CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Health is a dynamic process of adjustment and readjustment to the continuous changing environmental conditions. Among the various factors which play an important role in the maintenance of health of which nutrition is one (Kasturawar and Mohd, 2012).

Food are rarely if ever sterile as they carry microbial associations whose consumptions depend upon which organisms gains access and how they grow, survive and interact with food over time (Anio *et al*., 2011), therefore an adequate supply of safe, wholesome, and healthy food is essential to the health and well-being of humans and consumption of contaminated or un safe food may result in illness also referred as food-borne disease (Ifeadike, *et al*., 2012), which represent a persistent global health burden (Simsek *et al*., 2009). According to world health organization (WHO) there are 30% of population suffer from food-borne disease each year in developed countries, whereas in developing countries there are up to 2 million deaths are estimated per year (Dagnew *et al*., 2012).

To date, around 250 different food-borne diseases are described (Florence and Michel, 2002), and these can be caused by a wide variety of etiological agents such as viruses, bacteria, and parasites (Noor *et al*., 2012), among them the leading causative agent is *S. aureus*, the later is the abundant pathogen isolated from objects associated with food processing and food preparation according to screening study in the Omdorman area,

Sudan (Saeed and Hamid, 2010), and this due to it is ability to produce enterotoxins which result in sickness when ingested in sufficient amount (Edet *et al*, 2009) called staphylococcal food poisoning (SFP), which is the common intoxication that results from consumption of foods containing sufficient amounts of one or more pre-formed enterotoxins (Argudin *et al*, 2012). SFP may also caused by community aquired mithicillin resistant *S. aureus* (MRSA); this is according to study done by (Timothy *et al*, 2002), which investigated an outbreak in which a food handler, food specimen, and three ill patrons were culture positive for the same toxin-producing strain of MRSA.

Food may be contaminated from many sources, but food handlers with poor personal hygiene is the potential sources of infection which can be during slaughter and processing of livestock or by cross contamination during food preparation (Doyle *et al.*, 2012). Center for Disease Control and Prevention (CDC) stated that poor personal hygiene is third most commonly reported food preparation practice contributing to food-borne diseases (Zagllol, *et al.*, 2011).

1.2. Rationale

The bacteria *S. aureus* is commonly found on the skin and hair, as well as in the noses and throats of people and animals. It is present in up to 25% of healthy people. *S. aureus* can cause food poisoning and considered as the leading cause of food-borne diseases. Food handlers contaminate food by transferring pathogens that they are carrying in or on their body during production, processing, storage and food preparation. This study expected to investigate *S. aureus* nasal carriers among food handlers working in

cafeterias in Sudan University of Science and Technology (SUST), focusing on the role that food handling personnel play in food contamination and as an important sources of infection and intoxicaton, particularly staphylococcal food poisoning.

1.3. Objectives

1.3.1. General 0bjectives

To detect methicillin-resistant *S. aureus* carrying mecA gene among food handlers in Khartoum Province.

1.3.2. Specific Objectives

1. To isolate *S. aureus* from nasal cavity and hands of food handlers.

2. To identify methicillin-resistant *S. aureus*.

3. To detect *mec*A gene among methicillin-resistant *S. aureus*.

CHAPTER TWO
LITERATURE REVIEW

2.1. Entrance

Foods, microorganisms and humans have had a long and interesting association that developed long before recorded history. Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth. Microorganisms can be used to transform raw foods into gastronomic delights, including chocolate, cheeses, pickles, sausages, and soy sauce. On the other hand, microorganisms can degrade food quality and lead to spoilage. Importantly, foods also can serve as vehicles for disease transmission. The detection and control of pathogens and food spoilage microorganisms are important parts of food microbiology. During the entire sequence of food handling, from the producer to the final consumer, microorganisms can affect food quality and human health (Willey *et al.*, 2008).

2.2. Food-Borne Illnesses

Food-borne illness is an acute gastrointestinal infection caused by consuming food contaminated with pathogenic, bacteria, toxins, viruses, prions or parasites. Such contamination was caused by improper food handling, preparation or storage of food. Contacts between food and pests, especially flies, cockroaches and rodents are a further cause of contamination of food. Food-borne illness can also be caused by adding pesticides or medicine to food or consuming or by accidentally consuming naturally

poisonous substances. That is why food-borne illness can also be called food poisoning (http://www.123helpme.com /view.asp?id=65999).

Food-borne illnesses impact the entire world. In the United States, based on recent information from the Centers for Disease Control and Prevention, annual incidences of food-related diseases involve 76 million cases, of which only 14 million can be attributed to known pathogens. Food-borne diseases result in 325,000 hospitalizations and at least 5,000 deaths per year. Since 1942, the number of recognized food-borne pathogens has increased over fivefold, all of these food-borne diseases are associated with poor hygienic practices. Whether by water or food transmission, the fecal-oral route is maintained, with the food providing the vital link between hosts. Fomites, such as sink faucets, drinking cups, and cutting boards, also play a role in the maintenance of the fecal-oral route of contamination (Willey *et al.*, 2008). There are two primary types of food-related diseases: food-borne infections and food intoxications.

2.3. Food-Borne Infections

It is an infection occurs when a pathogen enters the gastrointestinal tract and multiplies. Infections of the GI tract characterized by a delay in the appearance of gastrointestinal disturbance while the pathogen increases in numbers or affects invaded tissue. There is also usually a fever, one of the body's general responses to an infective organism. Some pathogens cause disease by forming toxins that affect the GI tract (Tortora *et al.*, 2010).

2.4. Food-Borne Diseases

2.4.1. Salmonellosis It is a localized gastroenteritis caused by Nontyphoidal *Salmonella* serovars, particularly *Typhimurium* and *Enteritidis*. The symptoms of salmonellosis result from causative bacteria proliferating in the intestine of affected individuals. Transmission is usually via food, especially chickens, eggs, and egg products (Harvey *et al.*, 2013).

2.4.2. Acute Gastroenteritis caused by *Campylobacter jejuni*

C.jejuni is transmitted to humans primarily via the fecal–oral route—throug direct contact, exposure to contaminated meat (especially poultry), or contaminated water supplies.The disease lasts days to several weeks, and generally is self-limiting. Symptoms may be both systemic (fever, headache, myalgia) and intestinal (abdominal cramping and diarrhea, which may or may not be bloody). Bacteremia may occur, most often in infants and older adults (Harvey *et al.*, 2013).

