

CHAPTER ONE

1. INTRODUCTION

1.1 Introduction

Foodborne diseases encompass a wide spectrum of illnesses in developed and developing countries. They are growing public health problem. They are the result from ingesting contaminated foodstuffs, by many different pathogens, chemical hazards or other harmful toxins that present in food (Kibret and Abera, 2012). The World Health Organization (WHO) estimated that in developed countries, up to 30% of the population suffers from food borne diseases each year, whereas in developing countries up to 2 million deaths are estimated per year (WHO, 2007).

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in then environment (soil, water and air) and is also found in the nose and on the skin of humans(Dinges *et al.*, 2000)., *Staphylococcus aureus* is the third most common cause of confirmed bacterial foodborne disease in the United States . After an incubation period of 30 min to 8 h, consumption of staphylococcal enterotoxin-contaminated food results in symptoms of vomiting, diarrhea, and abdominal cramping (*Fatih et al.,2014*) ,In all cases of staphylococcal food poisoning, the foodstuff or one of the ingredients, was contaminated with an SE-producing *S. aureus* strain and was exposed, at least for a while, to temperatures that allow *S. aureus* growth. Most of the time the foodstuff reaches this temperature because of a failure in the refrigeration process, or because a growth-permissive temperature is required during processing (e.g., cheese making) (Paciorek *et al.*, 2007).. Many different foods can be a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning, including milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings(Le Loir *et al.,2003*),). Lacking personal hygiene among food handlers is one of the most commonly reported practices contributing to food-borne illness with poor hand and surface hygiene (Lancette and Bennett., 2013) .

1.2. Rationale

Staphylococcal food poisoning is one of the most common types of food borne disease worldwide. It has been identified as causative agent in numerous outbreak of food poisoning but is believed to be under reported due to self limited nature of illness, misdiagnosis and improper collection of specimen. Nasal and hand carriage of enterotoxigenic *S. aureus* by food handlers is an important source of staphylococcal food contamination in restaurants, cafeterias and fast food outlets. Therefore is important to detect enterotoxigenic *S. aureus* carriage among food handlers to prevent possible contamination by them which result in food poisoning. Food poisoning outbreak result in huge financial loss to restaurants, hospital expenses, loss of patient working day and productivity in addition to the loss of reputation and confidence among the public. (22) students of colleges of music and drama and communication sciences, Sudan University of science and Technology (SUST), poisoning after eating a bean gesture at breakfast near the university, were rushed to a hospital accident in Khartoum (last moment news paper, 2013) .Ministry of Health revealed for 227 poisoning case came to hospital and the reason is due to eating breakfast on the occasion of marriage at al-Sagai area (Al-Watan newspaper , 2012).

Staphylococcal food borne are estimated to cause 6-81 million illness and up to 9000 death and account for 14-21% of outbreak involving contaminated in the USA (Mead *etal.*, 1999) also there were reported in the European union 29 outbreak in 2008 (European food safety authority, 2010).

This study aims to determine the frequency and susceptibility pattern of s.aureus among foodhandlers.

1.3. Objectives

1.3.1. General objective

To study enterotoxigenic *Staphylococcus aureus* among food handlers in Cafeterias of Sudan University of Science and Technology, Khartoum hospital and Alestad cafeterias.

1.3.2. Specific objectives

1. To isolate *S. aureus* from nasal and hand of food handlers.
2. To determine most common site inhabit by *S. aureus* on food handlers.
3. To determine the susceptibility of isolated bacteria to antimicrobial agents.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Food borne diseases

Foodborne disease is often referred to generally as “food poisoning” which has in turn been defined by WHO as “any disease of an infectious or toxic nature caused by or thought to be caused by the consumption of food or water” (Dharod *et al.*, 2009).. This definition includes all food and waterborne illness regardless of the presenting symptoms and signs: it thus includes not only acute illnesses characterized by diarrhoea and/or vomiting, but also illnesses presenting with manifestations not related to the gastrointestinal tract, such as scombrototoxin poisoning, paralytic shellfish poisoning, botulism, and listeriosis. In addition, the definition includes illnesses caused by toxic chemicals but excludes illness due to known allergies and food intolerances (Thaker *et al.*, 2013) Bacteria are the causative agents of foodborne illness in 60% of cases requiring hospitalization (Mead *et al.*, 1999).

The microbes have been associated with food-borne illness for decades include *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. that they together with members of the genera *Listeria*, *Campylobacter*, *Bacillus* and *Clostridium* are the cause of illness and even death to many people each year, at immeasurable economic cost and human suffering (Bassyouni *et al.*, 2012).

