of food poisering. The Coagulase- positive *S. intermedius* was the predominant non-*S. aureus* species isolated from foods, which was the only species has clearly involved staphylococcal food poisoning outbreaks.

In this study *S. hyicus* was isolated and identified from different meat product at the prevalence rate of 13.5% except burger Kiss *et al.*, (1997) and Lilienbaum *et al.*, (1987) reported that *S. hyicus* is found most predominantly in milk and poultry products. Some authors have described its low distribution in food products 0.2% (Hooves, 1983) and 2% (Balaban and Rasooly, 2002).

Coagulase- Negative Staphylococcus was the third most frequently isolated species in the current study. The present result was higher than 3.4% as reported by Udo *et al.*, (1999). The Coagulase-Negative Staphylococcus inhabit the skin and mucous membranes of human beings and these

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**Table 1**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of Isolates Sensitive</th>
<th>No. of Isolates Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin-B</td>
<td>40/41(98)</td>
<td>1/41(2)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>36/41(88)</td>
<td>5/41(12)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>37/41(93)</td>
<td>4/41(10)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>35/41(80)</td>
<td>6/41(15)</td>
</tr>
</tbody>
</table>

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

**percent sensitive to the antibiotic tested**
microorganisms can contaminate foods if not handled properly (Bergdoll, 1995). This may be due 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. Wienke et al., (1993) and in France the 9.5% by Haeghebart et al., (2002). Differently from other food items, *S. hyicus* was found to be the most common contaminant of roasted chicken followed by *S. hyicus* may be due to frequent handling, improper roasting and outside and not properly covering the cooked products.

In the 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. intrmedius* from cooked meat products; the present finding also indicated the similar prevalence rate.

In the current study, out of 116 sample of roasted meat 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 State. 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 rousted meat without heat treatment.

Burger was found relatively less frequently contamination 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 species reported 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 frequency found bacteria as the normal skin flora of dogs is *S. intrmedius* 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 organism from dogs to meat producing animals, as farmers most commonly keep their domestic animals in close contact with dogs.

Antibiotic resistant bacteria strains are increasingly emerging worldwide because of in discrimination 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, (1986). This may be due to many of the board spectrum antibiotics tested in the study are frequently used for treatment in animals and man.

**CONCLUSIONS AND RECOMMENDATION**

The result of the present study indicated that

- All five ready-to-eat meat products yield *Staphylococcus* species
- 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, and 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.

**REFERENCE**


Isolation and Identification of Staphylococcus Species from Ready-To-Eat Meat Products in and Around Debre-Zeit, Ethiopia

Senait G & Pro.A.R.S.Moorty


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