2.4.3. Listeriosis*,* It is caused by *Listeria monocytogenes,* was responsible for the largest meat recall in U.S. history—27.4 million pounds. In 2002, a seven-state listeriosis outbreak was linked to deli meats and hot dogs produced at a single meat-processing plant in Pennsylvania. Pregnant women, the young and old, and immunocompromised individuals are especially vulnerable to *L. monocytogenes* infections. In this outbreak, seven deaths, three stillbirths, and 46 illnesses were caused by consumption of contaminated meats (Willey *et al.*, 2008)

2.4.4. Shigellosis (bacillary dysentery), It is a human intestinal disease that occurs commonly among young children caused by *Shigella* species. Shigellae are typically spread from person to person, with food or water contaminated with fecal material serving as major source of organisms. Shigellosis characterized by diarrhea with blood, mucus, and painful abdominal cramping. The disease is generally most severe in the very young and elderly, and among malnourished individuals, in whom shigellosis may lead to severe dehydration, and sometimes death (Harvey *et al.*, 2013).

2.4.5. *Escherichia coli* **diarrhea** is an important food-borne disease organism. Enteropathogenic, enteroinvasive, and enterotoxigenic types can cause diarrhea. *E. coli* O157:H7 with its specific LPS O-antigen (O) and flagellar (H) antigen, is thought to have acquired enterohemorrhagic genes from *Shigella,* including the genes for shigalike toxins. This produced a new pathogenic strain, first discovered in 1982 and now known around the world. The pathogen is spread by the fecal-oral route, and an infectious dose appears to be only 500 bacteria. Enterohemorrhagic *E. coli* has been found in meat products such as hamburger and salami, in unpasteurized fruit drinks, on fruits and vegetables, and in untreated well water. Prevention of food contamination by *E. coli* O157:H7 is essential from the time of production until consumption. Hygiene must be monitored carefully in larger volume slaughterhouses where contact of meat with fecal material can occur. Even fruits and vegetables should be handled with care because disease outbreaks have been caused by domestic and imported produce (Willey *et al.*, 2008).

2.4.6. Cholera

Vibrio cholerae secretes a toxin which causes cholera, an infection in the small intestine. The cholera toxin causes an outflowing of ions and water to the lumen of the intestine. After an incubation period ranging from hours to a few days, profuse watery diarrhea ("rice-water" stools) begins. Untreated, death from severe dehydration causing hypovolemic shock may occur in hours to days, and the death rate may exceed 50 percent. Appropriate treatment reduces the death rate to less than 1 percent. Transmission occurs primarily by drinking water or eating food that has been contaminated by the feces of an infected person, including one with no apparent symptoms. Worldwide, cholera affects 3–5 million people and causes 100,000–130,000 deaths a year as of 2010. This occurs mainly in the developing world (Harvey *et al.*, 2013).

2.5. Staphylococcal Food Poisoning (SFP)

The SFP is a food-borne intoxication that develops in people who ingest food that has been improperly prepared or stored, (Schelin *et al.*, 2011), it acquired from eating enterotoxin-contaminated food, and it considered as the second most commonly reported types of food-borne diseases, this due to the insufficient pasteurization/decontamination of originally contaminated product source or its contamination during preparation and handling by individuals who are carriers of the organism. Also, since *S. aureus* grows over a wide range of temperatures and pH, the bacteria may grow in a wide assortment of foods. Therefore, food that is contaminated with SE-producing strains, if left at

temperatures that allow rapid growth of the bacteria (*i.e.*, inadequate refrigeration) is a common source of SE-outbreaks, (Pinchuk *et al.*, 2010). SFP can be caused by as little as 20–100 ng of enterotoxin (Schelin *et al*, 2011), The disease has a short incubation period that ranges from just a few minutes to hours since the toxin is preformed (Pinchuk *et al.*, 2010) after ingestion, symptoms appear rapidly and abruptly, consistent with diseases caused by preformed toxins. The symptoms include copious vomiting, diarrhea, abdominal pain or nausea. Ingested bacteria do not produce toxin, and the symptoms therefore normally wear off within 24 h. The severity of the illness depends on the amount of food ingested, the amount of toxin in the ingested food and the general health of the victim (Schelin *et al.*, 2011). Patients with this illness are not contagious. Toxins are not transmitted from one person to another (CDC, 2010). Toxin-producing *Staphylococcus aureus* can be identified in stool or vomit, and toxin can be detected in food items. Diagnosis of staphylococcal food poisoning in an individual is generally based only on the signs and symptoms of the patient. Testing for the toxin-producing bacteria or the toxin is not usually done in individual patients. Testing is usually reserved for outbreaks involving several persons (CDC, 2006). The disease is usually self-resolving, is rarely lethal and the elderly are more susceptible (Pinchuk *et al.*, 2010), for most patients, staphylococcal food poisoning will cause a brief illness. The best treatments for these patients are rest, plenty of fluids, and medicines to calm their stomachs. Highly susceptible patients, such as the young and the elderly, are more likely to have severe illness requiring intravenous therapy and care in a hospital. Antibiotics are

not useful in treating this illness. The toxin is not affected by antibiotics (Hennekinne *et al.*, 2010).

