PREVALENCE OF *Staphylococcus aureus* AND ITS ANTIMICROBIAL SUSCEPTABILITY PATTERN AMONG FOOD HANDLERS WORKING IN HOTELS AND RESTAURANTS IN JIMMA TOWN, SOUTHWEST ETHIOPIA

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Prevalence of *Staphylococcus aureus* and its antimicrobial susceptibility pattern among food handlers working in hotels and restaurants in Jimma town, Southwest Ethiopia, 2017

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ABSTRACT

Background: *Food handlers with poor personal hygiene practices serve as major sources of pathogenic S. aureus which potentially causes to food intoxication. Researchers reported that 30-50% of the general human populations are carriers of S. aureus more of in their nasopharynx. This study aimed to evaluate nasal and hand prevalence of S.aureus and its antimicrobial susceptibility pattern among food handlers. In Ethiopia, there were few researches conducted in community setting similar to this topic.*

Methods and materials: *A community based cross sectional study was conducted from Feb 30, to May 30, 2017 among food handlers working in restaurants and hotels of Jimma town. Data was collected by structured questionnaire and observation checklist. Swab specimens was collected from nares and hands of food handlers and microbiologically examined for the presence of S.aureus then drug sensitivity tests also performed. Food samples microbial quality was examined using standard microbiologic protocols. Finally data was entered into an Epi data 3.1 and SPSS version-21.0 windows statistical software.*

Result: *Among 300 food handlers 86(28.7%) were positive for S.aureus. Factors significantly associated with this prevalence rate were unfavorable attitude towards safe food handling (P=0.020), wearing hand ornaments (P=0.040) and for waiters job category (P=0.044). S.aureus Isolates (90.7%) showed high resistance to Amoxicillin and Penicillin but most of the isolates were sensitive to (96.5%) Ciprofloxacin, Cefoxitin (95.3), Amoxicillin-Clavulanic* Acid (94.2%). *Certain isolates showed resistance to Cefoxitin, Oxacillin and Vancomycin by 4.7%, 7% and 7% respectively.*

Conclusion*: The isolated S.aureus on both nares 27(9%), both hands 34(11.3%), on both noses and hands was 25(8.3%). Most isolates were resistant against the commonly prescribed drugs and also showed resistant against Vancomycin. Majority 37(86.1%) of food samples microbial quality was unsatisfactorily contaminated by aerobic mesophilic bacteria (mean count CFU/g>10^5). Aerobic mesophilic bacteria found on food indicates the unhygienic activities and bare hand food contact of food handlers.*

Keywords: *Food handlers, Nasal & hand carriage, S. aureus, Hotels and Restaurants,*

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ABBREVIATIONS

APC: Aerobic Plate Count

ARS: Antibiotic Resistant *S. aureus*

AST: Antimicrobial Susceptibility Test

ATCC: American Type Culture Collection

BHI: Brain Heart Infusion

CDC: Center for Disease Control and Prevention

CFU: Colony Forming Unit

CSA: Central Statistics Agency

E.C: Ethiopian Calendar

EFSA: European Food Safety Authority

FHs: Food Handlers

H.C: Health Center

JU: Jimma University

KAP: Knowledge Attitude and Practice

MHA: Muller Hinton Agar

MIC: Minimal Inhibitory Concentration

MRSA: Methicillin Resistance *S.aureus*

PCR: Polymerase Chain Reaction

SE: Staphylococcal Enterotoxin

SPSS: Statistical Package for the Social Sciences

WHO: World Health Organization

CHAPTER ONE: INTRODUCTION

1.1. BACKGROUND

Staphylococcal food poisoning is a gastrointestinal inflammation caused by eating foods contaminated with enterotoxins produced by the bacterium *S.aureus*. The bacterium was found on the skin and nose 20 to 30% of healthy people and animals. However, Coagulase positive *S.aureus* on food handlers potentially causes to food-borne enterotoxins. Particularly, young children, old peoples, pregnant mothers and immune compromised peoples are more sensitive to staphylococcal enterotoxins when they ingest preformed enterotoxin with food(El-Shenawy *et al.* 2013).

Even though the sources of food contamination are diverse, 20% of food-related intoxications are due to food handlers either as carriers of pathogens or causes to cross contamination(Assefa *et al.* 2015; Eke *et al.* 2015). High bacterial load may indicate a potential for the presence of enterotoxin, due to lack of adherence to good hygienic practices. *S.aureus* is among the top etiologic agents in food borne diseases globally (Fetsch *et al.* 2014; Igbinosa *et al.* 2016).

Antibiotic resistant *staphylococcus* particularly methicillin-resistant *S.aureus* (MRSA) has been common problem globally. In the United States, it accounts more than half of this entire organism exist on food handler's nose or skin. United States Center for Disease Control and Prevention (CDC) reported that invasive MRSA infection cases are more than 94,000 a year and caused to high mortality rate in the U.S. than HIV/AIDS. Coagulase-positive staphylococcal strains are capable of producing thermostable enterotoxins and causes to inflammation of intestinal lining and disturb cell homeostasis leading to efflux of water and electrolytes by interaction with a cell surface receptor**.** Food handlers rubbing their runny nose with hands and touching any food contact materials can transfer *S.aureus* to cooked moist protein rich foods .When Cooked food put at 7^{o}C to 48^{o}C for more than two hours *S.aureus* can grow at considerable numbers. Coagulase positive *S.aureus* species have been implicated in food poisoning and the commonly outbreaks linked agent(Teramoto *et al.* 2016).

The anterior nasal region is also a primary ecological reservoir of *S.aureus* which able to produce enterotoxins on food. Protecting *S. aureus* from the food with safe food handling has to be effective than heat tolerant SEs from the food once released(van Belkum *et al.* 2009).

1.2 STATEMENT OF THE PROBLEM

Now a day's food preparation at homes decreased and number of meals eaten out of the home increased especially among urban dwellers following life-style and food consumption behavior change (DeBess *et al.* 2009). On the other hand, the ever-increasing number of food-related health problems has been informs us to be alert for better hygiene and quality practices. A study in the USA suggested that food handlers' improper practices contributed to approximately 97% of food borne illnesses in food service establishments and homes(Baş *et al.* 2006).

Food handlers' bad personal habits like touching body parts, poor hand wash practices and the lack of sanitation facilities in food establishments can escalates the incidence of food poisoning. On the other hand, *S. aureus* mostly colonizes the skin and the anterior nares of a significant proportion of the populations(Castro *et al.* 2016).

S.aureus remains a considerable cause of mortality and morbidity in tropical countries mainly by its virulence factors like inhibiting phagocytosis, blood plasma clotting and multiple antibiotic resistance (Eke *et al.* 2015).Food borne illnesses by pathogenic toxin produced by coagulase positive *S.aureus* results death, illness, hospitalization, and economic losses.

Staphylococcal food poisoning occurs when food is consumed with Staphylococcal Enterotoxins (SEs).Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands is regarded as the main source of food intoxication via direct contact or through respiratory secretions. *S.aureus* strains are capable of producing more than 22 SEs with symptoms sudden onset of nausea, vomiting, diarrhea and abdominal cramp within 1–6 h (El-Shenawy *et al.* 2013).

On the other hand, *S.epidermidis* and *S.saprophyticus* are found on the skin of humans, but less virulent than S.*aureus* and which considered as a normal flora on the skin and girls' sexual organ respectively. However, if any scratch or chance to enter the visceral cavity it leads to urinary tract infections (Alsamarai *et al.* 2015).

Furthermore, the toxigenic dose required to induce *staphylococcal* food poisoning in humans is about 0.1µg and it may vary with individuals physiologic factors like being young, old, pregnant, immunosuppressed are vulnerable sections of the population(Evenson *et al.* 1988).

There is a number of food-borne disease incidents reported every year in Ethiopia. According to the Federal Ministry of Health yearly report in 2011, dysentery and gastroenteritis were among the ten top diseases of outpatient visits (Assefa *et al.* 2015).

Furthermore, in Ethiopia, most of the food handlers did not have pre-employment medical examination and food safety related certifications. On the other hand, the food establishment inspection regulation is poorly performed as related studies revealed. Similarly, another study in Jimma town indicated that , unsafe food storage conditions and poor hygienic practices in food establishments are major contributing factors to food associated illnesses (Tassew *et al.* 2010).

A study conducted in Samara city in Ethiopia, showed that (28%) of the *S.aureus* isolated from food handlers, were resistant to methicillin(Alsamarai *et al.* 2015).

Currently, the increasing prevalence of antimicrobial-resistant S. *aureus* is attracting researchers' attention. Thus, identifying associated factors with *S.aureus* prevalence together with its drug sensitivity test is vital to reduce the rate of fast acting toxins and its complications (Castro *et al.* 2016).

Antimicrobial resistant in *S. aureus* has been increased considerably, where the rapid emergence of Methicillin Resistant *S. aureus* (MRSA) has left only one intravenous antimicrobial, vancomycin, as a treatment option. Previously conducted studies were limited to hospital settings and MRSA infections have been not well reported in the community among food handlers.

There were few researches conducted on *S.aureus* prevalence among food handlers in Ethiopia. The previous study conducted in Jimma university students' cafeteria on *S.aureus* prevalence did not perform antimicrobial susceptibility test to isolates. Therefore, this study was conducted to identify *S.aureus* prevalence in hotels and restaurants. Then after antimicrobial sensitivity test was done to commonly prescribed drugs.

In general, FAO/WHO Expert Committee on food safety declared that medical examinations and food safety regulations alone are not guarantee for safe food provision, whereas identifying the hygienic practices barriers, should take the top place because others are less feasible in terms of cost and high turnover of food handlers (Emmanuel 2014).

1.3 SIGNIFICANCE OF THE STUDY

The Federal Democratic Republic of Ethiopia issued its first proclamation on public health back in 1947. In recent times two new proclamations on Trade Practice and Consumers' Protection proclamation 685/2010 and Commercial Registration and Business license proclamation 686/2010 were announced with the aim to contribute towards achieving better results in food safety assurance.

Most of the time in Ethiopia, majority food establishment owners do not set a criteria of food safety training and pre-employment health checkup certificates for food handlers to those provide application to work in food establishments. However, food handlers are the great role player in food-borne diseases prevention.

Therefore, this study will be helpful in:

- Providing timely information about the prevalence of *S.aureus* among food handlers working in hotels & restaurants for health planners and decision makers to improve the food safety handling situations.
- Identifying the antimicrobial resistance pattern of *S.aureus* in order to manage drug administration conditions
- Provide current information about food handlers' knowledge, Attitude and Practices status towards safe food handling.
- Giving insights about sanitation and hygiene barriers that inhibit of food handlers safe food handling practices
- Assessing factors potentially associated with *S.aureus* prevalence.
- Providing bench mark for further researches in the same title in *S.aureus* enterotoxins type identification.

CHAPTER TWO: LITRATURE REVIEW

Nasal carriage of *Staphylococcus aureus* is a well-recognized risk factor for staphylococcal thermostable enterotoxins, food poisoning and contamination. For many years, three patterns of nasal colonization have been recognized: persistent, transient and non-carriage. Serological studies revealed that persistent nasal carriers have a significantly higher level of antibodies against some staphylococcal enterotoxins (SEs) than non-carriers (Ho *et al.* 2014).Therefore, it is important to detect *S. aureus* carriage among food handlers to prevent possible food contamination and resulting food poisoning (Vatansever *et al.* 2016).

The detection of staphylococci in food is often connected to poor hygienic practices during slaughtering, transportation, chopping, storage and points of sale by the individuals involved in the food value chain. Contaminated food can transfer large amounts of staphylococci to stainless steel and polyethylene surfaces with other consumables in the same polythene packs and bags from food handlers and finally severely affect elders, young and immune suppressed community groups (Igbinosa *et al.* 2016).

2.1 *Staphylococcus aureus*

Staphylococcus aureus is aerobic and facultative anaerobic, Gram-positive spherical bacteria in the Staphylococcoceae family. The bacteria, have a broad host and distribution range in nature and is commonly found in the nose, throat, hair and skin of humans and animals(Vatansever *et al.* 2016)

Staphylococcus aureus is one of the commonest food poisoning non-spore former, non-motile catalase and coagulase positive bacteria. The bacterium is able to produce a broad range of enterotoxins in the food. When ingesting food with one or more pre-formed enterotoxins that excreted by these bacteria causes to impaired or inflamed intestinal mucosa of consumers. Then it leads to violent vomiting, abdominal cramp and diarrhea within 30 min to 24hrs after ingestion of poisoned food(Castro *et al.* 2016).

Regarding *S.aureus* growth determining factors, a study in Spain argued that at 8 °C, *S. aureus* grew only at optimum levels of pH and water activity .While at temperatures above 13 °C with

pH 4.5; water activity should increase from 0.86 to 0.96. Otherwise at less than 7.5 °C and low pH and water activity there was no growth detected. Therefore, growth of *S. aureus* can be inhibited at refrigeration temperatures below 8 °C together with low pH and low a_w levels(Valero *et al.* 2009).

2.2Virulence factor of *S.aureus*

S. aureus enzymes or proteins (coagulase, catalase, DNase/ staphylococcal thermo nuclease, oxidase, urease) and thermostable endonuclease enzyme characters are virulent factors. The study in Benin City, Nigeria on MRSA, thermostable endonuclease gene (nuc gene) encodes the expression of the thermostable endonuclease enzyme, known to be an important pathogenic factor for *S. aureus*. The presence of such gene in the genome of the staphylococcal cell increases the organism's inherent capacity to initiate infection(Okojie *et al.* 2014). The use of antibiotics in humans and in animals for therapeutic, growth promotion and prophylactic purposes possibly led to the selective increase of resistance in bacterial populations. The **p**enicillin **b**inding **p**rotein 2a (PBP2a) has a reduced resemblance for beta-lactam antibiotics, resulting in resistance to most beta-lactam antimicrobial agents (Igbinosa *et al.* 2016).MRSA strains have the ability to induce biofilms and this resulted in increase in their virulence(Alsamarai *et al.* 2015).

2.3. *S.aureus* food poisoning

Food poisoning caused by *staphylococcal* enterotoxins is among the leading causes of food-borne outbreak in the European Union. *Staphylococcal f*ood poisoning is characterized by a sudden onset of symptoms, with vomiting, abdominal pain, and stomach cramps are being the most common. The pathogenesis of bacteria causing food borne-poisoning is depending on their capacity to produce toxins. This can be either after ingestion in the digestive tract or before toxins performed in the food stuffs(Tassew *et al.* 2010; Fetsch *et al.* 2014).

The ingestion of preformed toxins by enter toxigenic strains in food often leads to the development of food poisoning. *Staphylococcal* enterotoxins are highly heat resistant in food stuffs in the absence of sterilization or canning. Definitely, restaurants have been taking the second important place in priority for acquiring staphylococcal food poisoning and it accounts for 14–20% of outbreaks involving contaminated food in the USA and United

Kingdom(Eke *et al.* 2015). Studies in Ethiopia not able to identify the presence or the absence of enterotoxin producer strains because of lack of reagent enterotoxin kit, Phage typing and PCR techniques.