2.2 Staphylococcal Food Poisoning

S. aureus colonizes in 30% to 50% of healthy human population , and the anterior nares of the nose are the most frequent carriage site for the bacteria, In the National Health and Nutrition Examination Survey conducted in 2001-2002 in the United States, it was estimated that nearly one third (32.4%) of the non-institutionalized population including children and adults were nasal carrier (Montville and Matthews, 2008).

Staphylococcal food poisoning symptoms generally have a rapid onset, appearing around 3 hours after ingestion (range 1–6 hours).

Common symptoms include nausea, vomiting, abdominal cramps and diarrhoea. Individuals may not demonstrate all the symptoms associated with the illness. In severe cases, headache, muscle cramping and transient changes in blood pressure and pulse rate may occur. Recovery is usually between 1–3 days.

Fatalities are rare (0.03% for the general public) but are occasionally reported in young children and the elderly (4.4% fatality rate).

S. aureus can cause various non-food related health issues such as skin inflammations (e.g. boils and styes), mastitis, respiratory infections, wound sepsis and toxic shock syndrome (Stewart 2003; FDA, 2012).

2.3 Mode of transmission

Staphylococcal food poisoning occurs when food is consumed that contains SE produced by *S. aureus*. 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 (Argudin *et al.* 2010).

Large outbreaks of SFP are relatively rare in developed countries. SFP accounts in the United States for only 1.3% of the total estimated cases of foodborne illnesses caused by known agents. In contrast, in the early 1980's, SFP was reported to account for 14% of total foodborne outbreaks in the United States. Similar decreases in frequency have been reported in Japan. Before 1984, 25-35% of all cases of bacterial foodborne illness in Japan involved SFP, whereas in the late 1990's, only 2-5% of incidents involved SFP (Bhatia and Zahoor, 2007). There is no documented report of incidence of SFP in Sudan.

Foods that are frequently incriminated in staphylococcal food poisoning include meat and meat products, poultry and egg products, salads, bakery products, milk and dairy products. mainly include food that rich in salt and carbohydrates and foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning.

Many types of foods have been implicated as vehicles of SFP, but typically such foods are high in protein, sugar, or salt which provide a good medium for the growth of staphylococci. In a study of food poisoning in England, the most frequent products contaminated were meat (ham), poultry (chicken) or their products (75%) followed by fish/shellfish (7%) and milk products (8%) such as cream, cheese, and custards (Bhatia and Zahoor, 2007). The foods that are most often involved in staphylococcal food poisoning differ widely from one country to another. In the United Kingdom, 53% of the staphylococcal food poisonings reported were due to meat products, meat-based dishes, and especially ham; 22% of the cases were due to poultry, and poultry based meals, 8% were due to milk products, 7% to fish and shellfish and 3.5% to eggs (Wieneke *et al.*, 1993). In France, things are different. Among the staphylococcal food poisonings reported in a two-year period (1999-2000), among the cases in which the food involved had been identified, milk products and especially cheeses were responsible for 32% of the cases, meats for 22%, sausages and pies for 15%, fish and seafood for 11%, eggs and egg products for 11% and poultry for 9.5% (Haeghebaert *et al.*, 2002). In the United States, among the staphylococcal food poisoning cases reported between 1975 and 1982, 36% were due to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and only 1.4% to milk products and seafoods. In 17.1% of the cases, the food involved was unknown (Genigeorgis *et al.*, 1989). Thus, the food involved in staphylococcal food poisoning differs widely among countries; this may be due to differences in the consumption and food habits in each of the countries.

Prevention of staphylococcal food poisoning from the infected food handlers may be difficult as carriers are asymptomatic. Other studies also reported high prevalence of enterotoxin-producing *S. aureus* in food handlers.

2.4 Food handlers the main source of food contamination:

Food handlers include those individuals employed directly in the production and preparation of food stuffs, including the manufacturing, processing, catering, and hospitality and retail industries.

However, the definition can also encompass workers undertaking maintenance work or repairing equipment in food-handling areas, and visitors to food-handling areas.

Food handling involves all aspects of treatment and storage of food from receipt of raw materials to the delivery of the prepared product.

Infected food handlers are those individuals who carry infection either with or without symptoms. Food handlers have transmitted both enteric and non-enteric infections via the food that they handled. The current consensus is that ensuring personal hygiene, particularly hand washing, is the most effective tool in preventing the spread of food-borne infections. Long nails harbor more bacteria under them than short ones; however, bacteria are best removed below long nails by liquid soap and a nail brush than by alcohol scrubs (María Ángeles *et al.*, 2010).

Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions. In fact, *S. aureus* is common commensals of the skin and mucosal membranes of humans, with estimates of 20–30% for persistent and 60% for intermittent colonization (Kluytmans, 2005) or even if the human infected can shed the bacteria in the food. However, *S. aureus* is also present in food animals, and dairy

cattle, sheep and goats, particularly if affected by subclinical mastitis, are likely contaminants of milk (Stewart, 2005) Also animal carry this bacteria naturally on their skin and mucous membranes. Air, dust, and food contact surfaces can also serve as vehicles in the transfer of *S. aureus* to foods.

Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment and environmental surfaces (Bhatia and Zahoor, 2007).

2.5 *Staphylococcus aureus*:

S. aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (Montville and Matthews 2008; FDA 2012).

S. intermedius, a *Staphylococcus* species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning , The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity, pH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains (Jay, 1986).

The temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C.

S. aureus is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *S. aureus* is readily killed during pasteurisation or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (ICMSF 1996; Stewart 2003).

S. aureus is uniquely resistant to adverse conditions such as low pH, high salt content and osmotic stress (Montville and Matthews 2008).

S. aureus is a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured meats that do not support the growth of other foodborne pathogens (Montville and Matthews 2008).

S. aureus is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart, 2003).

For a non-sporing mesophilic bacterium, *S. aureus* has a relatively high heat resistance (Stewart, 2003). The pathogenicity of *S. aureus* is due to the toxins, invasiveness and antibiotic resistance. *S. aureus* is major cause of nosocomial and community acquired infections. It is present as a normal flora of human beings and colonizes skin, but may become pathogenic and result in minor skin infections and abscesses, to life threatening diseases such as pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS), septicemia, mastitis, phlebitis, urinary tract infections, osteomyelitis and endocarditis (Bhatia and Zahoor, 2007).

The invasion of host tissues by staphylococci apparently involves the production of a huge amount of extra cellular, proteins, toxins, and enzymes like Coagulase, Staphylokinase, lipase and deoxyribonuclease (DNase). Also proteins having affinity for immunoglobulin (Igs) like Protein A (Bhatia and Zahoor, 2007).

α , β , δ and leukocidins are the common toxins found in *S. aureus* Besides, this given toxins *S. aureus* expresses a number of factors that have the potential to interfere with host defense mechanisms e.g., Capsular Polysaccharide, Protein A and antibiotic resistance. *S. aureus* secretes two types of toxin with superantigen activity, enterotoxins (SE's) and toxic shock syndrome toxin (TSST) which leading to cause of toxic shock syndrome (TSS). Besides this, the exfoliatin toxin which associated with scalded

skin syndrome (SSS), causes separation within the epidermis, between the living layers and the superficial dead layers (Bhatia and Zahoor, 2007).

2.6 Staphylococcal enterotoxins:

The *S. aureus* enterotoxins (SEs) are potent gastrointestinal exotoxins synthesized by *S. aureus* throughout the logarithmic phase of growth or during the transition from the exponential to the stationary phase (Derzelle *et al.*, 2009). They are active in high nanogram to low microgram quantities (Larkin *et al.*, 2009) and are resistant to conditions (heat treatment, low pH) that easily destroy the bacteria that produce them, and to proteolytic enzymes, hence retaining their activity in the digestive tract after ingestion (Letertre *et al.*, 2003).

The ingestion of foodcontaining pre-formed SE (Argudin *et al.* 2010). There are several different types of SE; enterotoxin A is most commonly associated with staphylococcal food poisoning. Enterotoxins D, E and H, and to a lesser extent B, G and I, have also been associated with staphylococcal food poisoning (Seo and Bohach 2007; Pinchuk *et al.* 2010).

SEs is produced during the exponential phase of *S. aureus* growth, with the quantity being strain dependent. Typically, doses of SE that cause illness result when at least $10^5 - 10^8$ cfu/g of *S. aureus* are present (Seo and Bohach 2007; Montville and Matthews 2008). Most genes for SEs are located on mobile elements, such as plasmids or prophages.

As such, transfer between strains can occur, modifying the ability of *S. aureus* strains to cause disease and contributing to pathogen evolution (Argudin *et al.* 2010; Pinchuk *et al.*, 2010).

S. aureus produces SEs within the temperature range of 10–48°C, with an optimum of 40–45°C. As the temperature decreases, the level of SE production also decreases (Normanno *et al.*, 2007). However, SEs remains stable under frozen storage. SEs is extremely resistant to heating and can survive the process used to sterilise low acid canned foods. SE production can occur in a pH range of 4.5–9.6, with an optimum of 7–8. Production of SEs can occur in both anaerobic and aerobic environments; however, toxin production is optimum in aerobic conditions (ICMSF 1996; Stewart 2003).

SEs is resistant to the heat and low pH conditions that easily destroy *S. aureus* bacteria.