2.6. *Staphylococcus aureus* and Food Handlers Carriers

S. aureus is carried by about one third of the general population; it colonizes humans as well as domestic animals, and is a common opportunistic pathogen. It is estimated that *S. aureus* is persistent in 20% of the general population, while another 60% are intermittent carriers, and the anterior nares is the most frequently site of colonization in humans (Pinchuk *et al*., 2010). In a cross sectional study conducted among food handlers working in University of Gondar, Northwest Ethiopia, Forty one (20.5%) food handlers were positive for nasal carriage of *S. aureus*, of these 4(9.8%) was resistant for methicilin (**Dagnew *et al*., 2012**). Another study done in Omdurman area of Sudan reported that; *S. aureus* is the most abundant pathogen, and was most prevalent in storekeepers (44.6%), followed by restaurant workers (25%), bakers (17.9%), butchers (5.4%), milk distributors (3.6%), and fruit/vegetable sellers (3.6%) (Saeed and Hamid, 2010). In another Cross-sectional study done in Kuala Pilah, Negeri Sembilan area of Malaysia, Fifteen (23.4%) of food handlers out of 64 were positive for *S. aureus* nasal carriage and all isolates were susceptible to oxacillin (Noor-Azira, *et al*, 2012**).** In a cross sectional study done in Province of Misiones, Argentina, a total of 37.5 % food handlers were positive for *S. aureus* from 88 nasal swabs take from 88 food handlers and there are 4 isolates were resistant to methicillin (Jordá *et al.,* 2012). In another study conducted by Çepoglu and his colleagues, they reported that a total of 92 isolates of staphylococcal species

consisting of 7 coagulase positive staphylococci (CPS) and 85 coagulase negative staphylococci (CNS) were isolated from hands of the 25 food handlers in different restaurants in turkey, similarly, 13 (CPS) and 96 (CNS) isolates were cultured from the nasal cavity of the workers (Çepoglu *et al*., 2010).

2.7. Food handlers as a Source of infection

The term 'food handler' mainly refers to people who directly touch open food as part of their work. They can be employed or agency staff. However, it also includes anyone who may touch food contact surfaces or other surfaces in rooms where open food is handled. This is because they can also contaminate food by spreading bacteria for example to surfaces that food will come into contact with, e.g. work tops and food packaging before it is used. They can also contaminate other surfaces such as door handles which can then contaminate the hands of people who handle food directly. The term can therefore apply to managers, cleaners, maintenance contractors and inspectors for example. It is the effect of their presence that is important, not the reason for them being there (Food Standards Agency). Food handlers carrying enterotoxin-producing strains in their noses or hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions (Argudín *et al.,* 2012). Food handlers that carry *S. aureus* in their nares or skin can be a potential source of infection; that they can be a source of contamination for food during producing and preparation especially food that requires no additional cooking, such as Salads, such as ham, egg, tuna, chicken, potato, and

macaroni, and bakery products, such as cream-filled pastries, cream pies, and chocolate

éclairs and Sandwiches. Other sources include milk and dairy products, as well as meat,

poultry,ggs,   and related   products

(http://www.foodsafety.gov/poisoning/causes/bacteriaviruses/*Staphylococcus*/).

CHAPTER THREE
MATERIALS AND METHODS

3.1. Study design and area

A cross sectional study was conducted among food handlers working in Sudan University of Science and Technology (SUST) cafeterias from June, 2013 to July, 2013.

3.2. Study population

All food handlers working in SUST cafeterias in period from June, 2013 to July, 2013.

3.3. Inclusion criteria

Food handlers working in the SUST cafeterias and agreed voluntary to participate in the study, were included.

3.4. Exclusion criteria

Food handlers working in SUST who had taken antibiotics within the three weeks prior to the study were excluded.

3.5. Sampling procedure

 Nasal swabs and hand swabs (left and right)) were collected from 53 food handlers included in the study.

3.6. Data collection

A pretested questionnaire was used collect information regarding age, sex, marital status, service years, educational status, status of training and habits of hands washing.

3.7. Ethical consideration

The data were collected after informed consent obtained from all participants.The study was approved by the Research Ethics Committee of the Sudan University of Science and Technology.

3.8. Collection of specimens

Nasal swabs were collected aseptically from food handlers nostrils rolling six times by applicator stick tipped with cotton moistened with normal saline. Hand swab was collected aseptically from food handlers by swabbing their fingers and area between the fingers using applicator stick tipped with cotton moistened with normal saline.

3.9. Culture of specimens

The swabs were streaked on Mannitol Salt Agar (MSA) and incubated aerobically at 37°C for 24 to 48h.

3.10. Identification of *S. aureus*

The identification of *S. aureus* was based on colonial morphology, Gram stain, mannitol fermentation, catalase and coagulase tests.

3.11. Gram stain

After emulsified a portion of growth in physiological saline and spread evenly in clean dry slide, let to dry to form dried smear, then smear was fixed by passing over the flame for seconds.

Crystal violet was added to cover fixed smear for one minute, then washed by tab water, lugol's iodine added for one minute and washed by tab water, then decolorized by using acid alcohol for 15-20 seconds and also washed by tab water, finally saffranin added for 2 minutes and washed by tab water then wiped the back of slide, let to dry and examined under microscope (Carl Zeiss,Germany) by oil immersion lens (x100).

3.12. Biochemical tests

3.12.1. Catalase test

Two ml of 3% hydrogen peroxide was transfer into sterile test tube and by using wooden stick apportion from growth of organism under test was added so release of air bubbles indicate positive result, no air bubbles indicate negative result. Positive results appear as formation of air bubbles but in negative result no air bubbles are formed (Cheesbrough, 2006).

3.12.2. DNase test

By using of sterile straight loop under aseptic condition the organism under test was inoculated in the DNase agar plate and making heavy spot, the plate were incubated at

37ºC for overnight at incubater (GALLENKAMP,U.K). In the end of incubation period the plate cover with hydrochloric acid, the presence of clear zone around the spot indicates positive result (Cheesbrough, 2006).

3.12.3 Coagulase test

This test was used to differentiate between *S. aureus* (positive) from other *Staphylococci* (negative) the test was performed by emulsifying portion of colonies from pure growth in a drop of undiluted plasma. Formation of clot indicates positive result (Cheesbrough, 2006). *S. aureus* ATCC 29213 used as positive control.

3.13. Preservation of culture organism

About 2-3 colonies of isolates were transferred into crayon tube contain 1 ml of nutrient broth with 20% glycerol and preserve at -20ºC for future analysis.

3.14. Detection of methicillin-resistant *S. aureus*

3.14.1. Phenotypic method

The sensitivity patterns of the isolates were performed using disk diffusion method according to the Clinical and Laboratory Standards Institutes (CLSI, 2010). Antibiotics disc used in this study was, oxacilin 1 µg. The turbidity of the tested microorganisms was set according to 0.5 McFarland Turbidity Standard. The entire Muller-Hinton agar was streaked with overnight culture and antibiotic disc were applied on the agar and incubated at 35ºC for 24 h. The zones of inhibition were interpreted according to CLSI (2010).