2.4 Nasal carriage of *S.aureus*

A study carried out in Hong Kong -China, stated that from a total of 434 participants, 99 (22.8%) were nasally colonized with *S. aureus(Ho et al. 2014).* Similarly from the prevalence of *Staphylococcus aureus* in food handlers in Kars City-Turkey described that, 56 (20 %). Out of this *S. aureus* prevalence rate , 32 (57 %) isolates from the nasal cavity, 13 (23 %) isolates from mouth and 11 (20 %) isolates from hands of workers (Vatansever *et al.* 2016).

A study in Ekpoma, Edo State, Nigeria reported that, the nasal carriage of *Staphylococcus aureus* among food handlers in restaurants were (60%) from 100 nasal swab samples. With regards to sex, 35(58%) were males and 25(42%) were females. In terms of age the prevalence of *S. aureus* ,within the age range of 26-30 had the highest prevalence (67%) followed by within the age range of ≤25(Eke *et al.* 2015).

The study undertaken in Egypt to determine the prevalence and risk factors associated with nasal carriage of *S. aureus* among 200 food handlers working in three different food processing plants revealed that 61 (31%) persons were found to be carriers of *S. aureus* of which 21 (34.4%) harbored enterotoxigenic staphylococcal strains(El-Shenawy *et al.* 2013).

A study at Samara city in Ethiopia revealed that the prevalence of *S.aureus* was 28% among food handlers working in restaurants. unhappily, 21.5% the isolates were resistant to methicillin and similarly this percentile are found on both nares of food handlers(Alsamarai *et al.* 2015).

Another study conducted on food handlers working in the cafeterias of University of Gondar and the Gondar Teachers' Training College reported that among 127 food-handlers 41.7% of them were infected with coagulase-negative staphylococci predominantly followed *by Staphylococcus aureus* (16.5%). The findings indicated that food handlers served as potential sources of infections and suggested that health institutions need to work a lot for appropriate hygienic and sanitary practices (Andargie *et al.* 2008).

Survey of nasal carriage of *Staphylococcus aureus* among the 200 healthy food handlers working at Gondar University students' cafeteria, reported that, the prevalence of nasal carriage of *S. aureus* was 41(20.5%) (Dagnew *et al.* 2012).Similarly, another study at Jimma University

students' cafeteria food handlers, the corresponding prevalence rates of *Staphylococcus aureus* from hand rinse were 54(23.5%).It is higher than reported from Gondar University students' cafeteria food handlers' *S.aureus* status(Assefa *et al.* 2015).

However, the types of toxins produced by *Staphylococcus aureus* were not identified at both study areas. Though following handling of food by persons who carry this bacterium in their noses, hand and skin, the carriers are potentially can causes to cross-contamination. The discrepancy in socioeconomic status, type of food establishment, and lack of personal hygiene practices explained this difference. Therefore, isolation of *Staphylococcus aureus* reflect improper hygiene practices such as pocking fingers into the nose (Assefa *et al.* 2015)

2.5 *S.aureus* isolation and identification

a) Culture: Different media helps to isolate of *Staphylococcus aureus* from swab samples onto Manitol Salt Agar (Oxoid, England), Baird-Parker Agar, Columbia blood agar and Nutrient Agar. After incubation at 37 °C for 24 h, suspected colonies of *S. aureus* was transferred into tubes containing Brain Heart Infusion (Valero *et al.* 2009).

b) Biochemical test: *Staphylococcal colonies* could be identified by basic biochemical tests which included Gram-staining, catalase testing (using 3% hydrogen peroxide), indole, oxidase, coagulase, citrate, urease, Voges-Prosakauer, DNase tests, sugar fermentation and the oxidation and fermentation of Mannitol Salt Agar(Igbinosa *et al.* 2016).

C) Microscopically identification: All isolates confirmed to be *Staphylococcus aureus*, characteristically, by their typical colonial appearance (displaying golden-yellow colonies), a positive coagulase test, and staining as Gram-positive cocci in clusters. Other *staphylococci* species were culturally and morphologically identified as coagulase-negative s*taphylococci* (CNS) forming non-golden yellow and non-beta-hemolytic colonies. For further isolation of genomic DNA extraction was carried out in PCR to identify Staphylococcal isolates (Igbinosa *et al.* 2016).

2.6 Antimicrobial resistance pattern of *S.aureus*

A study in Nigeria reveals that the antibiotic resistance by *Staphylococcus aureus* was high for Ampicillin 60 (100%), ampiclox 45 (75%) and pefloxacin 15 (25%)(Eke *et al.* 2015). Moreover, in Kars City, Turkey , antimicrobial resistance of nasal carriage *S. aureus* isolates from 32 food

handlers showed that 2(6%) of them were at equal magnitude intermediate resistant and resistant to only for Trimethoprim but sensitive to all antimicrobial drugs(Vatansever *et al.* 2016).

In contrast to Kars City findings; a study conducted at Gondar University students' cafeteria the result of drug susceptibility pattern, reveals that all isolates of *S.aureus* were sensitive to vancomycin. But, half of the rate of the isolates of *S. aureus* 21 (51.2%) were resistant to penicillin and 19(46.3%) were resistant to ampicillin. Sixteen (39%) of the isolates were resistant to amoxicillin. Thirteen (31.7%) and 11 (26.8%) of the isolates were resistant to tetracycline and cotrimoxazole respectively. Six (14.6%) of the isolates were resistant to erythromycin, whilst 4 (9.8%) of the isolates were resistant to methicilin and ciprofloxacin respectively (Dagnew *et al.* 2012).

In this study antimicrobial sensitivity was conducted specially to identify whether vancomycin, Oxacillin and Cefoxitin resistance *S.aureus* strain isolates presence or not. These drugs are commonly prescribed drugs to penicillin, ampicillin and methicilin resistance strains.

2.7 Knowledge Attitude and Practice (KAP) of Food handlers

In Turkey Knowledge, Attitude and Practice assessment revealed that almost half of respondents (47.8%) had not taken food safety training. The self-reported hygienic practices showed that only 9.6% of food handlers handle food by protective gloves during their working activity. Concerning the habit of hand washing (8.1%) washed their hands before putting gloves and (3.8%) after removing it (Baş *et al.* 2006)

In contrast, a study done in restaurants of King Saud University - Saudi Arabia reported that food handlers have extremely good personal hygiene practices whereby (96.6%) maintained safe practices, such as wearing uniforms and caps. Majority of the respondents (75.9%) removed personal stuffs like watches, rings and jewelry that can contaminate foods while working. Majority (85%) of food handlers responded as awaked about the danger in touching food with cut hands or fingers and almost 51.5% were aware of danger of wearing adornments (Al-Shabib *et al.* 2016).

Similarly, evaluation of basic Knowledge, Attitude and Practice in Peninsular Malaysia food establishment revealed that majority (94.3%) of respondents had basic knowledge about the importance of hand washing after coughing and sneezing. But few (10%) of food handlers went to health facilities health checkups every six months(Woh *et al.* 2016).

In Ethiopia, Addis Ababa University Students' cafeteria, all food handlers were literate but only 52.3% of them had good hygienic practices. This implied that there are other factors that determine food handlers' hygienic practices in addition to educational status(Meleko 2013). Similarly, a study conducted in 105 food and drink establishments in Dangila town revealed that only 213 (52.5%) of food handlers had good food handling practice (Andargie *et al.* 2008).

In Gondar University students' cafeteria, a study reported that, above half 105 (52.5%) of the food handlers had education above primary school. In hand washing practices, 148 (74%) of food handlers washing their hand with soap and water, the rest 52(26%) only used water for their hand after toilet. Again, 92(46%) food handlers had a habit of hand washing after touching nose between handling of food items. About half 93(46.5%) of food handlers had no medical check-up. The amount of service years less than a year observed the lowest rate of colonization by *S. aureus* which was 8.3%(Dagnew *et al.* 2012).

2.8 **Microbiological testing of food sample**

The microbial quality of all food is not equally evaluated for different microorganisms in it. This microbial quality of food could be evaluated by Standard Plate Count (SPC).There are three levels of standard plate count or aerobic plate count.

Level 1: applies to ready-to-eat foods in which all components of the food have been cooked in the manufacturing process/preparation.

Level 2: applies to ready-to-eat foods which contain some components that have been cooked and then further handled (stored, sliced or mixed)

Level 3: This applies to foods such as fresh fruits and vegetables (including salad vegetables). SPCs were not applicable to these types of foods. Colony counts only show the degree of contamination level but identification of organism is advisable(Sagoo *et al.* 2001).

2.8 Conceptual framework

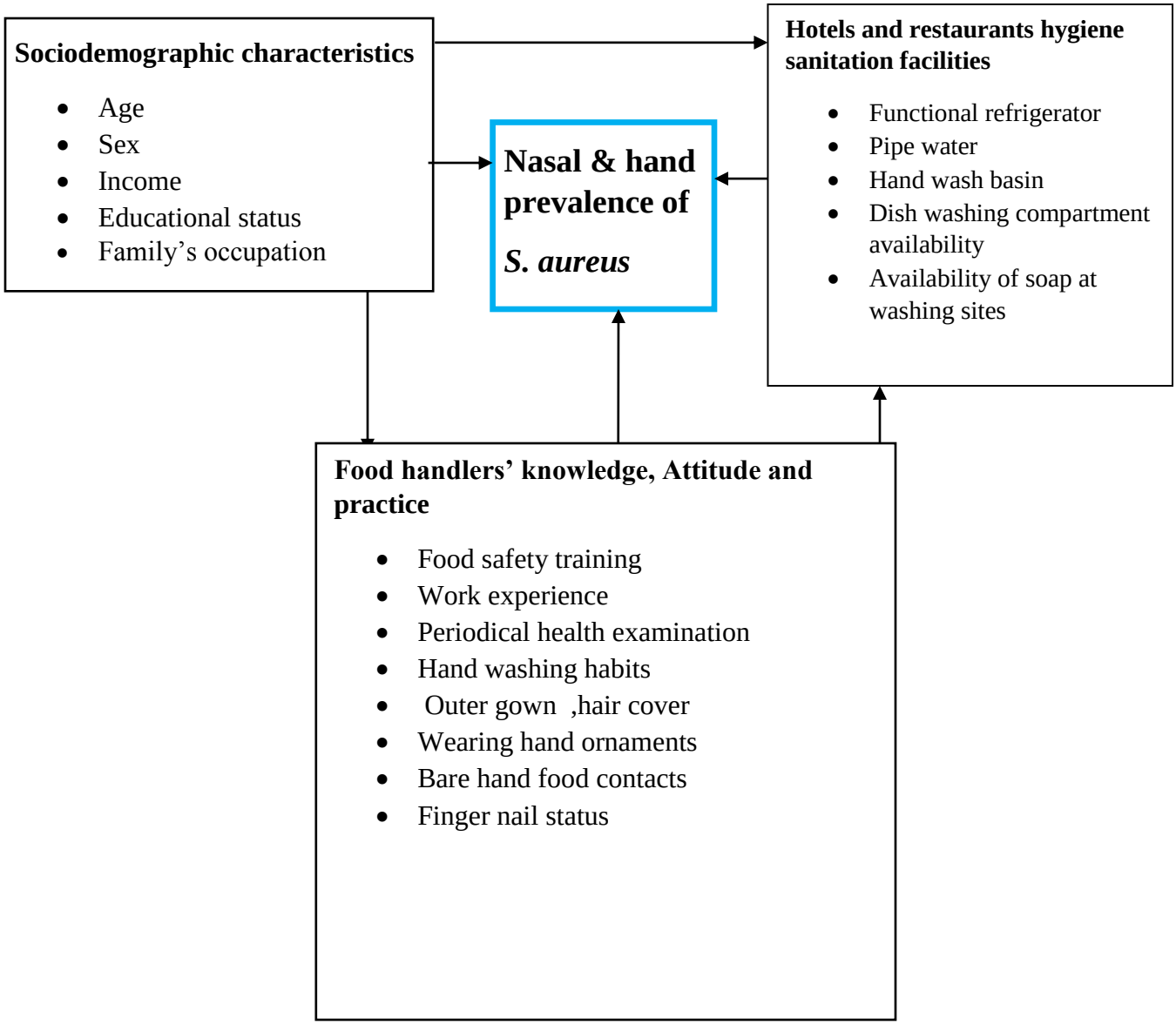

Figure 1: Conceptual framework after reviewing different literatures

CHAPTER THREE: OBJECTIVES

General Objective

The aim of this study was to assess the prevalence of *S.aureus*, its antimicrobial susceptibility pattern among food handlers working in hotels and restaurants in Jimma town, southwest Ethiopia, 2017.

Specific objectives

- The specific objectives of this study was to:

1. Identify the prevalence of nasal and hand carriage of *Staphylococcus aureus* among food handlers
2. Determine antimicrobial susceptibility pattern of *S. aureus* isolated from food handlers' nose and hand swab
3. Identify the associated factors regarding safe food handling.
4. Identify microbiological quality food samples collected from hotels and restaurants in CFU/g.

CHAPTER FOUR: METHODS AND MATERIALS

4.1Study area

The study was conducted in Jimma town. Jimma town is located in southwest Ethiopia at 355 km far from Addis Ababa at 70 13' and 80 56' N latitude and 35^0 52' and 37^0 E longitude. The area has an altitude of 1700-1750m above sea level. The total projected population of the town from 2007 Central Statistical Agency (CSA) census report was 120,960, of whom 60,824 are males and 60,136 females. Jimma town is divided into 13 urban administration kebeles and has four governmental health centers and two hospitals. There are also 79 hotels and 89 restaurants in these kebeles in the report document of Jimma town health office in 2016.

4.2 Study design and period

A cross-sectional study was conducted from February 30 to May 2017 among food handlers working in hotels and restaurants in Jimma town

4.3 Source population

All food handlers working in restaurants and hotels in Jimma town

4.4 Study population

Selected food handlers working in the restaurants and hotels in Jimma town.

4.5 Sample Size

Sample size (n) was calculated using single population proportion formula. Both nares of nasal carriage prevalence of *S.aureus* 21.5% among restaurant food handlers in Samara city was taken as proportion(Alsamarai *et al.* 2015).

Therefore, p=0.215, **Z**=95 %, d=5% and q=1-p

$$\boldsymbol{n = \frac{(za/2)^2) * p(1-p)}{d^2}}$$

Where:

n=the required sample size

p= prevalence for *S.aureus* nasal carriage contamination rate (21.5%)

z = confident interval at 95% with standard value of 1.96.

q= 1.0 – p

d = degree of absolute precision/ error tolerated (0.05)

$$\boldsymbol{n = \frac{(1.96)^2) * 0.215(0.785)}{(0.05)^2}} = \mathbf{259.}$$

= Including 16% non-response rate, the sample size for this study was **300.** For Face-to-face survey , 80-85% response rate is good(Tourangeau *et al.* 2007).