The SEs are also resistant to proteolytic enzymes, hence SEs retain their activity in the gastrointestinal tract after ingestion. SEs range in size from 22–28 kDa and contain a highly flexible disulphide loop at the top of the N-terminal domain that is required for stable conformation and is associated with the ability of the SE to induce vomiting (Argudin *et al.*, 2010).

It has been suggested that SEs stimulate neuroreceptors in the intestinal tract which transmit stimuli to the vomiting centre of the brain via the vagus nerve (Montville and Matthews 2008).

In addition, SEs is able to penetrate the lining of the gut and stimulate the host immune response. The release of inflammatory mediators, such as histamine, causes vomiting. The host immune response also appears to be responsible for the damage to the gastrointestinal tract associated with SE ingestion, with lesions occurring in the stomach and upper part of the small intestine. Diarrhoea that can be associated with staphylococcal food poisoning may be due to the inhibition of water and electrolyte reabsorption in the small intestine (Argudin *et al.* 2010). However, staphylococcal enterotoxins have been proposed to be named according to their emetic activities. Only SAGs that induce vomiting after oral administration in a primate model will be designated as SEs (Argudin, 2010).

In contrast to the case of many other bacterial enterotoxins, specific cells and receptors in the digestive system have not been unequivocally linked to oral intoxication by a SE. It has been suggested that SEs stimulate the vagus nerve in the abdominal viscera, which transmits the signal to the vomiting center in the brain. SEs is able to penetrate the gut lining and activate local and systemic immune responses.

Release of inflammatory mediators (including histamine, leukotrienes, and neuroenteric peptide substance P) causes vomiting and the emetic response can be eliminated by H₂- and calcium channel-blockers, which also block the release of histamine. Local immune system activation could also be responsible for the gastrointestinal damage associated with SE ingestion. Inflammatory changes are observed in several regions of the gastrointestinal tract, but the most severe lesions appear in the stomach and the upper part of the small intestine. The diarrhea sometimes associated with SEs intoxication may be due to the inhibition of water and electrolyte reabsorption in the small intestine. In an attempt to link the two distinct activities of SEs, superantigenicity and enterotoxicity, it has been postulated that enterotoxin activity could facilitate transcytosis, enabling the toxin to enter the bloodstream and circulate through the body, thus allowing the interaction with antigen presenting- and T-cells that leads to superantigen activity. In this way, circulation of SEs following ingestion of SEs as well as their spread from a *S. aureus* infection site, could have more profound effects upon the host *versus* if the toxin remains localized (Argudin, 2010).

2.7 Diagnosis

Laboratory procedures to assist in diagnosis of staphylococcal infections are quite simple. Staphylococci grow overnight on blood agar and manitol salt agar incubated aerobically. Catalase and coagulase tests performed directly from the colonies are sufficient for identification. Antibiotic susceptibility tests are indicated because of the emerging resistance of *S. aureus* to multiple antimicrobics, particularly methicillin and vancomycin (James *et al*, 2004).

2.8 Treatment

Penicillin and cephalosporins are active against *S. aureus* cell wall peptidoglycan and vary in their susceptibility to inactivation by staphylococcal beta Lactamases.

Although penicillin G is the treatment of choice for susceptible strains, the penicillinase resistant penicillins (methicillin, nafcillin, oxacillin) and first generation cephalosporins are more commonly used because of resistance. For strains resistant to these agents or patients with beta-lactam hypersensitivity, the alternatives are vancomycin, clindamycin, or erythromycin. Synergy between cell wall active antibiotics and the aminoglycosides is present when the staphylococcus is sensitive to both types of agents. Such combinations are often used in severe systemic infections when effective and rapid bactericidal action is needed, particularly in compromised hosts (James *et al*, 2004).

2.9 Prevention

Preventive measures are aimed at controlling reinfection and, if possible eliminating the carrier state. Clothes and bedding that may cause reinfection should be washed at a sufficiently high temperature to destroy staphylococci (70°C or higher) or dry cleaned. In adults, the use of chlorhexidine or hexachlorophene soaps in showering and washing increases the bactericidal activity of the skin. In such individuals, or persons found to be a source of an outbreak, anterior nasal carriage can be reduced and often eliminated by the combination of nasal creams containing topical antimicrobics (eg, mupirocin, neomycin, and bacitracin) and oral therapy with antimicrobics that are concentrated within phagocytes and nasal secretions (eg, rifampin or ciprofloxacin). Attempts to reduce nasal carriage more generally among medical personnel in an institution are usually fruitless and encourage replacement of susceptible strains with multiresistant ones.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study design

3.1.1 Type of study

This is a cross sectional study

3.1.2 Study area

Data of this study were collected from different cafeterias (n=3) of Sudan university of Science and Technology at Khartoum State, Khartoum hospital and Alestad cafeterias.