3.14.2. Genotypic method (Detection of the *mecA* gene)

3.14.2.1.DNAextraction

Bacterial culture was grown overnight in nutrient broth and 2 mL of the culture was transferred into a microcentrifuge tube and spun for 2 minutes. The pellet was resuspended in 567 µL of Tris EDETA (TE) buffer to which 30 µL of 10% Sodium Dedocyl Sulphate (SDS) and 3 µL of 20 mg/mL proteinase K were added, mixed gently and incubated for 1 hour at 37°C. Following this, 100µL of 5M NaCl was added and mixed thoroughly (Rallapalli *et al*., 2008). After addition of 80µL of 10% CTAB-0.7M NaCl solution and the tubes were incubated for 10 minutes at 65°C. Equal volume of chloroform/isoamyl alcohol (24:1) was added, mixed well and centrifuged at 10,000 RPM for 10 phenol/chloroform/isoamyl alcohol (25:24:1) was added and centrifuged at 10,000 r.p.m for 10 minutes. The upper aqueous phase was transferred to a new tube and 0.8 volumes of isopropanol was added, mixed gently until the DNA was precipitated. The DNA was washed with 70% ethanol and resuspended in 50 µL TE buffer (Rallapalli *et al*., 2008).

3.14.2.2. Polymerase Chain Reaction (PCR)

3.14.2.2.1. Primer Sequncies

The primer used in study was *mec*A F5-'GTAGAAATGACTGAACGTCCGATGA 3' *mec*A R 5'CCAATTCCACATTGTTTCGGTCTAA 3' with product size 310 bp (Geha *et al.,*1994).

3.14.2.2.2. Preparation of reaction mixture

Intron's Maxime PCR premix kit was used in this study

PCR reaction mixture component	volume
Template DNA	2 µl
Primer F	1 µl
Primer R	1 µl
Distilled water	16 µl
Total volume	20 µl

3.14.2.2.3. PCR Protocol Used for Amplification *mecA* gene

The protocol used in this study is a modified one of protocol used by Geha *et al.,* (1994), in this protocol DNA thermocycler was programmed with initial denaturation at 94ºC for 5 minutes, 30 cycles with 30s denaturation step at 94ºC , 30s annealing step at 57 and 30s extention step at72ºC followed by final extention at72ºC for 7min.holding step at 4ºC until the sample was analysed. Amplified products were analyzed using horizontal 1·5% agarose gel electrophoresis, and visualized with UV light transilluminator. The positive test have shown PCR product of 310 bp. ATCC 29123 strain was used as control strain.

CHAPTER FOUR
RESULTS

A total of 165 specimens (55 nasal swabs, 110 hand swabs (left and right)) were collected using sterile cotton swabs. These swabs were collected from 55 food handlers working in cafeterias of Sudan University of Science and Technology (SUST) (Table 1). Among them, 51(93%) were males and 4(7%) were females (Table 2). The targeted food handlers were divided into four age groups as follows; $\leq$ 20 years (32.7%), 21-31 years (40%), 32-41 (20%), and $\geq$ 42 year (7.3%), (Table 3). The majority 45(81.8%) of respondents food-handlers had a middle educational level, primary and secondary, while among the others there are three (5.4%) respondents had a certificate and 7(12.7%) were illiterates (Table 4).

The data in this study showed clearly the existence of *S. aureus* in nasal swabs 21/55(38%), in right hand 17/55(31%) and in left hand 10/55(18%) (Fig. 1, 2 and 3).

The results revealed that out of 48 tested isolates, 4 (8.3%) were methicillin resistant by oxacillin disk diffusion method, 5 (10.5%) were intermediate and 39 (81.3%) were sensitive to oxacillin (Table 6).. PCR technique indicated that 21(43.8%) were *mecA* positive as MRSA strains (a band typical in size (310bp)), and 56.2% (n=27) were *mecA* negative (Table 7), when oxacillin disk diffusion method was used.

Results compared with the PCR based method (Table 8), oxacillin disk method sensitivity and specificity was 19%, and 81% respectivly (Table 9).

Table 1. Types and Frequency of each specimen collected during this study

Type of specimen	Frequency	%
Nasal swab	55	33.3
swab from right hand	55	33.3
swab from left hand	55	33.3
Total	165	100.0

Table 2. Distribution of specimens according to sex

Sex	frequency	%
Male	51	93
Female	4	7

Table 3. Distribution of specimens according to age group

Age group(years)	Frequency	%
lowest through 20	18	32.7
21-31	22	40.0
32-41	11	20.0
42 through highest	4	7.3
Total	55	100.0

Table 4. Food-handlers educational levels frequencies

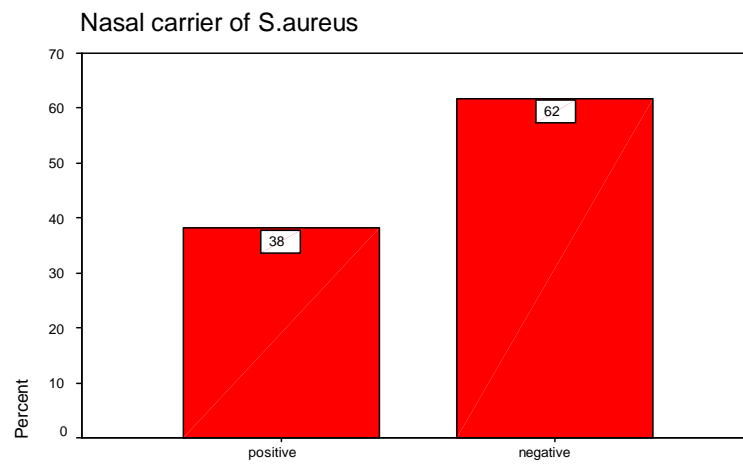| Educational levels	frequency	%
Illiterate	7	12.7%
Primary	23	41.8%
Secondary	22	40%
Certificate	3	5.4%