4.6 Sampling Frame

According to Jimma town Municipality, Trade & Market Development Office and Health Office's health related services and products quality control work process; the sampling frame is about 79 hotels and 89 restaurants in Jimma town (unpublished report, 2016).

4.7. Sampling technique

In order to get an average number of food handlers, preliminary survey was conducted among 50% of 168 hotels & restaurants using Simple Random Sampling Technique (lottery method) with their specific trade name and drawn from a box without replacing the drawn trade name. Therefore, the average number of food handlers per establishment was seven. Therefore,46 food establishment were required to get 300 food handlers (Huang 2016). Three hundred food handlers working in these 46 food establishments were selected by simple random sampling technique (lottery method) by drawing their identity code when their number exceeds seven per food establishment. Otherwise taken all since a food establishment first selected by SRS (Tassew *et al.* 2010).

4.8 Inclusion and Exclusion Criteria

4.8.1 Inclusion criteria

Food handlers who were engaged in food preparation, serving, and utensils washing in the selected hotels and restaurants.

4.8.2 Exclusion criteria

Food handlers who had scaled skin syndrome, eczema, cutaneous impetigo and wound

4.9 Study variables

Dependent variables

Prevalence of *S. aureus* on food handlers' nares and hands
Antimicrobial susceptibility test

4.9.2 Independent variables

Socio-demographic characteristics

- Age
- Sex
- Educational status
- Income
- Family's occupation
- Working experience in Years
- Food safety training

Knowledge

Attitude

Practices

- Periodical medical check up
- Outer protective gown and Hair cover
- Hand ornaments of food handlers
- Bare hand food contacts
- Finger nail status

4.10 Data and specimen collection Procedure

4.10.1 Data collectors

Two laboratory technicians and two environmental health professionals were recruited for Knowledge, Attitude and Practice data collection from food handlers and food establishments' observational findings.

4.10.2 Questionnaire and observational check list

A structured questionnaire was used to collect data regarding knowledge, Attitude and Practice of food handlers. The questionnaire was adapted from related researches done before this study(Sharif *et al.* 2013).

The assessment tool was divided into five sections as followed:

Part I (background section): Includes 8 socio-demographic characteristics

Part II (Practice section) includes 6 questions. The score range was between 0 – 6 and results were converted to 100 points. Food handlers who was scored >50% was categorized as safe practice and ≤50% it considered as unsafe practice (Sharif *et al.* 2013(Akabanda *et al.* 2017).

Part III (knowledge section): Includes 8 questions. The score range for knowledge of food handlers was between 0 - 8 and the sum of it was converted to 100 points. Food handlers who scored above 75% was categorized as good and below it as poor(Meleko 2013).

Part IV (attitude sections): Includes 9 questions. All questions evaluate attitude and put in a degree of agreements scored on a five-point scale (1 to 5) with options of (5)strongly agrees, (4)agree, (3)not sure, (2)disagree and (1)strongly disagree. For dichotomous classification the scores 1,2 & 3 was categorized as unfavorable attitude or ≤75% while the scores 4 and 5 was categorized as a favorable attitude or >75% (Sharif *et al.* 2013).

Part V (Observation check list section): Includes 11 attributes. The score of an establishment was between 0-11 and the sum of results was converted to 100 points. A score of "1" was assigned for the presence of appropriate facility while a score of "0" was assigned for its absence. When these establishments score ≤50% of 11 assessment parameters the result was classified as poor hygienic & sanitation facilities in restaurant or hotel while >50% was classified as good (Okojie *et al.* 2014).

4.10.1 Nasal and hand swab sample collection

Participants were informed prior to giving a nasal swab. After getting their willingness a swab specimen was taken by cotton tipped sterile applicator stick moistened with saline solution. The tips inserted 1–2 cm inside the anterior nares in both nostrils and rotating six times. Then the swabs immediately insert into a sterilized screw capped tubes containing 5ml normal saline. During specimen collection, data collectors were worn disposable gloves (Dagnew *et al.* 2012;Vatansever *et al.* 2016).

For hand swab, notification was not given in advance for extra hand hygiene during the hand swab sample collection. A sterile cotton tip applicator stick technique was employed to collect the hand swab samples. Participants was asked to stretch their hands and swabbed with normal saline moistened swab at their palm, between finger nails and at finger tips or under nails then put the swab in test tube with tight screw cover and labeled. The specimen was collected at both shifts of a day and weekends. Then the test tubes were immediately transported in ice box within 1-3 hours duration to Jimma University Specialized Hospital bacteriology laboratory for examination(Assefa *et al.* 2015; Castro *et al.* 2016,Woh *et al.* 2016).

4.10.2 *S.aureus* isolation and identification

The nasal and hand swab specimens were inoculated on Mannitol Salt Agar (Oxoid, England) and the plates were incubated at 37^{o}C for 24 hours under aerobic conditions. After this the growth of *S.aureus* results the change of Phenol red pH indicator were regarded as presumptive detection of the salt tolerant Staphylococci. Therefore, formation of yellow Golden colony on fermented Mannitol to yellow background was taken as suspected positive for further tests. Then basic identification tests explicitly, Gram staining, catalase and coagulase tests was performed (Vatansever *et al.* 2016) (Graham *et al.* 2006; Dagnew *et al.* 2012; Ho *et al.* 2014; Castro *et al.* 2016; Vatansever *et al.* 2016; Woh *et al.* 2016).

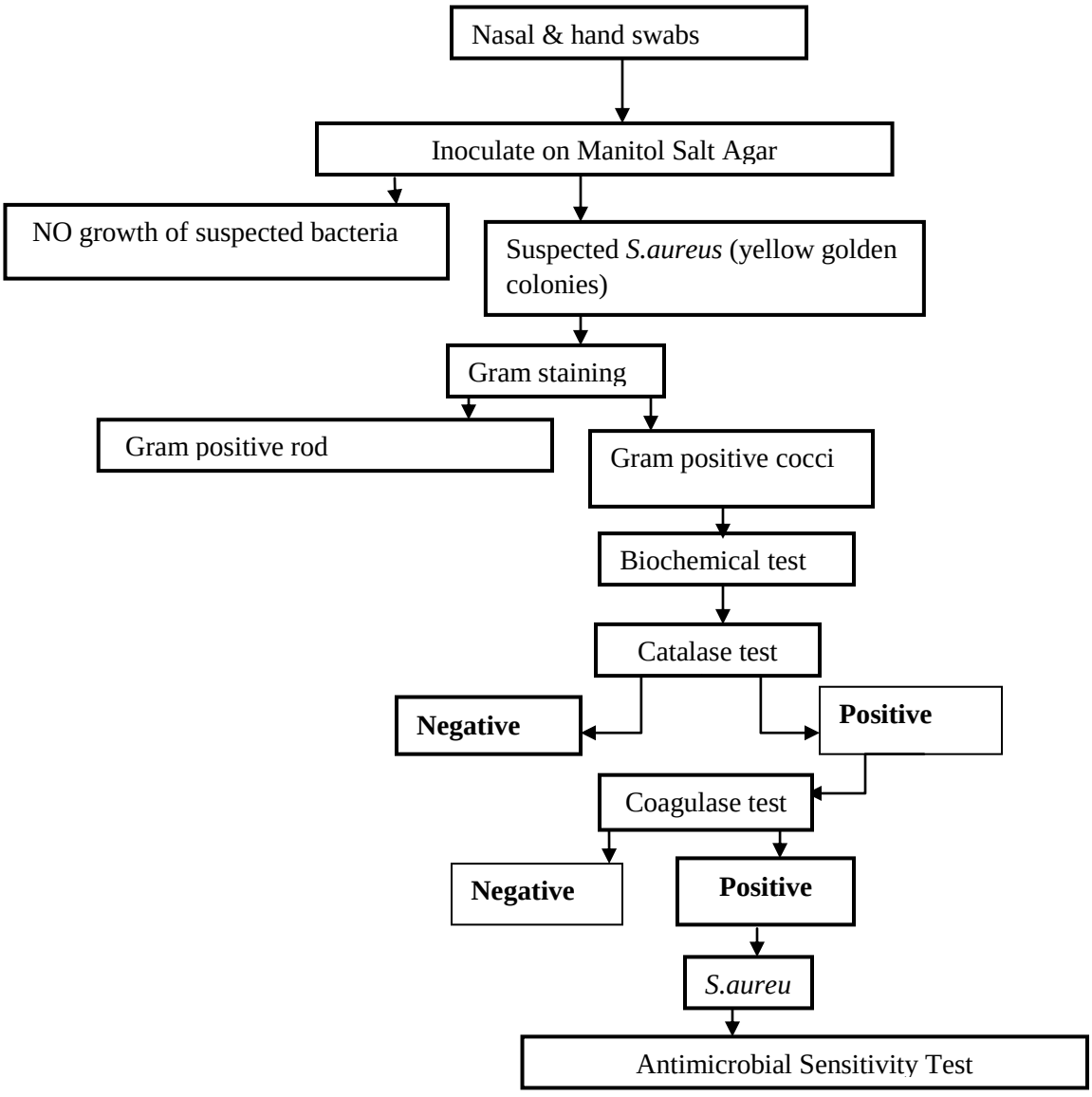

Figure 2: Simplified laboratory flow chart for identification of nasal & hand prevalence *S.aureus* from swab specimens.

4.11 Food sampling methods

Clean, dry, leak-proof, wide-mouthed, sterile, polyethylene plastic screw caped containers were used for food sample collection. More than 100 gram food from each 43 establishment were delivered to the laboratory within 1 to 3 hours in cold (0 to 5^{o}C) transporting box (Holland *et al.* 2003). Food samples were collected after knowing the S.aureus status of food handlers. This is because of foods for microbial quality should be taken from *S.aureus* positive food handlers for

the sake of checking whether the aerobic mesophilic bacteria and *S.aureus* bacteria can transfer to food they have prepared or not. The sample should be protected against extraneous contamination by the air, the sample container, the sampling devices used and improper handling. A sample container should not be filled more than three-quarters in order to avoid leakage and to allow proper mixing of the sample in the laboratory. Food items were packed with separate sterile polyethylene plastic bag and after sealing and labeling it were transported at 0-5^{o}C temperature to Jimma University specialized hospital microbiology laboratory within 1 to 3h (Van Egmond *et al.* 2007).

About 100 gram of prepared foods all components have been cooked and final food products was collected from each 43(50%) food handlers who had *S.aureus* on their nose and hand. The sample collection time was all the day in a week and both morning and afternoon. From aseptically collected as well as labeled food samples, 25g of each sample was well mixed individually into a sterile homogenizing beaker containing 225ml peptone water, homogenize with 8000-10000 rpm for 1-2min. Then after 1h, 1ml of each food homogenate sample was transferred aseptically to 9ml sterilized peptone water in test tube and mixed thoroughly. The homogenates were serially diluted from 10^{-1} to 10^{-8} and then a volume of 1ml aliquot was spread on solid Manitol Salt Agar (Oxoid, England) particularly for *S.aureus* and on Standard Plate Count Agar for aerobic mesophilic bacteria count at the same time in the same dilution factor. Then all plates turned up- side down and incubated at 36$\pm$1^{o}C for 48h$\pm$2h (Bolton *et al.* 1996; Van Egmond *et al.* 2007).

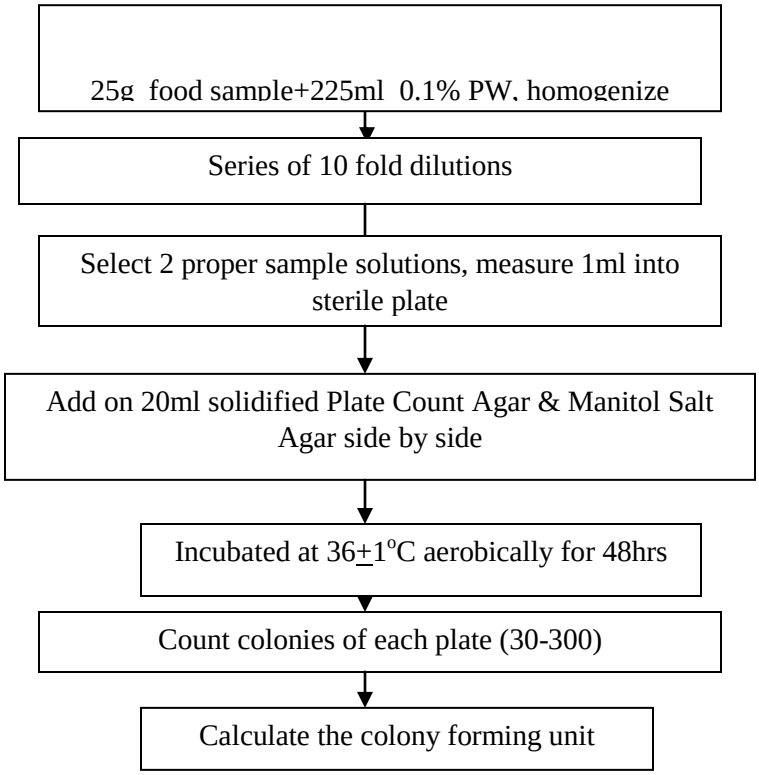

Figure 3: The Examination Procedures of plate count**(Jia *et al.* 2013)**

4.11.1 Microbiological quality of food samples

The significance of Standard Plate Count markedly varies according to the type of food products and the processing it has passed on.

Microbiological quality of cooked ready to eat food was examined by the average counts of CFU/g of food samples compared with the ranges put in the standard as satisfactory, marginal, unsatisfactory and potentially hazardous to consumers' health. On Standard Plate Count (SPC), an average aerobic mesophilic bacteria CFU/g in ready to eat foods per gram categorized as satisfactory ($<10^5$) Marginal (10^5) and Unsatisfactory ($\geq 10^{5)}$.

On the other hand, for Coagulase +ve *S.aureus* bacteria by selective Manitol Salt Agar the average CFU/g food samples were distinguished as $<10^2$ satisfactory, 10^2-10^3 marginal, 10^3-10^4 unsatisfactory and $\geq 10^4$ potentially hazardous to health of consumers by producing enterotoxins(Sagoo *et al.* 2001).

Aerobic Mesophilic Bacteria CFU/g calculation method

A tenfold serial dilution 1ml of aliquot was spread on pre-dried Standard Plate Count (SPC). Total plate counts with colony number 30-300 was Selected. Plates with <30 CFU/g, recorded as

too few to count (TFTC) while >300 CFU/g recorded as too numerous to count (TNTC). Colony counting was performed by colony counter and multiplied by the corresponding plate dilution factors then reported in CFU/g of food samples (Mekonnen *et al.* 2003).

SPC was calculated for two continuous dilutions with two replicates as:

$N = C/V [n_{1+} (0.1n_2)] d_1$.