3.1.3 Study population

Sample in this study were collected from males food handlers their ages range from 15-40years.

3.1.4 Inclusion criteria

Food handlers working in the cafeterias of Sudan university of Science and Technology at Khartoum state (n:7), Alestad cafeterias (n:5) and cafeteria of Khartoum hospital (n:8) and given informed consent were included in the study.

3.1.5 Exclusion criteria

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

3.1.6 Sample size

The study was conducted on swabs from nasal cavity, hand and fingernails of 20 of food handlers from 3 different cafeterias of Sudan university of Science and Technology, Alestad cafeterias and cafeteria of Khartoum Hospital.

3.1.7 Ethical consideration

(Approval of study by university Institutional Review Board), informed consents were signed by food workers who agreed to have nasal mucosal and hand samples taken.

3.2 collections of specimens

A total of 20 nasal swabs, 20 swabs from right hands and 20 from left hands were collected. A pretested structured questionnaire was used for collecting information on age, sex, service years, educational status, status of training and habits of hand washing of each food-handler. Nasal swab was collected aseptically from food handlers' nostrils rolling several times by applicator stick tipped with cotton and moistened with sterile normal saline. Hand swab was collected by rolling several times inside and outside the hand, between fingers and under nail from right and left hand using separate applicator stick tipped with cotton and moistened with sterile normal saline. Swabs were labeled with patients name and age. All specimens were transferred during one hour to the laboratory.

3.3 Culture

By using sterile bacteriological loop Manitol Salt Agar (MSA) media (**appendix1**) plates were inoculated under aseptic condition near the flame. The plates were incubated aerobically at 37°C for overnight incubation in incubator. Only specimens yield growth of yellow colonies were identified by Gram stain and biochemical tests, manitol fermenter appear as yellow colonies but non manitol fermenter appear as red to pink colonies.

3.4 Identification

3.4.1 Gram stain

After emulsified a colony in physiological saline (**appendix 2**) and spread evenly in clean dry slide we 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 tap water, lugol's iodine added for one minute and washed by tap water, then decolorized by using acid alcohol for 15-20 seconds and also washed by tap water, finally safranin added for 2 minutes and washed by tap water then wiped the back of slide, let to dry and examined under microscope (Carl Zeiss, Germany) by oil immersion lens (x100).

3.4.2 Biochemical tests

3.4.2.1 Catalase test

2ml of 3% hydrogen peroxide (**appendix 2**) 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.

3.4.2.2 DNase test

By using of sterile straight loop under aseptic condition the organism under test was inoculated in the DNase agar (**appendix 4**) plate and making heavy spot, the plate were incubated at 37 °C for overnight at incubator. In the end of incubation period the plate cover with hydrochloric acid (**appendix 2**), the presence of clear zone around the spot indicates positive result.

3.4.2.3 Coagulase test

This test 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

3.4.3 Susceptibility techniques

The susceptibility techniques testing was done by NNCLs disc diffusion technique. .

3.4.3.1 Disc diffusion technique

Kirby-bauer diffusion disc method was used for antimicrobial Disc susceptibility testing on Muller hinton Agar.

The diameter of the zone of inhibition of the test is measured and compared with previously prepared table (makki and macCartney.1996).

3.4.3.2 Method of susceptibility:

- 1.preparation of Agar:The media was prepared and sterilized as instructed by the manufacture.
- 2.Preparation of inoculation and turbidity standard McFarland :two to three colonies of the test organism was emulsified in a small volume of the sterile saline the 0.5 McFarland was prepared by adding 0.5ml of 1.75%(w/v) barium chloride solution to 99.5ml of 1%(v/v) sulphuric acid . the turbidity standard was liquated into test tube identical to those used to prepare the inoculums suspension .McFarland standard tube was sealed with wax and some other means to prevent evaporation . Befor any use shake well .Then suspension compared with McFarland standard to adjust the turbidity.
- 3.seeding of the inoculums: Sterile cotton swab was impregnated inside the suspension of test organism, excess was removed by passing it in the tube . it was then applied to the center of sensitivity agar plate . the inoculums were spread evenly across the plate . the inoculums then allowed to drying for a few

minutes. Then antimicrobial disc were placed on the plate using sterile plate forceps the plate were incubated aerobically at 73°C for over night . Then inhibition zone were read and compared with NCCLS standard tables.

Reaction of the test organism were read and reported for eash Anibiotic as sensitive or resistant.

3.4.3.3 Antimicrobial disc:

Commercial discs 6mm in diameters were used.

The following antibiotics were used :

Erythromycin , vancomycin ,Methicillin , gentamicin.