Nasal carrier of S.aureus

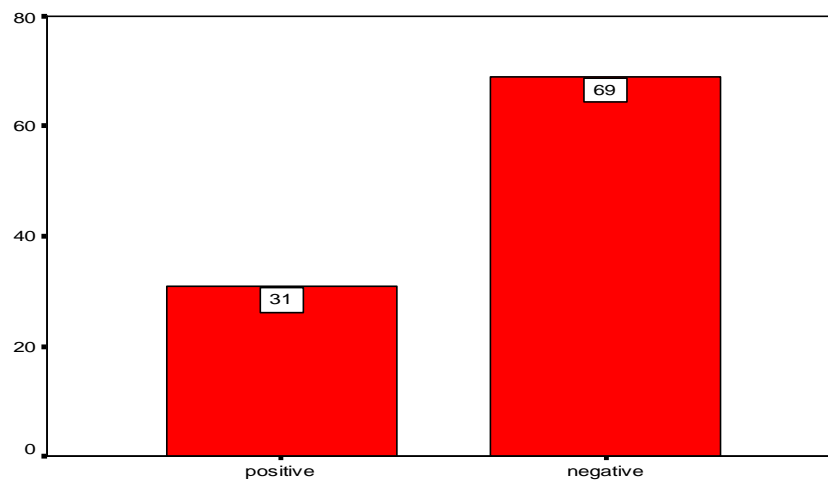**Figure 1.** Percentage of nasal carrier of *S. aureus* among food handler

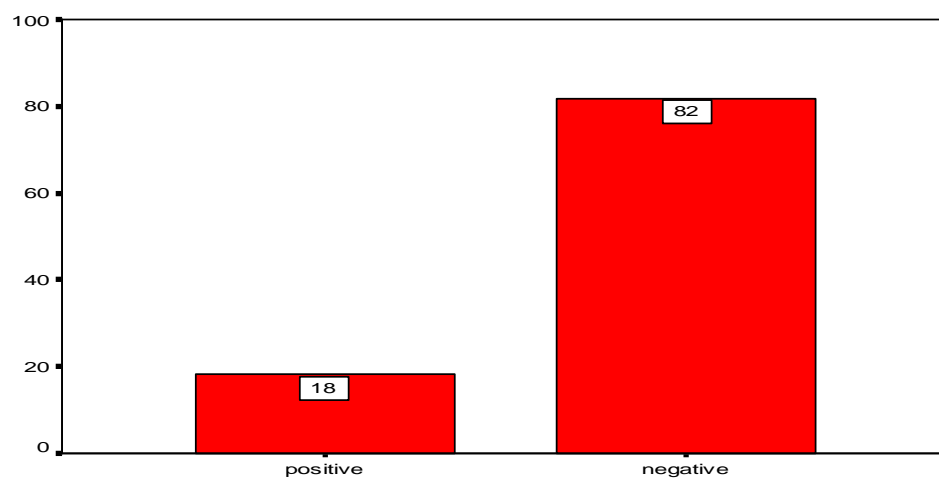**Fig. 2.** Percentage of Right hand carrier of *S. aureus* among food handlers

Fig. 3. Percentage of Left hand carrier of *S. aureus*
among food handlers

Table 5. Sociodemographic characteristics in relation to *S. aureus* detected in nostrils, right hand and left hand of food handlers

Characteristic	% of *S. aureus* in nostrils		AssociationX2 and p value	% of *S.aureus* in right hand		AssociationX2 and p value	% of *S.aureus* in left hand		AssociationX2 and p value
	+ve	-ve		+ve	-ve		+ve	-ve	
Education									
Illiterate	2(28.6%)	5(71.4%)	X2 =0.031 p value=0.882	2(28.6%)	5(71.4%)	X2 =0.324 p value=0.995	2(28.6%)	5(71.4%)	X2 =1.676 p value=0.642
Primary school	9(39.2%)	14(60.8%)		8(34.8%)	15(65.2%)		5(21.7%)	18(78.3)	
Secondary school	9(41%)	13(59%)		6(27.3%)	16(72.7%)		3(13.6%)	19(86.4)	
Certificate	0(0%)	3(100%)		1(33.3%)	2(66.7%)		0(0%)	3(100%)	
Sex									
Male	18(35.3%)	33(64.7%)	X2 =0.347 p value=0.556	16(31.4%)	35(68.6%)	X2 =.071 p value=0.791	9(17.6%)	42(82.4%)	X2 =.135 p value=0.714
Female	2(50%)	2(50%)		1(25%)	3(75%)		1(25%)	3(75%)	
Age in years									
≤20	9(50%)	9(50%)	X2 =3.536 p value=0.316	8(44.4%)	10(55.6%)	X2 =4.117 p value=0.243	5(27.8%)	13(72.2%)	X2 =5.670 p value=0.129
21-31	5(22.7%)	17(77.3%)		5(22.7%)	17(77.3%)		2(9%)	20(91%)	
32-41	4(36.4%)	7(63.6%)		4(36.4%)	7(63.6%)		1(9%)	10(91%)	
≥42	2(50%)	2(50%)		0(0%)	4(100%)		2(50%)	2(50%)	

X^2 = chi-square

Table (6): Frequencies of suscbtibility testing

Suscbtiblity to oxacillin	frequency	%
Resistant	4	8.3
Intermediate	5	10.4
Sensitive	39	81.3
Total	48	100

Table (7): *mecA* frequency

mecA present	frequency	%
Negative	27	56.2%
positive	21	43.8%
Total	48	100%

Table 8. Susceptibility of *S.aureus* isolates tested with oxacillin disk diffusion method in comparison with mecA PCR

		Oxacillin method		
mecA PCR resistant	Number of isolates	susceptible	intermediate	
Negative	27	22	3	2
positive	21	17	2	2
Total	48			

Table 9. Sensitivity and specificity of phenotypic and genotypic methods used for the detection of MRSA strains.

Method	sensitivity (%)	specificity%	PPV%	NPV%
Oxacillin disk diffusion	**19**	**81.5**	**44**	**56.4**
PCR for *mecA* gene	**100%**	**100%**		

PPV: Positive Predictive Value.