Where

N =Plate Count in sample

C = is the sum of the colonies counted from two successive dilutions.

n_1= the number of colonies on the plates of the first dilution.

n_2=the number of colonies on the plates of the second dilution.

d_1 =Dilution Factor (the first dilution)

V=Diluents volume (1% peptone water)

S.aureus colony count

In the same way of the Standard Plate Count Agar, technique appropriate dilution of 1ml aliquot was spread on pre-dried Manitol Salt Agar (Oxoid, England) plate and incubate at 37^{o}C for 48hs.This method determines the presence of *S. aureus* by plating known quantities of (dilutions of) a food sample onto a selective agar. After incubation, presumptive staphylococcal colonies were selected, and subjected to confirmatory tests. From the results of these tests, the number of *S. aureus* colonies per gram of food sample was calculated(Mekonnen *et al.* 2003).

Coagulase Test

Coagulase test was done using slide test and tube test procedures. In slide test, a colony of the purified isolates was emulsified in a drop of distilled water on two ends of clean glass slide to make thick suspension. The first slide labeled as test and the second also labeled as control. Then a drop of rabbit plasma was added to the first suspension and mixing gently and wait about 10 seconds. Coagulation within 10 seconds was observed when the organism is *S.aureus*. Like wise to identify *S.aureus* the colony of suspected *S.aureus* was taken from culture media and

suspension made on two test tubes. The first test tube labeled as test and the second as control. The control test tube only normal saline and plasma suspension. On the test tube 0.8ml was made from 0.2 ml rabbit plasma and 0.6ml normal saline suspension and then incubated at 37^{o}C $\pm$2 for 4 hrs. Finally the control was compared with test result. When the organism is *S.aureus* there was coagulation(Mekonnen *et al.* 2003; Cheesbrough 2006).

4.12 Antimicrobial Susceptibility testing of *S.aureus*

Antimicrobial susceptibility test was done using 13 different disk diffusion methods (Kirby Bauer *et al.*, 1966) and reported according to Clinical Laboratory Standards Institute guidelines(Humphries *et al.* 2016).

The 13 drugs with their respective concentration were used:- *Ampicillin (AMP,10 µg), Amoxicillin-Clavulanic Acid (AMC,30 µg), Ceftriaxone (CRO,30 µg), Ciprofloxacin (CIP,5 µg), Chloramphenicol (C,30 µg), Erythromycin (E,15 µg), Gentamicin (CN,10 µg), Kanamycin K,30 µg),Oxacillin(OX1C,1*µg*) Cefoxitin (CXT,30 µg), Tetracycline (TE,30 µg), and Trimethoprim-Sulphamethoxazole (TMP-SXT,1.25/23.75µg),*Vancomycin (VA,30 µg) and Penicillin G (,P10 units). The plates were incubated at 37^{o}C for 24 hours. A diameter of the zone of inhibition around the disc was measured to the nearest millimeter using a ruler. Antimicrobial interpretive criteria was described as Susceptible(S), Intermediate (I) and Resistant(R).For positive control; *Staphylococcus aureus* ATCC 6538, was taken from Ethiopian Health and Nutrition Research Institute/Pastor(Mekonnen *et al.* 2003; Humphries *et al.* 2016)

Test performed by Kirby-Bauer method

A sterile swab was dipped into the bacterial suspension; any excess fluid was expressed against the side of the tube then, the surface of a Mueller-Hinton agar plate was inoculated by bacterial isolate. The whole surface of the plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another 90º and streaked once more.

Antimicrobial disc was picked up by a sterile forceps and placed on the surface of the inoculated Muler Hinton Agar (MHA) plate. The disc was pressed gently into full contact with the agar. All antimicrobial discs under the test spaced evenly a way from each other. Then, the plates were incubated at 35ºC for 24 hours.

Finally, after 24 hrs. of incubation the plates were examined for the presence of inhibition zone of bacterial growth around 13 antimicrobial discs, if there was no inhibition zone the organism was reported as resistant to the antimicrobial agent in that disc. If a zone of inhibition surrounded the disc, the diameter of the zone of inhibition was measured (by millimeters) and compared their sizes with listed in a CLSI, 2016(Humphries *et al.* 2016).

Table 1: Zone Diameter Interpretation standards(Humphries *et al.* 2016).

Antimicrobial Agents	Disc potency (µg/ml)	Zone diameter nearest whole mm		
		Resistant	Intermediate	Susceptible
Ampicillin (AMP)	10	≤28	-	≥29
Amoxicillin-Clavulanic Acid(AMC)	30	≤21	-	≥22
Ceftriaxone (CRO)	30	≤21	-	≥22
Ciprofloxacin(CIP)	5	≤15	16-20	≥21
Chloramphenicol (C)	30	≤12	13-17	≥18
Erythromycin (E)	15	≤13	14-22	≥23
Gentamicin (CN)	10	≤13	14-22	≥23
Cefoxitin (CXT)	30	≤21	-	≥22
Tetracycline (TE)	30			
Trimethoprim-Sulphamethoxazole (TMP-SXT)	*1.25/23.75*	≤10	11-15	≥16
Vancomycin (VA)	30	≤15		≥15
Penicillin G (P)	10	≤28	-	≥29
Oxacillin(OX1C)	1	≤21	-	≥22
Ampicillin (AMP)	10	≤28	-	≥29

4.13 Operational definitions

Food Safety: Conditions and measures that are necessary during production, processing, storage, distribution and preparation of food to ensure that it is safe, sound, wholesome and fit for human consumption(WHO, 1984).

Food borne diseases: All intoxication, infection, or illness contracted by the consumption of contaminated food (Assefa *et al.*, 2016).

Food handler -any person who directly handles packaged or unpackaged food, food equipment and utensils, or food contact surfaces and is therefore expected to comply with food hygiene requirements(Meleko 2013).

Personal hygiene : refers to those protection measures primarily with the responsibility of the individual, which promote and limit the spread of infectious disease, like hand washing using soap and water, keep body clean etc(Meleko 2013).

Safe food handling: Handling or storing of food with inhibiting conditions for the growth of pathogenic microorganisms

Practice: To assess the level of practices accustomed ways and habits employed by food handlers during cooking, handling and serving(Sharif *et al.* 2013(Akabanda *et al.* 2017).

Knowledge: To assess the level of awareness, ideas and facts about food preparation, handling and serving by food handlers(Meleko 2013)

Attitude: To assess the predisposition or a tendency to respond positively or negatively towards a certain idea, object, person, or situation. Attitude influences an individual's choice of action, and responses(Sharif *et al.* 2013).

Functional latrine: latrine with slab and superstructures and that provided services at the time of data collection even if the latrine required maintenance(Awoke *et al.* 2013).

Hand washing facilities: its availability and presence of soap during data collection around toilets and dining rooms.

Functional refrigerator: perishable foods stored in a deep freeze in separated container with tight cover and in a clean condition during data collection.

Restaurants: are businesses which prepare and serves food and beverages to customers in exchange for money. Meals are generally served and eaten on the premises, but many restaurants also offer take away .It vary greatly in appearance and offerings (Mysen *et al.* 2014).

Hotels: are public establishments offer two major types of services: (a) accommodation and (b) dining services. Services delivered are private bath, telephone, radio, and television, laundry, valet, cleaning and pressing ballrooms, health spas, coffee shops, dining rooms, cocktail lounges or night clubs, gift shops or newsstand-tobacco counters, and business centers for social occasions, health buffs, and business conferences(Edralin *et al.* 2001).

4.14 Data Processing and analysis procedures

Each questionnaire's consistency and completeness was checked daily and corrected immediately. Data analysis was performed using Epi data 3.1 and exported to SPSS window 21.0 versions. Data analysis done by:

Univariate analysis was used to examine the frequency and proportion of independent variables (Socio-demographic characteristics, variables in relation to Knowledge, Attitude, Practice, food samples' aerobic mesophilic bacteria and *S.aureus* bacteria CFU/g, food establishments' sanitation facilities).

Bivariate analysis also used to identify the relation between an independent and dependent variables. Independent variables are: Socio-demographic characteristics, variables in relation to Knowledge, Attitude, Practice, food establishments' sanitation facilities with dependent variable Food handlers' nasal and hand prevalence of *S. aureus).*Here p-value < 0.25 was taken as candidate to multivariate analysis.

Multivariate analysis was the last screening method to identify relationships between multiple independent variables & a dependent variable to *S.aureus* presence or not. When the association was less than 0.05 p-values the result was concluded as significantly associated.

4.15 Data and specimen collection quality control

4.15.1 Questionnaire

All data and specimen collectors were trained for two days. The training was focusing on the way of communication with participants and specimen handling from nasal swab and hand rinse from each food handler working in restaurants and hotels in Jimma town.

Five percent of the questionnaire was pre-tested in Jimma zone Agaro town. Questions were adapted from related articles and adjusted in relation to this study objective and finally, English version was translated to Amharic and Afan Oromo then back to English. All members of the study team were trained for two days to obtain consistent interviewing standards. Data were checked for consistency and completeness during data collection, data entry and analysis. Epi-data templates was prepared to enter data then exported to SPSS for analysis.

The consistency of likert scale items were checked by Cronbatch Alpha which was 0.82. All items with Cronbatch Alpha $\geq$ 0.6 were taken for analysis.

Laboratory Procedures quality control

To manage the quality of the study standard laboratory procedures were strictly followed during processing of each sample. All the instruments used for sample processing were checked for proper functioning. Control strains of *S. aureus* ATCC 6538 used as standard and *Escherichia coli* ATCC 25922 also for MSA media inhibition for gram negative bacteria were used throughout the procedure. Both strains were obtained from Ethiopian Health and Nutrition Research Institute Laboratory. Culture media also were also prepared based on the manufactures instruction. Then the sterility of culture media was checked by incubating the batch at 35-37^{o}C overnight and observed for bacterial growth. Those media showed any growth was discarded (Assefa *et al.* 2015).

For Antimicrobial Sensitivity Test control

From a pure culture 3-5 colonies of bacteria was taken by using a sterile inoculation loop and transferred to a tube containing 5 ml sterile normal saline and mixed gently to a homogenous suspension and the turbidity of the suspension was adjusted to a McFarland standard 0.5 which

corresponds to cell density of about 10^7-10^8 CFU/g. McFarland is a $BaSO_4$ standard against which the turbidity of the test. This standard was made by mixing 1% v/v solution of H_2SO_4 and 2^{nd} solution of 1% v/v solution of $BaCl_2$ .An amount of 0.5ml $BaCl_2$ mixed with 99.5ml of H_2SO_4 gives 0.5 McFarland in a screw cap bottle to compare the turbidity of test suspension.(Piot *et al.* 1991).

4.16 Ethical consideration

This research project was conducted after getting approval from Jimma University, Institute of Health Research Ethics Committee. Next permission was obtained from Jimma town Municipality and Health office. During data collection the aim of the study was communicated to the respondents and the owner of food establishments then they were signed on the consent form (Annex II) prepared for this purpose. Food handlers who were found to be positive for *S.aureus* were advised to contact health professionals before they go back to the usual work. Despite keeping results confidentiality, the health information related to food borne diseases and performing the necessary good hygienic practices when handling food were strongly advised.

4.17 Dissemination plan

The finding of the study will be presented to Jimma University, Institute of Health, department of environmental health science and technology as a requirement for partial fulfillment of post graduate study. The copy of the thesis will also be given to Jimma town Health Office JU's health science library and Department of Environmental Health Sciences and Technology. Finally, an attempt will be made to publish on peer review journals.

CHAPTER FIVE: RESULTS

5.1 Sociodemographic characteristics and *S.aureus* prevalence

A total of 300 food handlers participated in this study making a response rate of 100%. Of these 197(65.7%) were females and the rest were males. Among the total participants 160 (53.3%) were found in the age range of 20 to 29 years. The average age of food handlers was 22.51 years. Regarding their job position 138(46%) were Cook, 122(40.7%) were waiters and 40(13.3%) were utensils cleaners. Majority 166(55.3%) of food handlers completed primary school and 32(10.7%) of them hand no formal education (Table 1).

Regarding their monthly salary 170(56.7%) of food handlers earn 500 to 1000 Eth. Birr but 101(33.7%) of food handlers get less than 500 Eth. Birr. Concerning working experiences, majority 162 (54%) of food handlers had 2-5 years of experience followed by 76(25.3%) for less than two years and 21(7%) for 10 years and above (Table 1).

Table 2: *S.aureus* prevalence in terms of socio-demographic characteristics of food handlers working in hotels and restaurants in Jimma town, Feb 30 to May 30/2017.

Variable		*S.aureus* positive		*S.aureus* negative		Total participants	
Sex	Male	Frequency	%	Frequency	%	Frequency	%
		26	25.2	77	74.8	103	34.3
	Female	60	30.5	137	69.5	197	65.7
	Sub total	86	28.7	214	71.3	300	100
Age	< 20 year	32	30.5	73	69.5	105	35.0
	20-29 year	45	28.1	115	71.9	160	53.3
	30-39 year	8	30.7	18	69.3	26	8.7
	≥ 40 year	1	11.1	8	88.9	9	3.0
	Sub total	86	28.7	214	71.3	300	100
Job category	Cook	50	36.3	88	63.7	138	46.0
	Dish cleaner	14	35	26	65	40	13.3

	Waiter	22	18.1	100	81.9	122	40.7
		86	28.7	217	71.3	300	100
Educational status	University	1	33.3	2	66.7	3	1.0
	College	5	35.7	9	64.3	14	4.7
	High school	20	23.5	65	76.5	85	28.3
	Elementary school	50	30.1	116	68.9	166	55.3
	Can't read and write	10	31.2	22	68.8	32	10.7
	Sub total	86	28.7	214	71.3	300	100
Income in Eth. Birr/month	< 500	28	27.7	73	72.3	101	33.7
	500-1000	45	26.5	125	73.5	170	56.7
	1000-1500	6	37.5	10	62.5	16	5.3
	1500-2000	3	75	1	25	4	1.3
	above 2000	4	44.4	5	55.6	9	3.0
	Sub total	86	28.7	214	71.3	300	100
Work Experience	<2 years	27	35.5	49	64.5	76	25.3
	2-5 years	44	27.2	118	72.8	162	54.0
	6-9 years	11	26.8	30	73.2	41	13.7
	≥10 years	4	19.1	17	80.9	21	7.0
	Sub total	86	28.7	214	71.3	300	100

5.2 Nasal and hand carriage of *S.aureus*

Among (n=300) food handlers, those working in hotels and restaurants, 86(28.7%) were colonized by *S. aureus* (Table 2). Total, *S.aureus* prevalence of food handlers in terms of their body parts showed that nasal, hand and both nose and hand were 27, 34 and 25 respectively(Fig 4). Female food handlers were the majority 60(69.8%) of the total *S.aureus* positive (Table 1). According to the age category in the age range between 30-39 years 26(8.7%) food handlers

8(30.7%) were colonized by *S.aureus*. This finding was the highest among other age categories in this study. Moreover, regarding the job positions, among 138(46%) Cooks 50(36.3%) was carrying *S.aureus.* Relatively, among 122(40.7%) waiters, only 22(18.1%) were carry *S.aureus*. In relation to food handlers' educational status, the highest findings was among 14(4.7% total participants there were 5(35.7%) were positive for *S.aureus.* Regarding food handlers' monthly average income, among 170(56.7%) participants who earn 500-1000 Eth. Birr per month was 45 (26.5%) which was the highest prevalence of *S.aureus* Service years also showed a relation with prevalence rate of *S.aureus*. Food handlers who had <2 years working experience were 76(25.3%), among these participants 27(35.5%) were positive for *S.aureus* (Table2). Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via direct contact or through respiratory secretions. Food handlers rubbing their runny nose with hands and touching any food contact materials can transfer *S.aureus* to cooked moist protein rich foods. *S.aureus* on hand(11.3%) was higher than in nose(9%),could be due to hands of food handlers' may have contamination out of their body parts from other contaminated surfaces(Fig:4).