3.5 Statistical analysis:

Some of the table were constructed and calculated manually and others done by statistical package for social sciences (SPSS-s).

Chapter Four

4. Results

A total of (60) samples of nasal and hand (right and left) swabs were collected from (20) food handlers work in cafeterias of Sudan University of Science and Technology (n:7), Alestad cafeterias (n:5) and cafeteria of Khartoum Hospital (n:8) (**Table 1**). All food handlers were classified into 4 group, age group one 4(20%), age group two 11 (55%), age group three 3(15%) and age group four 2(10%) (**Table 2**). The (table3) show that *S.aureus* isolated from left hands were highly sensitive to vancomycin (100%), Erythromycin (100%) and Gentamicin (100%), but less sensitive to Methicillin (33%), The (table4) show that *S.aureus* isolated from right hands were highly sensitive to vancomycin (100%), Erythromycin (100) and Gentamicin (100%), but less sensitive to Methicillin (22.5%). The table5 show that *S.aureus* isolated from Nasal carriers were highly sensitive to vancomycin (100%), Erythromycin (100%) and Gentamicin (100%), but less sensitive to Methicillin (18%). The data in this study confirmed clearly the existence of *S.aureus* in nasal swab 11/20(55%) (**Figure1**), in right hand 8/20(40%) (**Figure 2**) and in left hand 6/20(30%) (**Figure 3**).

(**Table 1:** Shows frequency of each sample enrolled in this study:

Type of sample	Frequency	Percent%
Nasal swab	20	33.3%
swab from right hand	20	33.3%
swab from left hand	20	33.3%
Total	60	100.0%

Table 2: Shows the distribution of each age group among the study population

Age group(years)	Frequency	Percent%
lowest through 20	4	20%
21-31	11	55%
32-41	3	15%
42 through highest	2	10%
Total	20	100%

Table3: Antimicrobial sensitivity of *Staphylococcus aureus* isolated from left hand:

Bacterial growth	Sensistive(NO)	Percent%	Resistant(NO)	Percent%	Total
Vancomycin	6	100%	0	0%	6
Gentamicin	6	100%	0	0%	6
Erythromycin	6	100%	0	0%	6
Methicillin	2	33%	4	67%	6

Table4:Antimicrobial sensitivity of *Staphylococcus aureus* isolated from right hand:

Bacterial growth	Sensistive (NO)	Percent%	Resistant (NO)	Percent%	Total
Vancomycin	8	100%	0	0%	8
Gentamicin	8	100%	0	0%	8
Erythromycin	8	100%	0	0%	8
Methicillin	1	12.5%	7	88%	8

Table5:Antimicrobial sensitivity of *Staphylococcus aureus* isolated from Nasal carriers:

Bacterial growth	Sensitive(NO)	Percent%	Resistant(N O)	Percent %	Total
Vancomycin	11	100%	0	0%	11
Gentamicin	11	100%	0	0%	11
erythromycin	11	100%	0	0%	11
Methicillin	2	18%	9	81%	11