NPV: Negative Predictive Value.

lane(1) lane(2) lane(3) lane(4) lane(5) lane(6) lane(7) lane (8)

310
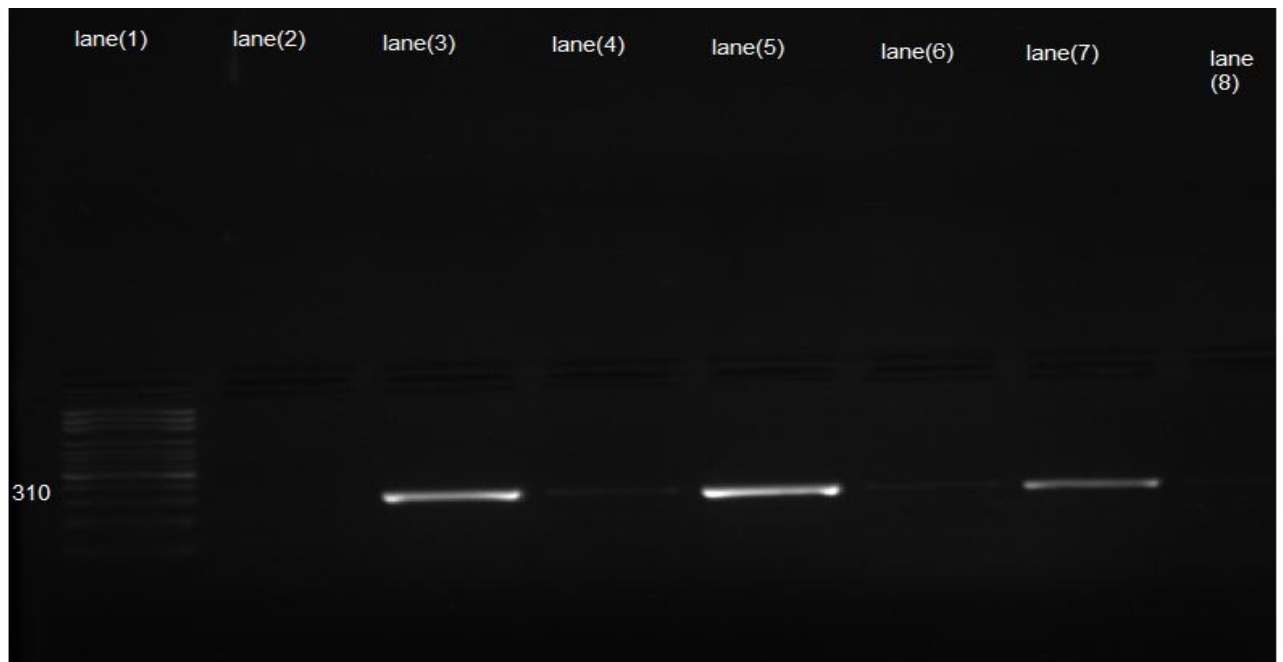
*mec*A PCR results for some of the tested isolates. There are positive bands at 310 bp(lane3-lane8). MSSA ATCC 29213 (negative control) at lane 1.

CHAPTER FIVE
DISCUSSION

5.1. Discussion

In this study out of 55 food handlers tested, 38% were found to be positive *S. aureus* nasal carriers. The prevalence of *S. aureus* nasal carriage among the respondents was higher than that reported by Souza and Santos, (2009) (29.5%), Acco *et al*., 2003 (30%), and Oteri *et al*. 1989 (24%) Neela *et al*. (2008), which was 20.9%. This study revealed also that the percentage of *Staphylococcus* hand carrier among food handlers is 49%. This result is higher than that reported by Loeto *et al*., (2007) which was 30.9%.

In present study 4(8.3) isolated *S. aureus* strain out of 48 was susceptable to methicillin when using oxacillin agar diffusion method. This result nearly similar to the result obtained by Dagnew *et al* (2012) which was (9.8%), but higher than that reported by Noor-Azir *et al*., (2012), which was 0%.

The comparison between disk diffusion method and the result by molecular method; revealed that there was 2 isolates gave positive result by OXD (resistant to oxacillin) and gave negative result by PCR (not having *mecA* gene). The explanation of this result is that this isolates may have gene-other than *mecA* gene-that responsible for their resistant to methicillin, and this gene could be a mecA homologue, mecA(LGA251) which has recently been described in MRSA from human and dairy cattle (Paterson *et al*, 2012). Comparison revealed also that the sensitivity and specificity of oxacillin agar diffusion method which is a routine phenotypic method for detection of MRSA strains is (19%) and (81%) respectively and this was low percentage when compared with Baddour *et al*.

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(2007) who reported that the sensitivity and specificity of the oxacillin disk diffusion test were 87.5 and 79.2%; respectively. In another study by Jain *et al*., (2008) the sensitivity and specificity of the oxacillin disk diffusion test were 95.83 and 58.33%; respectively which was also high compared with this study.

5.2. Conclusion

The study concluded that:

- The prevalence of *S.aureus* among food handlers is high, so food handlers can be a potential source of food contamination and *Staphylococcus* food poisoning.

- The prevalence of methicillin resistance among *S.aureus* isolates from food handlers is considerable.

- Oxacillin disk diffusion method compared to PCR is not a valid phenotypic method for detecting MRSA.

5.3. Recommendations

1. The findings of this study necessitate improvements in regional carrier detection, infection control, and food hygiene.

2. Anyone working in a food business who is likely to come into contact with food must report to their manager immediately if they have an illness that is likely to be passed on through food or if they have certain medical conditions that could lead to this.

3. Very important to wash hands after**:**

   -Handling raw food, such as meat.

   -Changing a dressing or touching open wounds.

-Any contact with other people's faeces or vomit, e.g. changing nappies.

ouching animals / pets.

-Handling waste and touching bins.

-Cleaning.

-Breaks.

4. More studies about MRSA as a cause of SFP.

5. More studies in phynotypic methods for detection of MRSA.

6. Educational lectures for food handlers on how to prepare food in a safety way.

REFERENCES

1. **Acco, M., Ferreira, F. S., Henriques, J. A. P., & Tondo, E. C. (2003).** Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handlers. *Food Microbiology,* 20(5), 489-493.

2. **Ani. O. C, Uhu. A. C., and Nzenwa. N. J. (2011).** The Prevalence of *Staphylococcus aureus* in Food Samples At Selected areas of ABA, ABIA State, Nigeria. *Continental Journal of Biological Sciences*; 4(2):1.