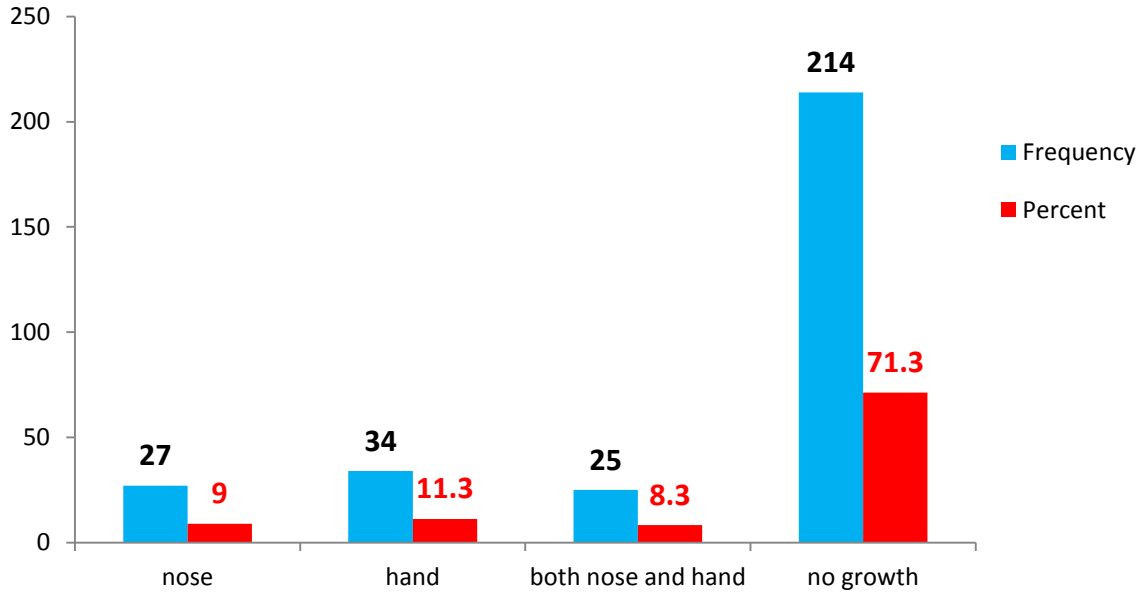

Figure 4:*S.aureus* carriage prevalence among food handlers in Jimma town, 2017.

Table 3: *Staphylococcus aureus* growth category among hotel and restaurant food handlers

Food establishments	*S.aureus* positive No (%)	*S.aureus* negative No (%)	Total No (%)
Hotel	46(53.5)	111(51.9)	157(52.3)
Restaurant	40(46.5)	103(48.1)	143(47.7)
Total	86(100)	214(100)	300(100)

Regarding the prevalence rate of *S.aureus* between hotel workers and restaurant workers there were not have significant differences. In this finding the number of food handlers who had *S.aureus* in hotels were 46(53.5%) and in restaurant workers 40(46.5%) (Table2).

5.3 Personal hygiene practices of food handlers

The following table showed that the actual activities performed during data collection. Food handlers practices were recorded just by observing what they were did. Therefore, food safety practices against *S.aureus* prevalence were described in table 3.

Table 4: Personal hygiene practices of food handlers working in Jimma town hotels and restaurants from Feb.30 to May 30, 2017.

Variables	Categories	*S.aureus* positive		*S.aureus* negative		Total participants	
		No	%	No	%	No	%
Food handler wear outer garments/gown during data collection	NO	21	23.3	69	76.7	90	30.0
	Yes	65	31	145	69	210	70.0
Food handler wear hair cover during data collection	NO	48	25.3	142	74.7	190	63.3
	Yes	38	34.5	72	65.5	110	36.7
Food handlers wash their hands after touching any soiled materials	NO	31	34.8	58	65.2	89	29.7
	Yes	55	26.1	156	73.9	211	70.3

Food handler wear hand ornaments during data collection	NO	27	22	96	78	123	41
	Yes	59	33.3	118	66.7	177	59.0
Food handlers practice bare-hand food contact during data collection	NO	61	27.7	159	72.3	220	73.3
	Yes	25	31.2	55	68.8	80	26.7
Food handlers finger nail presence during data collection	NO	23	28	59	72	82	27.3
	Yes	63	29	155	71	218	72.7
	Sub total	86	28.7	214	71.3	300	100
Food handlers have taken training about food safety	Yes	11	24.4	34	75.6	45	15.0
	NO	75	29.4	180	70.6	255	85.0

Regarding personal hygiene practices of food handlers, majority 210 (70%) were worn outer protective gown among these 65(31%) were positive for *S.aureus*. Similarly, 110(36.7%) were worn hair cover during their actual work but 38(34.5%) colonized by *S.aureus.* These findings were higher than among food handlers did not worn gown 21(23.3%) and not worn hair cover 48(25.3%).

Among 177(59%) food handlers who worn hand ornaments 59(33.3%)were positive for *S.aureus*. Likewise, among 80(26.7%) of food handlers who touch ready to eat foods with bare hands, 25(31.2%) of them were carry *S.aureus.* These findings were higher than not wear hand ornaments 27(22%) and not touch foods with bare hand 61 (27.7%).

Out of 218(72.7%) food handlers who did not trimmed their finger nails 63(29%) of them were positive for *S.aureus.* This result was higher than trimmed their finger nail 23(28%). Moreover, among 255(85%) food handlers who did not have ever training about safe food handling, 75(29.4%) had *S.aureus* but food handlers who have taking training before 11 (24.4%)(Table 3). Findings showed that wearing outer garment did not have significant effect on *S.aureus* prevalence but wearing hand ornaments and not trimming finger nails and not having training about food safety more likely causes to *S.aureus* colonization compared to not having hand ornament and trimmed finger nails.

5.4 Knowledge of food handlers

Among 300 study participants, 266 (88.7%) of food handlers were surely answered that proper hand washing can prevent food borne illnesses. Therefore, only 69(26%) of them were positive for *S.aureus.* This prevalence was lesser than not knowing and not sure answered. Similarly 213(71%) answered that coughing and sneezing towards foods potentially contaminate not covered food and 59(27.7%) of them carry *S. aureus*. This prevalence rate of *S.aureus* was less than those answered as not contaminate food by coughing and sneezing towards food *(*Table 4).

Table 5: Knowledge of food handlers' towards safe food handling questions in Jimma town

Variables	Response/ categories	*S.aureus* positive		*S.aureus* negative		Total participants	
		No	%	No	%	No	%
Hand wash prevent food borne disease	Yes	69	26	197	74	266	88.7
	NO	7	54	6	46	13	4.3
	Not sure	10	48	11	52	21	7
Coughing or sneezing contaminate food	Yes	59	27.7	154	72.3	213	71
	NO	21	35	39	65	60	20
	Not sure	6	22.2	21	77.8	27	9
Bare hand touch contaminate foods	Yes	69	27.2	185	72.8	254	84.7
	NO	10	38.5	16	61.5	26	8.7
	Not sure	7	35	13	65	20	6.6
Apparently healthy person may carry microorganisms	Yes	29	23.4	95	76.6	124	41.3
	NO	45	31.2	99	68.8	144	48
	Not sure	12	37.5	20	62.5	32	10.7
Fever, nausea, vomit and diarrhea could by symptom of food borne illnesses	Yes	19	28.8	47	72.2	66	22
	NO	66	28.9	162	71.1	228	76
	Not sure	1	16.7	5	83.3	6	2

5.5 Attitude of food handlers

From the summary of attitude questions, among 286(95.3%) food handlers who had favorable attitudes towards safe food handling activities only 77(26.9%) were colonized with *S.aureus*. Whereas from 14(4.7%) of total food handlers who had unfavorable attitude, majority 9(64.3%)

were colonized *S.aureus*. This showed that, unfavorable attitude has significantly associated with *S.aureus* prevalence rate. Because, the majority 77(89.5%) food handlers who had favorable attitudes the least *S.aureus* prevalence. This findings showed that alike hygienic practices, food handlers' favorable attitude did not influence the prevalence rate of *S.aureus* (Table 5).

Table 6: Summary of Knowledge, Attitude and practice of food handlers' towards safe food handling and its influence on prevalence of *S.aureus*

Variable		*S.aureus* positive		*S.aureus* negative		Total participant	
		No	%	No	%	No	%
Knowledge	Poor	59	68.6	119	55.6	178	59.3
	Good	27	31.4	95	44.4	122	40.7
Attitude	Favorable	77	26.7	209	73.1	286	95.3
	Unfavorable	9	64.3	5	35.7	14	4.7
Practice	Good	50	58.1	127	59.3	177	59
	Poor	36	41.9	87	40.7	123	41

5.6 Bivariate analysis

Bivariate analysis was conducted to identify candidate variables for multivariate analysis. The Bivariate analysis P-value <0.25 variables were food handlers unfavorable attitude, wearing gown, hair net, hand ornament and working experiences (Table 6).

Table 7: Factors associated with *Staphylococcus aureus* prevalence among food handlers in Jimma town, 2017(P-value <0.25).

Variables	Response/category	Frequency	B	S.E.	P-value	COR	95% C.I	
							Lower	Upper
Job category	Waiter	122	-.949	.295	.001	.387	.217	.690
	Cook	138				*1		
Attitude	Unfavorable	14	-1.586	.573	.006	0.205	0.067	0.630
	Favorable	286				*1		
Wear Gown	NO	90	-.387	.291	.183	.679	.384	1.200
	Yes	210				*1		
Wear hair cover	Yes	110	-.446	.261	.088	.640	.384	1.068
	NO	190				*1		
Hand ornament	Yes	123	-.575	.270	.033	.563	.331	.955
	NO	177				*1		
Work experience	<1 years	76	.391	.298	.190	1.478	.824	2.649
	2-5 years	162				*1		

N: B *1 is reference groups, all variables in Bivariate analysis were listed down in Annex III

5.7 Multivariate Analysis

All candidate variables from Bivariate result (<P-value 0.25) were entered into multivariate analysis. Accordingly job category, wearing hand ornaments and food hinders' unfavorable attitude were significant predictors of the *S. aureus* prevalence rate. These dependent variables were analyzed by multivariate analysis in relation to dependent variable *S.aureus* presence or absence. Those are <P- value 0.05 (Table 7).

Table 8: Factors analyzed in Multivariate logistic regression to identify risk factors significantly associated with prevalence of *S. aureus*

Variable	Category	Frequency	B	S.E.	P-value.	AOR	95% C.I.	
							Lower	Upper
Attitudinal responses	Unfavorable	14	1.481	.637	.020	4.399	1.262	15.335
	Favorable	286				*1		
Wearing Hand ornaments	Yes	123	-.624	.304	.040	.536	0.295	0.973
	NO	177				*1		
Job category	Waiter	122	-.815	.406	.044	.442	0.200	0.980
	Cook	138				*1		

N:B *1 Reference groups

Food handlers who had unfavorable attitude were 4 times more likely carrier for *S.aureus* compared to food handlers with favorable attitude. Among food handlers who had unfavorable attitude, the prevalence of *S.aureus* were 9(64.3%) much higher than have favorable attitude77 (26.9%). Food handlers who wear hand ornaments were 0.536 times more likely exposed to *S.aureus* compared to those food handlers not wear hand ornaments. Among food handlers who wear hand ornaments 59(33.3%) were positive for *S.aureus* test which were higher than not wear hand ornament 27(22%). Regarding job position, Waiters were less likely colonized by *S.aureus*. Among 122(40.7%) total waiters only 22(18.1%) were positive for *S.aureus* test.

5.8 Antimicrobial Sensitivity Test

Over all 86 isolates were tested for 13 different drugs (antimicrobial discs).The majority78 (90.7%) of the isolates of *S. aureus* were resistant to penicillin and ampicillin. But there were less isolates were resistant to amoxicillin 5(5.8%). And high number of isolates were resistant to tetracycline 32(37.2%) and cotrimoxazole18 (20.9).On the other hand the current prescribed drug to MRSA Vancomycin 6(7%) resistant were observed in this study.

Twelve (14%) of the isolates were resistant to erythromycin, whilst 4 (4.7%) of the isolates were resistant to *Cefoxitin* and 6(7%) were resistant to Oxacillin. *Cefoxitin* and Oxacillin are

methicilin drugs .Therefore, resistance to Cefoxitin and Oxacillin are Methicilin Resistance. (Table8).

Table 9: Antimicrobial Sensitivity pattern of *S.aureus* isolated from food handlers in Jimma town southwest Ethiopia, 2017.

Antimicrobial agent	Disc potency (µg/ml)	Resistant		Intermediate		Sensitive	
		No	%	No	%	No	%
Ampicillin (AMP)	10	78	90.7			8	9.3
Amoxicillin-ClavulanicAcid(AMC)	30	5	5.8			81	94.2
Ceftriaxone (CRO)	30	6	7.0			80	93.0
Ciprofloxacin(CIP)	5	2	2.3	1	1.2	83	96.5
Chloramphenicol (C)	30	12	14.0			74	86.0
Erythromycin (E)	15	12	14.0			74	86.0
Gentamicin (CN)	10	3	3.5	10	11.6	73	84.9
Cefoxitin (CXT)	30	4	4.7			82	95.3
Tetracycline (TE)	30	32	37.2			54	62.8
Trimethoprim-Sulphamethoxazole (TMP-SXT)	*1.25/23.75*	18	20.9			68	79.1
Vancomycin (VA)	30	6	7.0			80	93.0
Penicillin G (P)	10	78	90.7			8	9.3
Oxacillin(OX1C)	1	6	7.0			80	93.0

5.9 Average microbial counts on food samples

S.aureus on food samples

Pasta and minced meat were the first highly exposed foods to contamination by *S.aureus* bacteria 2(33.3%) among the seven types of food samples collected. But there was no *S.aureus* bacteria was identified on cooked meat, cheese and cooked egg. Moreover, all sampled foods were contaminated by Aerobic Mesophilic Bacteria (Table 9).