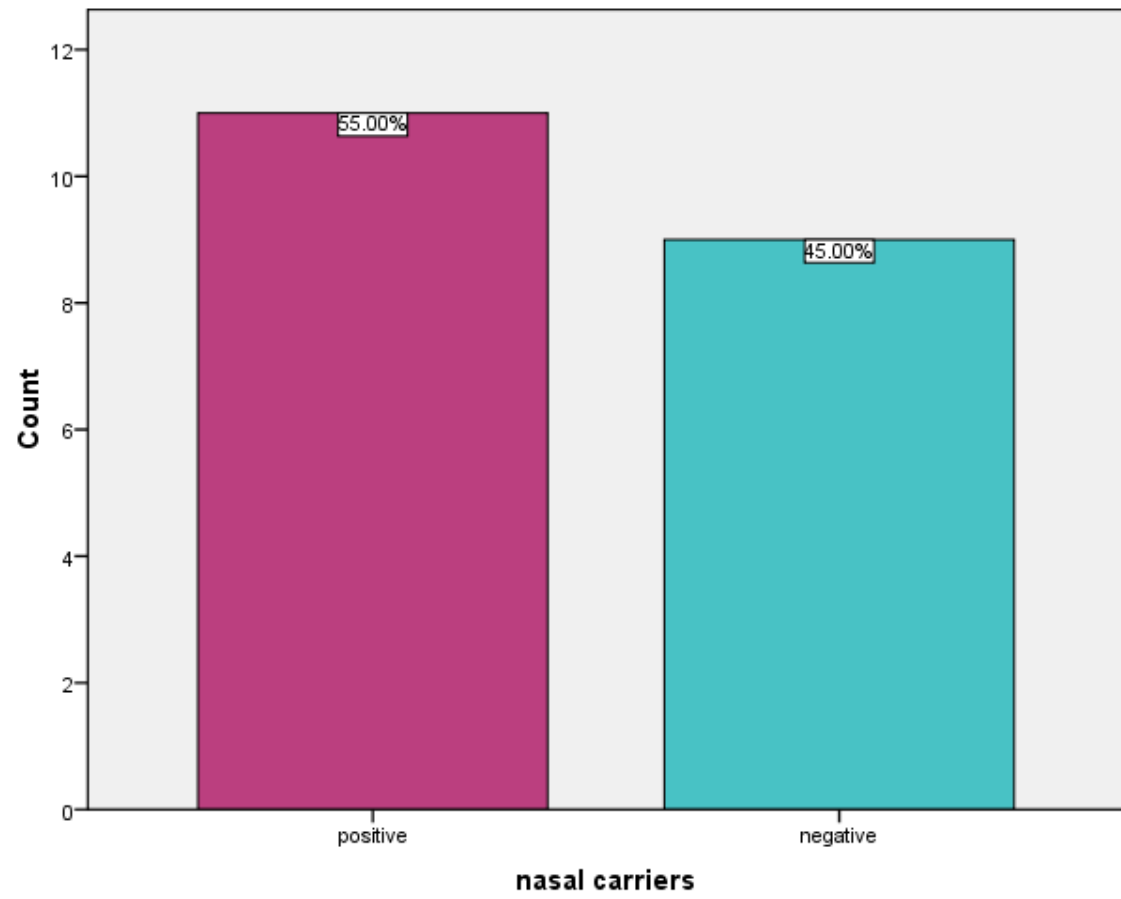


Figure 1: Shows the percentage of nasal carrier of *S.aureus* among food handler

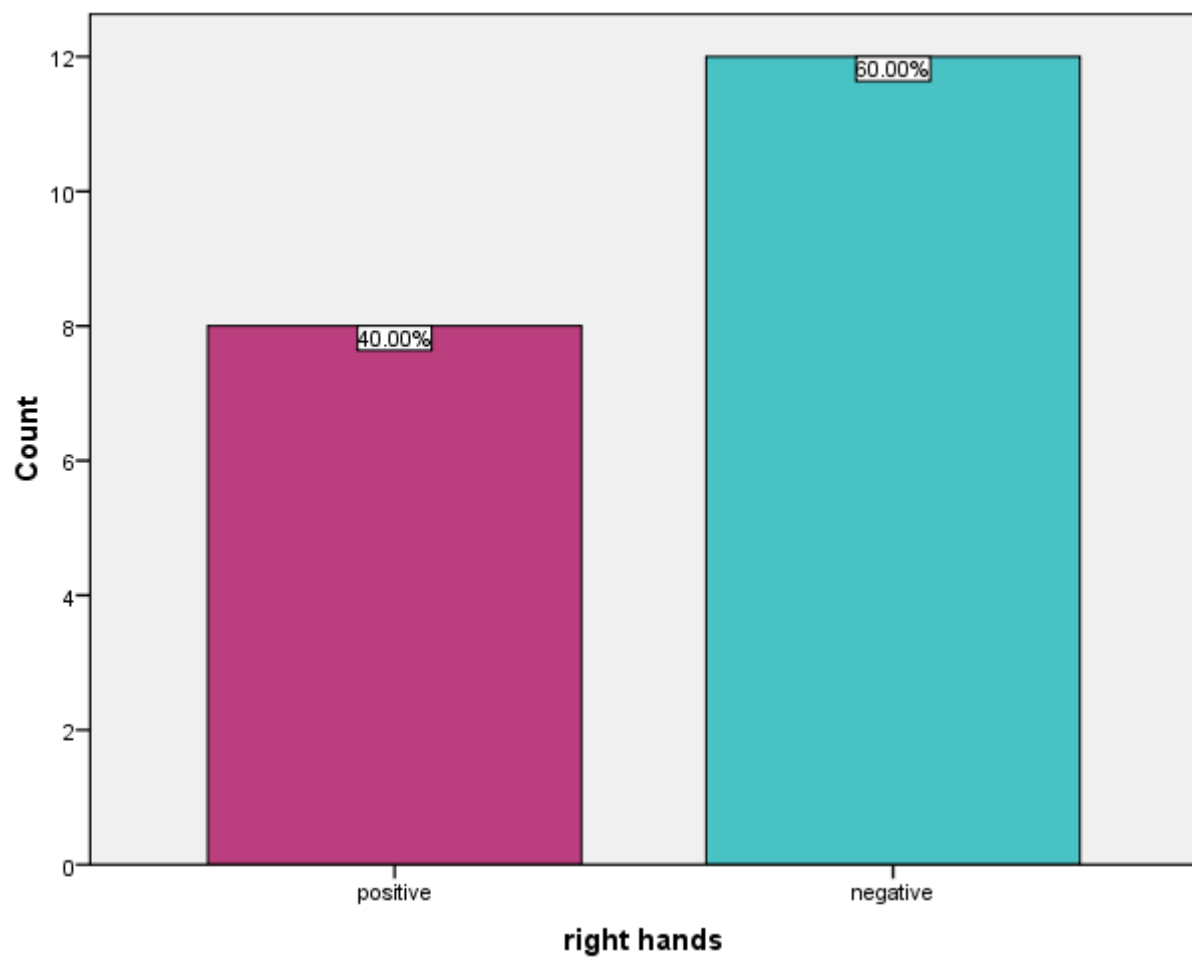