3. **Argudín. M. A., Mendoza. M. C., González-Hevia. M. A., Bances. M., Guerra. B., and Rodicio. M. R. (2012).** Genotypes, Exotoxin Gene Content, and Antimicrobial Resistance of *Staphylococcus aureus* Strains Recovered from Foods and Food Handlers, *Applied and Environmental Microbiology*; 78(8): 2930–2935.

4. **Baddour MM, Abuelkheir MM, and Fatani AJ (2007).** Comparison of mecA polymerase chain reaction with phenotypic methods for the detection of methicillin-resistant *Staphylococcus aureus. Current Microbiol*; 55: 473-479.

5. **Centers for Disease Control and Prevention (CDC).2006**. *Staphylococcal* Food Poisoning.

6. **Çepoğlu. H. Vatansever. L. and Oral. N. B. (2010).** Isolation of Staphylococci from Food Handlers and Investigation of Their Enterotoxigenicity and Susceptibility to Some Antibiotics. *Kafkas Univ Vet Fak Derg* 16 (Suppl-A): S1-S5, 2010.

7. **Cheesbrough. M. (2006).** District laboratory practice in tropical countries, part 2, second edition, Cambridge University Press.

8. **Clinical and Laboratory Standards Institute (CLSI). (2010).** Performance Standard for Antimicrobial Susceptibility Testing; 20*th* Informational Supplement. CLSI document M100-SW20 Clinical and Laboratory Standards Institute, Wayne, PA.

9. **Dagnew. M., Moges. T, Feleke. M, and Zinaye. T. (2012).** Survey of nasal carriage of *Staphylococcus aureus* and intestinal parasites among food handlers working at Gondar University, Northwest Ethiopia. http://www.biomedcentral.com/1471-2458/12/837.

10. **Doyle. M. E., Hartmann. F. A., and Lee Wong. A. C. (2012).** Methicillin-resistant staphylococci: implications for our food supply?. *Cambridge Journals.* 13(02): 157-180).

11. **Edet E Udo, Siham Al-Mufti, and M John Albert. (2009).** The prevalence of antimicrobial resistance and carriage of virulence genes in *Staphylococcus aureus* isolated from food handlers in Kuwait City restaurants, BioMed Central Research Notes; 2: 108.

12. **Florence. B., and Michel. G. (2003).** *Staphylococcus aureus* and Food Poisoning. Genetics and Molecular Rsearch; 2(1):63-76.

13. **Food Standards Agency.** Food Handlers Fitness To Work: Regulatary Guidance and Best practice Advice for Food Business Operators.

14. **Geha, D. J., Uhl, J. R.., Gustaferro, C. A., and Persing, D. H. (1994)**. Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. *Journal of Clinical Microbiology,* 32, 1768-1772.

15. **Harvey. R. A., Cornelissen. C. N., and Fisher. B. D. (2013).** Microbiology (Lippincott's Illusterated Review), third edition, LIPPINCOTT WILLIAMS AND WILKINS: A Wolter Kluwer Companey.

16. **Hennekinne. J .A., Ostyn. A., and Dragacci. S. (2010).** How Should Staphylococcal Food Poisoning Outbreaks Be Characterized?. *Toxins.* 2(8): 2106-2116.

17. **Ifeadike C. O., Ironkwe O. C., Adogu P. O. U., Nnebue C. C., Emelumadu O. F., Nwabueze S. A., and Ubajaka. C. F. (2012).** Prevalence and pattern of bacteria and intestinal parasites among food handlers in the Federal Capital Territory of Nigeria. *Nigerian Medical Journal*; 53(3): 166–171.

18. **Jain A, Agarwal A, Verma RK (2008).** Cefoxitin disc diffusion test for detection of meticillin-resistant Staphylococci. *J. Med. Microbiol.* 57:957-961.

19. **Jordá. G. B., Marucci. R. S., Guida. A. M., Pires. P.S., and Manfredi. E. A. (2012).** Carriage and Characterization of *Staphylococcus aureus* in Food Handlers, *Revista Argentina de Microbiología*, vol.44 no.2 Ciudad Autónoma de Buenos Aires Apr./June 2012.

20. *Kasturwar NB, and Mohd. Shafee. (2010).* Knowledge, Practices and Prevalence of MRSA among Food Handlers. *International Journal of Biological & Medical Research*: 2(4): 889-894.

21. **Loeto. D., Matsheka, M. I, and Gashe, B.A (2007).** Enterotoxigenic and Antibiotic Resistance Determination of Staphylococcus strains isolated from Food Handlers in Gaborone, Botswana. *Journal of Food Protection*; 70(12):276-8.

22. **Neela, V., Ehsanollah, G. R., Zamberi, A., Van Belkum, A., & Mariana, N. S. (2008).** Prevalence of panton–valentine leukocidin genes among carriage and invasive *Staphylococcus aureus i*solates in Malaysia. *International Journal of Infectious Diseases, 13,* 131-132.

23. **Noor-Azira, A. M. Mohammad-Faid, A. R. Shuhaimi, M. Syafinaz, A. N. Hamat, R. A and Malina, O. (2012).** *Staphylococcus aureus* in Food and Nares of Food Handlers in Kuala Pilah, Malaysia**,** Pertanika J. Trop. Agric. Sci. 35 (4): 853 - 862 (2012).

24. **Oteri, T., Staff Clinic, & Ekanem, E. E. (1989).** Food hygiene behaviour among hospital food handlers. *Public Health, 103(3*), 153-159.

25. **Paterson G. K., Larsen. AR., Robb A, Edwards GE, Pennycott. TW, Foster. G, Mot. D, Hermans, K, Peacock, SJ, Parkhill. J., Zadoks. RN., Holmes. MA(2012)**. The newly described mecA homologue, mecALGA251, is present in methicillin-resistant Staphylococcus aureus isolates from a diverse range of host species. *Journal of Antimicrobial Chemotherapy*; volume 67, issue 12.

26. **Pinchuk, I. V. Beswick, E. J and Reyes.V. E. (2010).** Staphylococcal Enterotoxins. *Toxins*; 2(8): 2177-2197.