Table 10: Bacteriological quality analysis of food samples in Jimma Town Hotels and Restaurants, 2017

S.No	Types of food samples	No samples	*S.aureus on MSA*		AMB on SPC	
			Mean CFU/g	SD	Mean CFU/g	SD
1	Rice	6	*$6.9x10^3$	$\pm 1.6x10^1$	**$1x10^8$	$\pm 1.5x10^3$
2	Pasta	6	**$5.8x10^3$	$\pm 1.2x10^2$	**$7.5x10^6$	$\pm 9.6x10^2$
3	cooked meat	11	-	-	*$6.4x10^4$	$\pm 1.1x10^1$
4	cooked vegetables	7	**$9.7x10^3$	$\pm 2.57x10^2$	**$9.7x10^7$	$\pm 1.3x10^3$
5	Cheese	3	-	-	**$1x10^8$	$\pm 1.70x10^4$
6	cooked egg	3	-	-	**$1x10^8$	$\pm 1.6x10^3$
7	minced meat	7	**$5.x10^3$	$\pm 1.3x10^2$	**$1.9x10^8$	$\pm 1.3x1^4$
	Total	43				

* Marginal * * Unsatisfactory *** potentially hazardous

5.10 Average Aerobic Mesophilic Bacteria colony counts on food samples

Based on an average $CFUg^{-1}$ of food samples microbial quality of food were classified into satisfactory, marginal, unsatisfactory and potentially hazardous to health. Satisfactory describes no need of prevention action and above marginal the foods needs an action to protect consumers' health. Therefore, Aerobic Mesophilic Bacteria (AMB) on Standard Plate Count Agar (SPC) categorized as satisfactory when average counts in $CFUg^{-1}$ less than 10^4, marginal 10^4 to 10^5 and greater than 10^5 considered as unsatisfactory.

Majority 37(86.1%) of food samples were unsatisfactory in microbial quality. Because the colony counts per gram food sample were outside of acceptable microbial limits (mean AMB $\geq 10^5 g^{-1}$).This indicated that there were poor hygiene or food handling practices in the food establishment. Only 6(13.9%) food samples were satisfactory (mean AMB, CFU/g < 10^4) (Sagoo *et al.* 2001).

5.11 Average *S.aureus* colony counts on food samples

Similarly, among the pathogenic bacteria, *S.aureus* colonies can be counted on Manitol Salt Agar (Oxoid, England) media. Based on the guide line, the food quality also classified upon the result of an average CFU/g of food. Due to this when coagulase positive *S.aureus* average CFU/g results less than 100,it categorized as Satisfactory, Marginal 10^2 to 10^3 ,Unsatisfactory 10^3 to 10^4 and $\geq 10^4$ was Potentially hazardous to consumers' health(Sagoo *et al.* 2001).

Moreover, the average colony count of *S.aureus* bacteria on Manitol salt Agar showed that majority 37(86.1%) of the completely cooked food samples were satisfactory. which was less than 100 per gram of food sample. However 1(25%) was marginal (10^2 to 10^3 CFU/g) and 5(11.7%) unsatisfactory (10^3 to 10^4 CFU/g) (Table 10).

Table 11: Microbial counts of (average CFU/g) of 43 food samples

Test		Microbiological quality (average $CFUg^{-1}$ and St. deviation)					
Media	Isolated bacteria	Satisfactory	Marginal	Unsatisfactory	Potentially hazardous	Mean	SD
*SPC	*AMB	6	0	37		$8.4x10^6$	$\pm 5.3x10^2$
*MSA	*S.aureus*	37	1	5	0	$9.4x10^3$	$\pm 1.3x10^2$

*MSA: Mannitol Salt Agar; *AMB: Aerobic Mesophilic Bacteria; *SPC: Standard Plate Count.

5.12 Food establishments' sanitation facilities

Among observed 23 hotels 73.9 % had functional and clean food storing refrigerators whereas less than half (47.8%) of restaurants had properly functioning refrigerators. During the observation period some of them had storing foods without any cover and in the same compartment of both cooked and raw food items.

All hotels and the majority of restaurants (73.9%) were had good hand washing facilities Moreover, Majority of hotels (95.6%) and all Restaurants had running tap water in their premises. Most hotels (86.9%) hotels and (82.6%) restaurants were put soaps at hand wash basins and utensils washing compartment during data collection period.

Table 12: Sanitation facilities in Hotels' and restaurants' Jimma town in 2017.

Sanitation facilities and food handlers documents	**Hotels**		**Restaurants**	
	Frequency	%	Frequency	%
Clean & functional refrigerator with separately stored and covered food staffs	17	73.9	11	47.8
Availability of soap at washing compartments	20	86.9	19	82.6
Three dish washing compartments	21	91.3	19	82.6
Food handler's medical document	2	8.6		
Food handler's training document	4	17.3		
Tap/running water availability	22	95.6	23	100
Clean and functional latrine	21	91.3	17	73.9
Clean and functional shower	21	91.3	9	39.1
Clean and functional hand washing basin	23	100	17	73.9
Solid waste storage bin with cover	4	17.3	1	4.34
Liquid waste from washing compartments disposal system(pit, septic tank, ditch in the ground)	19	82.6	15	65.2

CHAPTER SIX: DISCUSSION

In this study, the overall nasal and hand prevalence of *S.aureus* prevalence among food handlers was 86(28.7%). This result is lower than in Ekpoma Edo-Nigeria (60%) and in Egypt (31%) (El-Shenawy et al., 2013, Eke et al., 2015). These variations in nasal and prevalence rates were probably due to sample size differences between the studies populations.

On the other hand, this finding was higher than studies conducted in kars city-Turkey (20%), in Gonder University (20.5%), in Jimma University (23.5%) and Samara City (21.5%) (Dagnew et al., 2012, Ho et al., 2014, Alsamari et al., 2015, Vatansver et al., 2016).This inconsistency might be due to differences of pre-employment food safety trainings, study designs, socio-cultural differences and use of different assessment tools.

S.aureus prevalence among food handlers having working experiences less than 2 years were (35.5%) higher than among other categories service years. This is in line with study in Jimma university 67.2%(Assefa *et al.* 2015).

This study showed, food handlers wearing hand ornaments demonstrated that (33.3%) of them were carry *S.aureus*. This result was higher than not having hand ornaments. Moreover, food handlers who had unfavorable attitude accounts (64.3%) of *S.aureus* prevalence compared to food handlers who had favorable attitude towards safe food handling activities. On the other hand, in a job category waiters were less likely colonized by *S.aureus* compared to cooks and dish cleaners. Only (18.1%) of Waiters were positive for *S.aureus* test.

Drug sensitivity pattern among isolated *S.aur*eus showed that 78(90%) of isolates were resistant to amoxicillin and penicillin. But, majority isolates sensitive to Gentamicin, Ciprofloxacin, Cefoxitin and amoxaciline-clavulanic acid 83(96.5%), 83(96.5%), 82(95.3%) and 81(94.2%) respectively. The drug resistance development for these MRSA strains treatments may be due to mishandling and drug prescriptions out of the national drug prescription guide lines. This result is inconsistent with study in Ekpoma Edo state Nigeria , 100% resistant to ampicillin and 100% sensitive to Ciprofloxacin and Gentamicin respectively(Eke *et al.* 2015). The discrepancy might be due to difference of sample size and socio cultural situations.

In this study, isolates were resistance against Cefoxitin, Oxacillin and Vancomycin by 4.7%, 7% and 7% respectively. Oxacillin and methicilin are beta- lactam antibiotics .Therefore 6(7%) strains of *S.aureus* were resistant to methicillin. It is important to remind that the emergence and dissemination of MRSA (Methicillin Resistant *Staphylococcus aureus*) is an increasing global health problem that complicates the therapeutic management. Isolates resistance to vancomycin in this study was in line with 4(9.8%) reported in Gondar University and lower than reported in Samara City (28%). The variation could be due to sample size (Alsamarai *et al.* 2015). On the other hand, 6(7%) resistance against Vancomycin drug is unusual and needs attention. But Vancomycin has been prescribed to MRSA strains treatment. A study, conducted in Gonder University reported that there were no vancomycin resistant isolated *S.aureus*(Dagnew *et al.* 2012).

According to this study, 37(86.1%) food samples were unsatisfactorily contaminated by Aerobic Mesophilic bacteria. Therefore, an aerobic mesophilic bacteria count suggests that there were unhygienic activities done by food handlers .Meaning that excessively high ($>10^5$CFU/g) Standard Plate Count may reasonably an indicator of poor sanitation and utilization of non-functional refrigerators in food establishments (Sagoo *et al.* 2001).

In this study 21(91.3%) of hotels and 19(82.6%) of restaurants had three washing compartments or sinks for utensils and dishes. It was lower than Mekele town (98.1%) (Lalit *et al.* 2015) and nearly equal to Awash Sebat Kilo, Afar Region (90.9%), had piped drinking water supply(Kumie *et al.* 2016). The observation results revealed that 22(95.6%) hotels and 23(100%) of restaurants piped water supply access. All hotels and 17 (73.9%) of restaurants had functional hand washing facilities. Regarding the food storage mechanism, functional and properly used refrigerator was a problem especially for restaurants there was less than half 11(47.8%) of them had properly functioning and clean refrigerators. In the same way only 9(39.1%) restaurants had clean and functional shower rooms for their workers.

Limitation of the study

Since the study is cross sectional it does not claim causal relationship. There may social desirability bias during data collection because the interview was conducted in the compounds of food establishments.

CHAPTER SEVEN: CONCLUSION and RECOMMANDATION

7.1 CONCLUSION

On this study findings showed that, the prevalence of *S.aureus* among (n=300) food handlers working in 23 hotels and 23 restaurants in Jimma town was (28.7%). The isolated *S.aureus* on both nares 27(9%), both hands 34(11.3%), on both noses and hands was 25(8.3%). It was higher in comparison with similar studies reported before in Ethiopia. Factors significantly associated with *S.aureus* prevalence among food handlers' were food handlers' attitude (unfavorable), wearing hand ornaments and job category (being a waiter).

Most isolates 78(90%) were resistant against commonly prescribed drugs and 6(7%) of isolates were resistant against Vancomycin and Oxacillin. But, majority of isolates were sensitive to Ciprofloxacin and Cefoxitin, Amoxicillin-Clavulanic acid. The observed prevalence of MRSA and vancomycin were 6(7%).This implied that drugs were prescribed out of national drug prescription guide line.

The result of Aerobic Mesophilic Bacteria (AMB) and *S.aureus* bacterial colony count indicated that, the majority of food samples were unsatisfactory in microbiological quality. Majority 37(86.1%) of food samples were unsatisfactorily contaminated by aerobic mesophilic bacteria (mean count CFU/g>10^5). Aerobic mesophilic bacteria found on food indicates the unhygienic activities and bare hand food contact of food handlers. Similarly, 5 (11.6%) of food samples were also unsatisfactorily (10^3 -to-10^4 CFU/g) contaminated by *S.aureus*

All hotels and 73.9% of restaurants had hand washing facilities in the premises and 95% of hotels and all restaurants also had running tap water supply. But (73.9%) of hotels and less than half (47.8%) of restaurants had functional refrigerator. This create a comfortable environment for microbial growth in stored foods

7.2 RECOMMENDATION

Based on finding the following recommendations were made;

1. **Jimma town's: Municipality, Health Office, Tourism and Trade, Market Development offices**

It is better to conduct integrative monitoring and inspection on hotels and restaurants. In this study findings showed that, the prevalence of *S.aureus* among food handlers was higher compared to other studies in Ethiopia. The coagulase positive *S.aureus* potentially produce enterotoxins that leads to food borne out break.

2. **Hotels and restaurants owners**

   It is better to arrange periodical heath checkups and trainings about safe food handling practice for their workers. Majority of hotels and restaurants owners or managers did not have any document to medical certificates; even some of them did not know the obligatory regulations concerning about food handlers' regular health checkups.

3. **Food handlers**

   They should apply hygienic practices by conforming to favorable attitudes and identify what should be done and not done during their actual work. Finally, any therapeutic activity should follow the countries drug prescription guidelines.

4. **Researchers**

   Better to conduct molecular analysis to detect strains and type of enterotoxins.

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ANNEX I: INFORMATION SHEET AND CONSENT FORM

Title of the project: - "*staphylococcus aureus* prevalence and antimicrobial susceptibility test among food handlers working in hotels and restaurants in Jimma town, southwest Ethiopia".

INFORMATION SHEET

Name of researcher:-Girma Mamo (BSc in Environmental Health)

Organization: - Department of Environmental Health Science and technology, Institute of Health, Jimma University

Name of sponsor: - Department of Environmental Health Science and technology, Institute of Health, Jimma University

1. Purpose of the study

Isolation of *Staphylococcus aureus* from food handlers' nose and hand those working in hotels and restaurants in Jimma town

2. Procedure

Nasal and hand swab samples from food handlers and food samples from hotels and restaurants were collected. Then after, the specimens were transported to Bacteriological laboratory located in Jimma University specialized hospital for analysis

3. Risk and Discomforts

- There is no discomfort or risk while collecting samples**.**
- Only 100g of food was taken from each food establishment.

4. Expected benefit of the study

- A pathogenic bacterium *(S.aureus*) was detected.
- Based up on the study result, the concerned individuals who have very close association with the food items which are working in hotels and restaurants were communicated.

- Depending up on the situation the town health office & municipality could be communicated.

5. Confidentiality of information

- Records are kept confidential
- Food establishments are not mentioned by name
- The specimens were used only for this study purpose.

6. Voluntary participation

- Your participation is by willingness.
- The participant can with draw from the study at any time and he/she can't be asked the reason.
- Withdrawal from study doesn't have impact on participant or on his food establishment routine activity.

CONSENT FORM

I am working in a food establishment in Jimma town. I understand about this research title and its objective. Though, I'm in a position to accept or refuse to participate as respondent or participant. Therefore, I can confirm my agreement with my signature here.

Sig_____

ANNEX II: QUESTIONNAIRE AND OBSERVATION CHECK LIST

JIMMA UNIVERSITY

INSTITUTE OF HEALTH, DEPARTMENT OF ENVIRONMENTAL HEALTH

Questionnaire for food handlers

- Food establishment type : 1.Hotel code [H _____] 2.Restaurant code [R _____]
- Respondent's ID/ Questionnaire serial number:_____
- Respondent's Address: Town Jimma, Kebele_____,
  Day_____/_____/2009E.C

Part I. Socio-demographic data of a food handler

Instruction to the interviewers

- Encircle the number that hold respondent's answer from the given options
- Describe what the respondents said only on the blank space provided
- The questions are adapted and modified from (Sharif *et al.* 2013).

1. Sex: 1.Male 2.Female
2. Age in yrs: _____
3. Educational status: 1.University 2. College 3.High school 4.Elementary 5.Can't read & write
4. Food handlers' category: 1. Cook 2.Cleaner 3.waiter
5. Work experience in yrs:_____
6. What was your families' livelihood? 1. Farmers 2. Merchant 3. Factory workers 4. Office workers 5.Other
7. Have you ever get food safety training? 1 Yes 2 NO
8. How many Eth. Birr you earn in average monthly?_____

Part II. Food handlers working Condition in restaurants and hotels towards practice questions on food handling in Jimma town, 2017.