27. **Rallapalli. S., Verghese. S., Verma. RS. (2008).** Validation of multiplex PCR strategy for simultaneous detection and identification of methicillin resistant *Staphylococcus aureus, Indian Journal of Medical Microbiology*; 26(4): 361-364.

28. **Saeed. H. A, and Hamid. H. H. (2010)**. Bacteriological and parasitological assessment of food handlers in the Omdurman area of Sudan. *Journal of Microbiology, Immunology, and Infection*; 43(1):70-3.

29. **Schelin, Wallin-Carlquist, and Rådström. (2011).**The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Landes Bioscience, *Virulance*; 2(6): 580-592.

30. **Simsek Z, Koruk I, Copur AC, Gürses G. (2009).** Prevalence of *Staphylococcus aureus* and intestinal parasites among food handlers in Sanliurfa, Southeastern Anatolia. *Journal of public health management and Practice*; 15(6):518-23.

31. **Souza, P. A., & Santos, D. A. (2009).** Microbiological risk factors associated with food handlers in elementary schools from Brazil. *Journal of Food Safety, 29,* 424-429.

32. **Temothy F. Jones, Molly E. Kellum, William Schaffner (2002).** An Outbreak of Community-Aquired Foodborne illness caused by Methiciilin-Resistant *Staphylococcus aureus*. *Emerging Infectious Diseases*; 8(1): 82-84.

33. **Tortora. G. J., Funke. B. R., and Case. C. L. (2010).** Microbiology: An Introduction, tenth edition, Benjamin Cummings.

34. **Willey. J. M., Sherwood. L. M., and Woolverton. C. J. (2008).** Prescott,Harley, and Klein's Microbiology, seventh edition, Mc Graw Hill.

35. **Zaglool D. A., Khodari Y. A., Othman. R. A. M., and Farooq M. U. (2011).** Prevalence of intestinal parasites and bacteria among food handlers in a tertiary care hospital. *Nigerian Medical Journal*; 52(4): 266–270.

36. **http://www.foodsafety.gov/poisoning/causes/bacteriaviruses/staphylococcus/**

Appendices

Instruments, Requirements, Media and Reagents

Instruments

Incubator	Torre Pi CEARD,Italy.
Autoclave	Dixon,s U.S.A
Hot air oven	Leader engineering
Water bath	Scout Zarf DIN Co., Germany.
Dry Cabinet	Leader Engineering, U.K.
Microscope	Micros, Austria.
Sensitive balance	Adventure, China.
Refrigerator	Cold air, Sudan.
Distiller	Barnstead, U.S.A.
Safety Cabinet	DAIHAN LABTECH CO., LTD (made in Korea)

Requirements

Sterile disposable universal container s

Sterile cotton swabs

Sterile cotton	
Sterile disposable petri dishes	
Sterile gloves	
Sterile swabs	Sudan
Petri dishes	KIMAX, U.S.A
Slides	CITOGLAS, China
Cotton wool swabs	Ningboyuda, China
Wire (loop, straight)	CITO TEST Jab ware MFG.China
Microscope oil	Aigh med Laboratories, Co, India
Test tubes	Glass, U.SA
Wooden sticks	Ning bo yuda, china
Bunzen burner	Torre pienardi, Italy
Pasteur pipette	MIDC, Kingdom of Saudi arbia

Media

Manitol Salt Agar MSA

Ingredient

Meat extract	1g
Casein peptone	5g
Sodium chloride	75g
D.manitol	10g
Phenol red	.o25g
Agar	15g

Preparation

111g of powder dissolve in 1L of D.W then sterilized by autoclave(Gritten and George ltd,England) at 121°c for 15 minutes then cool and pour in petridishes. Final PH 7.4 .2

Nutrient agar

Ingredient	**g/litre**
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50

Yeast extract   1.50

Agar   15.00

Preparation

28g of powder dissolve in 1L of D.W and sterilize by autoclave(Gritten and George ltd,England) at 121°c for 15 minutes then cool and pour in petridishs. Final pH (at 25 ℃) 7.4$\pm$ 0.2.

DNase Agar

Ingredient

Casein enzymic hydrolysate   15g

Papic digest of soya bean meal   5g

Deoxy ribonucleic acid   2g

Sodium chloride   5g

Casein enzymic hydrolysate   15g

Papic digest of soya bean meal   5g

Deoxy ribonucleic acid   2g

Sodium chloride   5g

Agar   15g

Preparation

42g of powder dissolve in 1L of D.W and sterilize by autoclave(Gritten and George ltd,England) at 121c̊ for 15 minutes then cool and pour in petridishs. Final PH7.3 .

Muller-Hinton agar

Ingredient	g/litre
Beef heart infusion	2.00g
Casein acid hydrolysate	17.50g
Starch	1.50g
Agar	17g

Preparation

38.0g of powder dissolve in 1L of D.W and sterilize by autoclave(Gritten and George ltd,England) at 121c̊ for 15 minutes then cool and pour in petridishs.

Reagents

A. normal saline

Sodium	8.5g
D.W	1L

B. Alcohol 70%

Absolute alcohol	70ml
D.W	30ml

C.HCL

Concentrated HCL	8.6ml
D.W	100ml
Concentrated hydrochloric acid	50ml

E. MacFarland standard

Sulphuric acid solution	99.5ml
Barium chloride solution	0.5ml

Appendix-3

Gram's stain

A. Crystal violet

Crystal violet	20g
Ammonium oxlate	9g
D.W	1L

B. Loglos iodine

Potassium iodine	2g
Iodine	10g
D.W	1L

C. Safranine

Safranine	0.5g
D.W	0.5ml

Appendix 4

-Tris EDETA (TE)

preprration

Tris Base   12.11g

EDTA   3.72g

Distilled water   1000ml

10% Sodium Dedocyl Sulphate(10%SDS).

Preparation

SDS   10g

Distilled water   100ml

5M Sodium chloride (5M NaCl)

Preparation

NaCl   292.2g

Distilled water   1000ml

Tris Borate EDTA Buffer

Preperation

Tris Base   108g

Boric acid   55g

EDTA   7.5g

Distilled water   1000ml