For reliability purpose the questions are adapted and modified from (Sylvia *et al.*,2014)

Glossary: "1" means Yes and "0" means NO

Instruction: Encircle only the response of a respondent

S. No	Questions	Possible answers	
1	Does food handler wear outer garments/gown during inspection	1	0
2	Does food handler wear hair cover	1	0
3	Do the food handlers have hand washing habit after touching any soiled materials?	1	0
4	Does food handler finger nail trimmed and clean?	1	0
5	Does food handler wear hand ornaments during food Handling?	1	0
6	Does employee practice bare-hand contact with ready-to-eat food?	1	0

II. Response of food handlers working in Restaurants and Hotels to knowledge questions on food handling in Jimma town 2017.

Instruction: Encircle the response of a respondent

Glossary:" 1" Yes "2" NO and "3" I am not sure

No	Questions	Possible answers		
11	Does hand washing before preparing food can prevent food borne illnesses?	1	2	3
12	Would you think coughing or sneezing, towards foods causes to contamination food?	1	2	3
13	Do you think touching ready to eat food with bare hands	1	2	3

	cause to food contamination?			
14	Do you think fever, nausea, vomit diarrhea or abdominal cramp may be symptom of food borne illness?	1	2	3
15	Does apparently healthy food handler may carry food borne pathogens?	1	2	3
16	Do you ever get typhoid and bacillary dysentery diseases?	1	2	3
17	Do you ever take drugs for typhoid and bacillary dysentery without interruption in treatment period?	1	2	3

Part IV. Response of food handlers working in restaurants and hotels to attitudinal questions on food handling in Jimma town.

Instruction: Encircle the options based on respondents answer

Glossary: 5 strongly agree 4 Agree 3Neutral 2 Disagree 1strongly disagree

No	**Questions**	**Possible answers**				
19	Training about food safety is important to me	5	4	3	2	1
20	Food handlers can be causes to food borne outbreaks	5	4	3	2	1
21	Safe food handling is my duty	5	4	3	2	1
22	Repeated hand washing is appropriate to reduce food contamination	5	4	3	2	1
23	Food handlers should wear gowns and hair cover	5	4	3	2	1
24	Food handlers should not wear hand ornaments during food preparation and serving	5	4	3	2	1
25	Long fingernails may harbor pathogenic organisms	5	4	3	2	1
26	Food handlers should be medically examined every six months	5	4	3	2	1
27	Food handlers with hand injury or illness not work at food establishments	5	4	3	2	1

THANK YOU !

PART V. FOOD ESTABLISHMENTS' OBSERVATION CHECK LIST

- **Date of Observation**: _____
- Time: From_____ to_____
- Observed Food Establishment: Hotel Code H---- Restaurant Code R----
- Glossary: 1 stands for Present and 0 stands for Absence
- Instruction: Encircle "1 "for Present and "0" for Absence(Okojie *et al.* 2014)
- Adapted and modified from(Assefa *et al.* 2015).

S.No	Variables	Present	Absence
1	**Utensils and equipments sanitation**		
1.1	Functional refrigerator holding cooked and raw food items separately	1	0
1.2	Hand and utensils washing soap	1	0
1.3	Three utensils and equipments washing compartments	1	0
2	**Food handlers**		
2.1	Food handler's Periodical medical examination certificate	1	0
2.2	Food handler's Food safety training certificate	1	0
3	**Facilities**		
3.1	Tap water	1	0
3.2	Clean and functional latrine during observation	1	0
3.3	Clean and functional shower room during observation	1	0
3.4	Clean and functional hand washing basin during observation	1	0
3.5	Solid waste storage bin with cover in the premise	1	0
3.6	Liquid waste sewerage /seepage pit/storage tank	1	0

3.የአማርኛ ቃለ-መጠይቅ እና የምልከታ ቼክሊስት

የጅማ ዩኒቨርሲቲ ጤና ኢንስቲትዩት የአካባቢ ጤና አጠባበቅ ት/ት ክፍል ለምግብ አዘጋጆችና አቅራቢዎች የሚቀርብ ቃለ-መጠይቅ እና የምግብ ድርጅት የምልከታ ቅፅ

- ✓ **የምግብተቋሙዓይነት፡** 1.የሆቴል ኮድ 2.የሬስቶራንት ኮድ
- ✓ **የተጠያቂዉ/ዋ ኮድ**_____ H _____ R --------
- ✓ **የጠተያቂዉ/ዋ አድራሻ**_____
- ✓ **ቀን**_____/_____/2009 **ዓ.ም**

የመረጃ መሰጫ ቅጽ

የጥናቱ ርእስ፡ "የምግብአዘጋጆች እና አቅራቢዎችበአፍንጫቸዉ እናበእጃቸዉሊገኝ የሚችል ምግብን ሊመርዝ የሚችል ስታፊሎኮከስ ኦሪስ ባክቴሪያ መኖር አለመኖሩን እንዲሁም በምግብ ንጽህና አጠባበቅ ዙሪያ ያላቸዉን እዉቀት አመለካከትና ትግበራ መዳሰስ " በተሰኘ ርእሰ -ጥናት ዙሪያ ሲሆን

ተመራማሪ፡ ግርማ ማሞ (የመጀመሪያ ዲግሪ በአካባቢ ጤና ሳይንስ)

የግንዘብድጋፉንየሚሰጠውድርጅት፡ጅማ ዩኒቨርሲቲ የአካባቢ ጤና አጠባበቅ ሳይንስና ተክኖሎጂ ፤የጤና ሳይንስ ኢንስቲትዩት፤ ጅማ ዩኒቨርሲቲ

የጥናቱዓላማ፡-የምግብ ቤት ሠራተኞች በአፍንጫቸዉ ዉሥጥ እና በእጃቸዉ ላይ ምግብን ሊመርዝ የሚችል ስታፊሎኮከስ ኦሪስ ባክቴሪያ መኖር አለመኖሩን ማረጋገጥ እና ሠራተኞች ያላቸዉን ዕዉቀት አመለካከትና የትግበራ ደረጃ መለካት እንዲሁም ባክቴሪያዉ ለመድሃኒቶች ያለዉን ተጋላጭነትም በዚህ ጥናት ይዳሰሳል፡፡

የአሰራሩ ሂደት

- ከአፍንጫ እና ከእጅ ላይ ናሙና ይሰበሰባል
- ከተሰበሰበ በኋላ በጅማ ዩኒቨርሲቲ ስፔሻላይዝድ ሆስፒታል ስነ-ተህዋስን ላቦራቶሪ ውስጥ ይጠናል፡፡

1. **በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች**

   ከጥናቱ ጋር የተያያዘ ምንም ችግር አይከሰትም

2. **በጥናቱ በመሳተፍየሚገኝጥቅም**

   በጥናቱ መሳተፍ ነፃ የላቦራቶሪ ምርመራ ያገኛሉ እንዲሁም የላቦራቶሪ ምርመራዎ በሽታ አምጪ ተዋሲያን ከተገኘ በህክምና ዶክተር አማካኝነት ህክምና እንዲያገኙ ሁኔታዎች ይመቻቻሉ፡፡

3. **የጥናቱመረጃዎችሚስጥራዊነት**

   በጥናቱ ውስጥ የተሰበሰቡ ማናቸውም መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል፡፡በጥናቱ ስምዎ አይጠቀስም እንዲሁም የተሰበሰዉ ምላሽ ለተጠቀሰዉ ዓላማ ብቻ ይዉላል፡፡

4. **በፍቃደኝነት ላይ የተመሰረተ ስለመሆኑ**

ይህ ጥናት በፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛውም ወቅት በፈቃድዎ ከጥናቱ መዉጣት ይችላሉ፡፡ ከጥናቱ ቢወጡም እንኳን የተለመደውን የህክምና እርዳታ በጤና ተቋሙ ውስጥ በማንኛውም ጊዜ የማግኘት መብት አለዎት፡፡

የስምምነት ቅፅ

እኔ በሆቴል ወይም በሬሰቶራንት የምሰራ ሰራተኛ ስሆን፤ ከዚህ በላይ በተገለጸዉ የጥናትና ምርምር ርዕስና ዓላማ መሰረት በጥናቱ ለመሳተፍ መስማማቴን በፊረዬ አረጋግጣለሁ፡፡

- ✓ እስማማለሁ፡ ፊርማ----------

ክፍል 1: ግላዊ ገጽታዎች

መመሪያ: *ሀ. ምላሾችን የያዙትን ቁጥሮች ብቻ ይክበቡ*

ለ. በጽሁፍ የሚገለጹ መልሶችን ደግሞ በተዘጋጁት ክፍት ቦታዎች ላይ ይፃፉ

1. *ፆታ፡* 1. ወንድ 2.ሴት
2. *ዕድሜ በዓመት*----------
3. *የትምህርት ደረጃ፡* 1.ዩኒቨርስቲ 2. ኮሌጅ 3. ከ/ሁለተኛ ደረጃ 4. አንደኛ ደረጃ 5.ማንበብና መፃፍ የማይችል/የማትችል
4. *በምግብ ድርጅቱ ዉሥጥ የስራ ድርሻ፡* 1.የኩሽና ዉስጥ ሥራ 2. ፅዳት 3. አስተናጋጅ
5. *በምግብ ድርግጅቱ ዉስጥ የስራ ልምድ በዓመት*----------
6. *የቤተሰብ (የወላጆች) የኑሮሁኔታ፡* 1.ግብርና 2.ንግድ 3.ፋብሪካ 4.የቢሮስራ 5. ሌላ--------
7. *በምግብ ንፅህና አያያዝ ዙሪያ ሥልጠና ወስደህ/ሽ/ ታዉቃለህ/ ታዉቂያልሽ?* 1. አዎ 2. አላዉቅም
8. *ወርሃዊ ደመወዝህ/ሽ በአማካይ ስንት የኢትዮጵያ ብር ነዉ?*----------

ክፍል፡2 በጅማ ከተማ በሆቴል እና ሬስቶራንት የሚሰሩ ምግብ አዘጋጆችና አቅራቢዎች በምግብ ንፅህና አጠባበቅ ዙሪያ የሚተገብሯቸዉን ተግባራት የሚዳስሱ ጥያቄዎች

ትርጓሜ፡ "1" ማለት አዎ/አለ ሲሆን "0" ማለት ደግሞ አይደለም/የለም ማለት ነዉ

መመሪያ፡ተጠያቂዉ/ዋ የመለሰዉን/ችዉን ቁጥር ብቻ ክበብ/ቢ

ተ.ቁ	ጥያቄዎች	ምላሾች	
1	ምግብ አዘጋጅና አቅራቢዉ/ዋ ንፁህ የሥራ ገዋን ለብሷል/ለብሳለች?	1	0
2	ምግብ አዘጋጅና አቅራቢዉ/ዋ ንፁህ የፀጉር መሸፈኛ አድርጓል/አድርጋለች?	1	0

3	ምግብ አዘጋጅና አቅራቢዉ/ዋ ንፁህ ያልሆኑ ቁሶች ከነካ/ች ወዲያዉኑ ይታጠባል/ ትታጠባለች?	1	0
4	ምግብ አዘጋጅና አቅራቢዉ/ዋ ጥፍሩን/ሯን/ አሳጥሯል/ለች?	1	0
5	ምግብ አዘጋጅና አቅራቢዉ/ዋ በእጁ/ጇ ላይ ጌጣጌጥ አድርጓል/ጋለች?	1	0
9	ምግብ አዘጋጅና አቅራቢዉ/ዋ የተዘጋጁ ምግቦችን በባዶ እጅ ይነካል/ትነካለች?	1	0

ክፍል፡3 በጅማ ከተማ በሆቴል እና ሬስቶራንት የሚሰሩ ምግብ አዘጋጆችና አቅራቢዎች በምግብ ንፅህና አጠባበቅ ዙሪያ ያላቸዉን ዕዉቀት የሚዳስሱ ጥያቄዎች

ትርጓሜ፡ “1“. አዎ “2“. የለም/አይሆንም “3“.እርግጠኛ አይደለሁም ማለትነዉ፡፡

መመሪያ፡ ተጠያቂዉ/ዋ የመለሰዉን/ቺዉን ብቻ ክበብ/ቢ

ተ.ቁ	ጥያቄዎች	ምላሾች		
11	እጅን መታጠብ የምግብ ወለድ በሽታዎችን መከሰት ይቀንሳል?	1	2	3
12	እጅን በሳሙና መታጠብ በዉሃ ብቻ ከመታጠብ የበለጠ ጀርምን ከእጅ ያፀዳል?	1	2	3
13	በምግብ አካባቢ ማስነጠስ፣ መነጋገር ወይም መሳል ምግብን ይበክላል?	1	2	3
14	የተዘጋጁ ምግቦችን ቀጥታ በእጅ በመንካት ለተመጋቢ መሸጥምግቡን ይበክላል?	1	2	3
15	ትኩሳት፣ ማቅለሽለሽ፣ ተቅማጥ (ደምየተቀላቀለ/ያልተቀላቀለ)፣ ትውከት፣ የሆድ ቁርጠት ከምግብ መመረዝ የሚመጡ ምልክቶች ናቸዉ	1	2	3
16	በጤናማ ምግብ አዘጋጆችና አቅራቢዎች አካል ላይ በሽታ አምጪ ተህዋስያን ይኖራሉ?	1	2	3

ክፍል፡4 በጅማ ከተማ በሆቴል እና ሬስቶራንት የሚሰሩ ምግብ አዘጋጆችና አቅራቢዎች በምግብ ንፅህና አጠባበቅ ዙሪያ ያላቸዉን አመለካከት የሚለኩ ጥያቄዎች

መግለጫ፡ 5 = በጣምእስማማለሁ 4 = እስማማለሁ 3 = መወሰንአልችልም

2 = አልስማማም 1 = በጣምአልስማማም

መመሪያ፡ተጠያቂዉ/ዋ የመለሰዉን/ቺዉን ብቻ ይክበብ

ተ.ቁ	ጥያቄዎች	ምላሾች				
18	ስለምግብ ንፅህና አጠባበቅ መሰልጠን ለእኔ አስፈላጊ ነዉ፡፡	5	4	3	2	1
19	ምግብ አዘጋጆችና አቅራቢዎች ለምግብ ወለድ በሽታዎች መከሰት ምክንያት ሊሆኑ ይችላሉ፡፡	5	4	3	2	1
20	ምግብን በጥንቃቄ መያዝ የእኔ ሃላፊነት ነዉ፡፡	5	4	3	2	1
21	ደጋግሞ እጅን መታጠብ ምግብ ወለድ በሽታዎችን ለመቀነስ ተገቢ ተግባር ነዉ፡፡	5	4	3	2	1
22	ምግብ አዘጋጆችና አቅራቢዎች ሁል ጊዜ በሥራ ላይ ሳሉ ገዋንና የፀጉር መሸፈኛ መልበስ አለባቸዉ፡፡	5	4	3	2	1
23	ምግብ አዘጋጆችና አቅራቢዎች በሥራ ላይ ሳሉ የእጅ ጌጣጌጦችን በፍፁም ማድረግ የለባቸዉም፡፡	5	4	3	2	1
24	ረጃጅም የእጅ ጥፍሮች በሽታ አምጪ ተህዋስያንን ደብቀዉ ይይዛሉ፡፡	5	4	3	2	1
25	ምግብ አዘጋጆችና አቅራቢዎች በየስድስት ወራት ልዩነት የጤና ምርመራ የማድረግ ግዴታ አለባቸዉ፡፡	5	4	3	2	1
26	ምንም ዓይነት የቆዳ ቁስለት ወይም ተቅማጥና ትዉከት ያለበት/ባት ምግብ አዘጋጅና አቅራቢ በምግብ ቤት ዉስጥ መሥራት የለበትም/ባትም፡፡	5	4	3	2	1

አመሰግናለሁ !

ክፍል፡5 በምልከታ መመዘኛ መሰረት የሬስቶራንትና ሆቴል ንፅኅና እንዲሁም የንፅኅና ቁሳቁስ መኖር አለመኖሩን ማረጋገጫ ቅፅ

ሀ. የምልከታዉቀን---------- ሰዓት ከ----------እስከ----------

ለ.በምልከታዉ የተካተቱ የምግብ ተቋማት፡ የሆቴል ኮድ----------የሬስቶራንት ኮድ -------

መመሪያ፡ "1"ማለት አለ/አዎ ሲሆን"0" ማለት ደግሞ የለም/አይደለም ማለት ነዉ:: ቁጥሩን በመክበብ ይለዩ

ተ.ቁ	*መመዘኛዎች*	አለ/አዎ	የለም/አይደለም
1	**የምግብ ቁሳቁስ ንፅህና አጠባበቅ**		
1.1	ንፅህናዉ የተጠበቀ የበሰሉ እና ያልበሰሉ ምግቦችን ለየብቻ ተከድነዉ የተቀመጡበት ምግብን ሊያቀዘቅዝ የሚችል ፍሪጅ	1	0
1.2	የእጅና ዕቃ ማጠቢያ ሳሙና በመታጠቢያ ቦታዎች	1	0
1.3	ሦስት የምግብ ቁሳቁሶች ማጠቢያ ሳፋዎች/ሲንኮች	1	0
2	**ምግብ አዘጋጅና አቅራቢዎች**		
2.1	የሰራተኛዉ/ዋ ወቅታዊና መደበኛ የጤና ምርመራ ምስክር ወረቀት	1	0
2.2	የሰራተኛዉ/ዋ የምግብ ንፅህና አጠባበቅ ሥልጠና ምስክር ወረቀት	1	0
3	***መገልገያዎች***		
3.1	አገልግሎት እየሰጠ ያለ የዉሃ ቧንቧ	1	0
3.2	አገልግሎት እየሰጠ ያለ ንጹህ መጸዳጃ ቤት	1	0
3.3	አገልግሎት እየሰጠ ያለ ንጹህ የገላ መታጠቢያ ክፍል	1	0
3.4	አገልግሎት እየሰጠ ያለ የእጅ መታጠቢያ ባዚን/ሲንክ	1	0
3.5	ክዳን ያለዉ የደረቅ ቆሻሻ ማጠራቀሚያ ዕቃ	1	0
3.6	የፍሳሽ ቆሻሻ ማስረጊያ ጉድጓድ ወይም ቱቦ መስመር	1	0

የኦሮምኛዉ ትርጓሜ ቃለ-መጠይቅ

Af-gaffi fi dhaabbata nyaata cheeklistiimuldhisu hika affan oromottin

Yuniversiti Jimma,kutaa barnoota institiiwitti,nyaata quophestota fayaa finaano fayyaa

Af-gaaffi fidhiyaa sitootaaf guca dhaabbata nyaataa & dhiyaata

Akaakuu dhaabbata nyaataa: kodii hoteela (H)_____ kodii restoraanti (R)_____

Kodii gaafatamaa/mtuu ykn tartiba af-gaaffichaa_____

Teesoon gaafatamaa/mtuu_____

Guca raga kennaa

Kabajamaa hirmaata qorannoo kanaa:

Af-gaaffin kun kan qophaaye baakteeriyaan"staafilokookas oriis"jedhamu kan nyaata summessu danda'u funyaan nyaata qoophesitootaafi dhihesitoota keessa jiraatu fi dhabamu akkasmumas equmsa qulqullina nyaataa irratti beekumsaa fi hubannoo qaban fi haalarawii isaani ilaala" qo'annoo naanno kan kan fakaatu yoo ta'u.

Kaayyoon qorannichaa:-hojjatooni mana nyaataa funyaan isaani keessaa fi harka isaani irra baakteeriaan staafilokookas oriisjedhamuun kan nyaata summessu danda'u jiraachuu fi jiraachuu dhabu isaa mirkannesuu fi hojjitonni beekumsa,hubannoo fi ga'umsa raawii qaba madaaluu akkasumas haala baakteerichi qorichaaf(dawwaaf)saaxilamu qo'annoo kan keesatti hammatma.

Deebiin dhugaa keessani galma ga'insa qo'anichaaf murteessa dha. Kana waan ta'eef yaaddoo tokko male dhugaa fi shakkii isaa haala gaafitiin nu deebisa. Eenyumaan keessan gonkumaa ykn haala kamiinu qaama birraaf dabarsine hin ibsinu.

Guca waligaltee

Ani hojjiataan hoteela ykn restoraanti keessa kan hojjiadhu yoo ta'u qo'annoof qorrannoo mata dureef kaayyoo haala kanaa olii ibsamen fedhi kiyyaan qorranicha irratti hirmaachuuf waliigaltee elumuu kiyyaa mallattoon kiyyaan nimirkaneessa.

1.**Waligaltee kiyyaa**: mallatto_____ 2. **Waligaltee kiyyaa miti**: mallatto_____

Kutaa-1: Fuulota dhunfaa

Qajeel fammaa

A)lakkoofsota deebii qaban qofa maraa

B)deebii bareeffamaan bakka duwwaa kennaman irratti haa barreeffaman

1. Saala: 1.dhira 2. Durba
2. Umrii waggaan:_____
3. Sadarkaa barnootaa: 1. Yuniversitti 2.koolleejji 3. Sadarkaa lammaffaa 4. Sadarkaa tokkooffaa 5. Dubbisuu fi barreessu kan hin dandeenye.
4. Gahee hojji dhaabbata nyaataa keessa qabu 1.hojjiatu kushinnaa keessa 2. Qulqullesitu 3. Keessummesitu
5. Muxannoo hojii dhaabbata nyaataa keessa qobu waggaan_____
6. Haala jireenya maati(warra)isaa hojjataa. 1qonnaa 2. Daldalaa 3.warshaa 5.kan biro
7. Haala qabeenya qulqullina nyaataa irratti leenji fudhate beeka(beetaa). 1.eyye 2.hin beeku
8. Mindaan ji'atti argatu jiddugala qafshii itiyoophiyaan_____

Kutaa-2: Hojjattoota hoteelaaf restoraanti magaalaa Jimma keessa qophiif dhiyeessa nyaataa,naanoo equmsa qulqullina nyaataa irratti raawwatamu fi raawii isaa kan haammatu dha.

Hikkoo: “1” jechuun eeyee/jira yoo ta’u , “0” jechuun miti/hin jiru ta’a

Qajeelfama: gaaffatamaan deebii lakkoofsaa qoofa maru qaba

Tartiba lakk.	**Gaaffileewwan**	**Deebiiwan**	
1	Nyaata qopheesaan/tuun ykn dhiyeessan qaawanii hajii qulqullina qabu ni uffatu	1	0
2	Nyaata qopheesan/tuun ykn dhiyeessan/tuun uwwisa rifeensaa qulqullina qabu ni uffatuu	1	0
3	Nyaata qopheesan/tuun ykn dhiyeessan/tuun yoo xuurii tuqan dafanii dhiqatan	1	0
4	Nyaata qopheesan/tuun ykn dhiyeessan/tuun qeensa ofi ni qabaabsaa/sitii	1	0
5	Nyaata qopheesan/tuun ykn dhiyeessan/tuun faaya adda adda harka isaa/shee irrati ni qaba/bdi	1	0
6	Nyaata qopheesan/tuun ykn dhiyeessan/tuun nyaata dhiyaatu harka isaa/shee tiin ni tuga/qti	1	0

Kutaa-3: Hojjattoota hoteelaaf restoraanti magaalaa jimma keessa qophiif dhiyeessa nyaataa naannoo eegumsa qulqullina nyaata irratti beekumsa qaban kan haammatu dha.

Kikkoo: “1” jechuun eeyee , “2” jechuun miti “3” jechuun qabatama miti jechu dha.

Qajelfama: gaaffatamaan/tuun deebii lakkoofsa qafa maru qaba.

Tartiba lakk.	**Gaaffileewwan**	**Deebiiwan**		
		1	2	3
11	Harka dhiqatuu, dhibee nyaataan dhufu ni xiqqeessa?			
12	Harka saamunaan dhiqachuun bishaaniin dhiqachuu caalaa summii germii harka dhiraa qulqulleessa?			
13	Naannoo nyaataati haxxeessuu dubbachuun ykn qufaa’un nyaata summeessa?			
14	Nyaata qophaa’e kallattiin harkaan tuqu dhaan fayyamaaf gurguruun nyaata summeessa?			
15	Torbee lammaan darban keessa bawwoo ol hammachuu, baassa(dhiiga hamatu hin hamane),hoqisiisaa,garaa muraa,isiin/sin quna mee jira?			
16	Fayyumaan nyaata qophesitootaaf dhiyyesitootaa qaama isaani irrati baakteeriyaa dhibee fidu ni jiraa?			
17	Dhibee sakeeraa/tasiboo ykn baassaaa dhiiga makatee si qabe beektaa?			
18	Deebii hakkoofsa 17 irratt eeyee yoo ta’e dawwaa /qoricha fudahatee jirta?			

Kutaa-4: Hojjattta hoteelaaf restoraanti magaalaa jimma keessa qophiif dhiyeessa nyaataa naannoo eegumsa qulqullina nyaata irrattii qaban kan haamaatu dha

Ibsa:5.baayee waligala 4.waligala 3.muraasaan waligala 2.wal-hingalu 1.baayee wal-hingalu

Qajeelfama: gaafatamaan deebisuuf lakkofsa deebii qafa haa maru.

Tartiba Lakk	Gaaffileewwan	Deebiiwan				
		5	4	3	2	1
19	Haali eegumsa qulqullina nyaataa kennuun naaf barbaachisa dha					
20	Nyaata qophesitoonif dhiyeesitoon dhibewwan nyaataan dhufaniif sababa ta'u					
21	Nyaata qulqullinaan qabuun gahe itti gaafatamummaa kooti					
22	Harka irra dadeebi'un dhiqachuun dhibewwan nyaataan dhufan salphisuuf gahee sirri dha					
23	Yeroo hunda nyaata qophesitoonif dhiyesitoon hojjii irratti gaawanii fi uwwisa rifeensaa godhachu(uffachu) qaban					
24	Yeroo hunda nyaata qophesitoonif dhiyesitoon hojjii irratti faaya harkaissani gonkumaa godhachu hin qaban					
25	Qeensa dheeraa(guddaa) ta'un baakteeriyaa dhibee fidan ni dhoksu					

26	Nyaata qophesitoon fi dhiyesitoon garaa garuumaa ji’a jahaan dirqama qo’annoo fayaa gochu qaban					
27	Yeroo kamiyuu madaa qamaa ykn baasaa fi haqisisaa kan qabu nyaata qophessaaf dhiyeessan mana nyaataa kessa hojjachuu hin qabu					

ANNEX III. BIVARIATE ANALYSIS TABLE

Variable		Frequency	B	S.E	p-value	COR	95 % CI	
Sex							Lower	Upper
	Male	103	-.260	.275	.344	.771	.450	1.321
	Female	197	-.826	.155	*1			
Age	less 20 year	105	.114	.275	.680	1.120	.653	1.922
	30-39 year	26	.127	.460	.782	1.136	.461	2.797
	>= 40 year	9	-1.141	1.075	.288	.319	.039	2.628
	20-29 year	160	-.938	.176	*1			
Education	University	3	.148	1.236	.904	.904	1.160	.103
	College	14	.254	.583	.663	.663	1.289	.411
	High school	85	-.337	.307	.272	.272	.714	.391
	can't read and write	32	.053	.417	.899	.899	1.055	.466
	Elementary	166	-.842	.169	*1			
Job	Cleaner	40	-.054	.376	.886	.948	.454	1.980
	waiter	122	-.949	.295	.001	.387	.217	.690
	Cook	138	-.565	.177	*1			
Experience	≤ 1 year	76	.391	.298	.190	1.478	.824	2.649
	6-9	41	-.017	.394	.966	.983	.454	2.130
	≥10 years	21	-.460	.583	.430	.631	.201	1.979
	2-5 years	162	-.986	.177	*1			
Knowledge	Good	122	.556	.270	.039	1.744	1.028	2.962
	Poor	178	-1.258	.218	*1			
Attitude	Unfavorable	14	1.586	.573	.006	4.886	1.588	15.034
	favorable	286	-.999	.133	*1	.368		

Bare hand food contact	Yes	80	-.170	.284	.551	.844	.483	1.474
	NO	220	-.788	.241	.001	.455		
Training	Yes	45	-.253	.373	.498	.776	.374	1.613
	NO	255	-.875	.137	*1			
Hair cover	Yes	110	-.446	.261	.088	.640	.384	1.068
	No	190	-.639	.201	.001	.528		
Hand wash	No	89	.416	.272	.126	1.516	.889	2.585
	Yes	211	-1.043	.157	*1			
Hand ornament wear	Yes	123	-.575	.270	.033	.563	.331	.955
	NO	177	-.693	.159	*1			
Gown wear	NO	90	-.387	.291	.183	.679	.384	1.200
	YES	210	-.802	.149	*1			
Hygiene practices	GOOD	123	-.050	.259	.848	.951	.573	1.581
	POOR	177	-.882	.198	*1			

*1 reference group

THESIS APPROVAL DECLARATION

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the thesis project and for provision of required progress reports as per terms and conditions to Jimma University, Institute of Health and Department of Environmental Health Science and technology is forwarded as the result of this application.

Under the title" Staphylococcus *aureus* prevalence and antimicrobial susceptibility test among food handlers working in Jimma town, southwest Ethiopia".

By: Girma Mamo Zegene

Date._____ Signature _____

THESIS APPROVED BY:

EXAMINERS

	Name	**Date**	**Sig.**
1 .	**_____, _____, _____**		

ADVISORS

	Name	**Date**	**Sig.**
1.	_____	_____	_____
2.	_____	_____	_____
3.	_____	_____	_____
4.	_____	_____	_____

JUNE, 2017

JIMMA-ETHIOPIA