Figure 2: Shows the percentage of Right hand carrier of *S.aureus* among food handlers

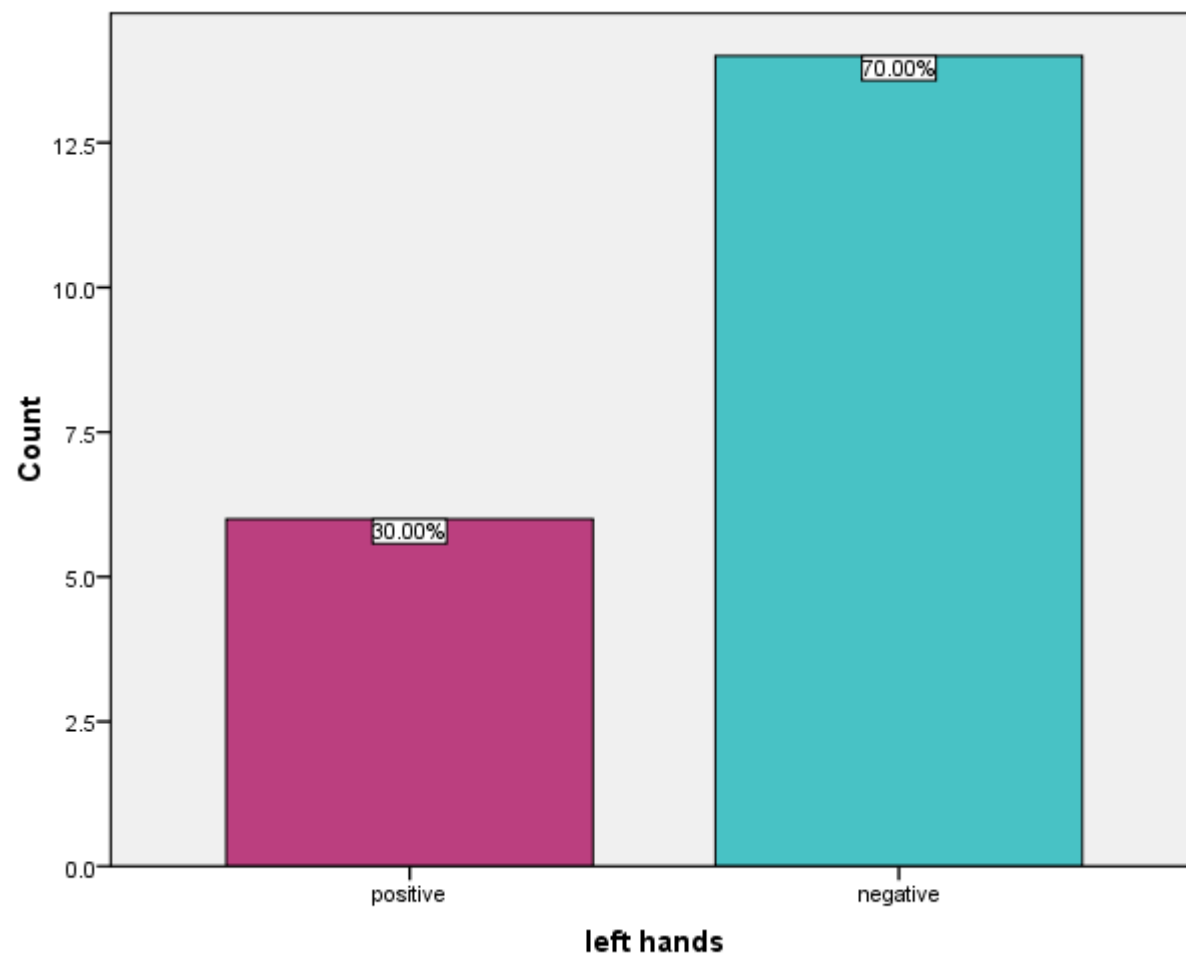
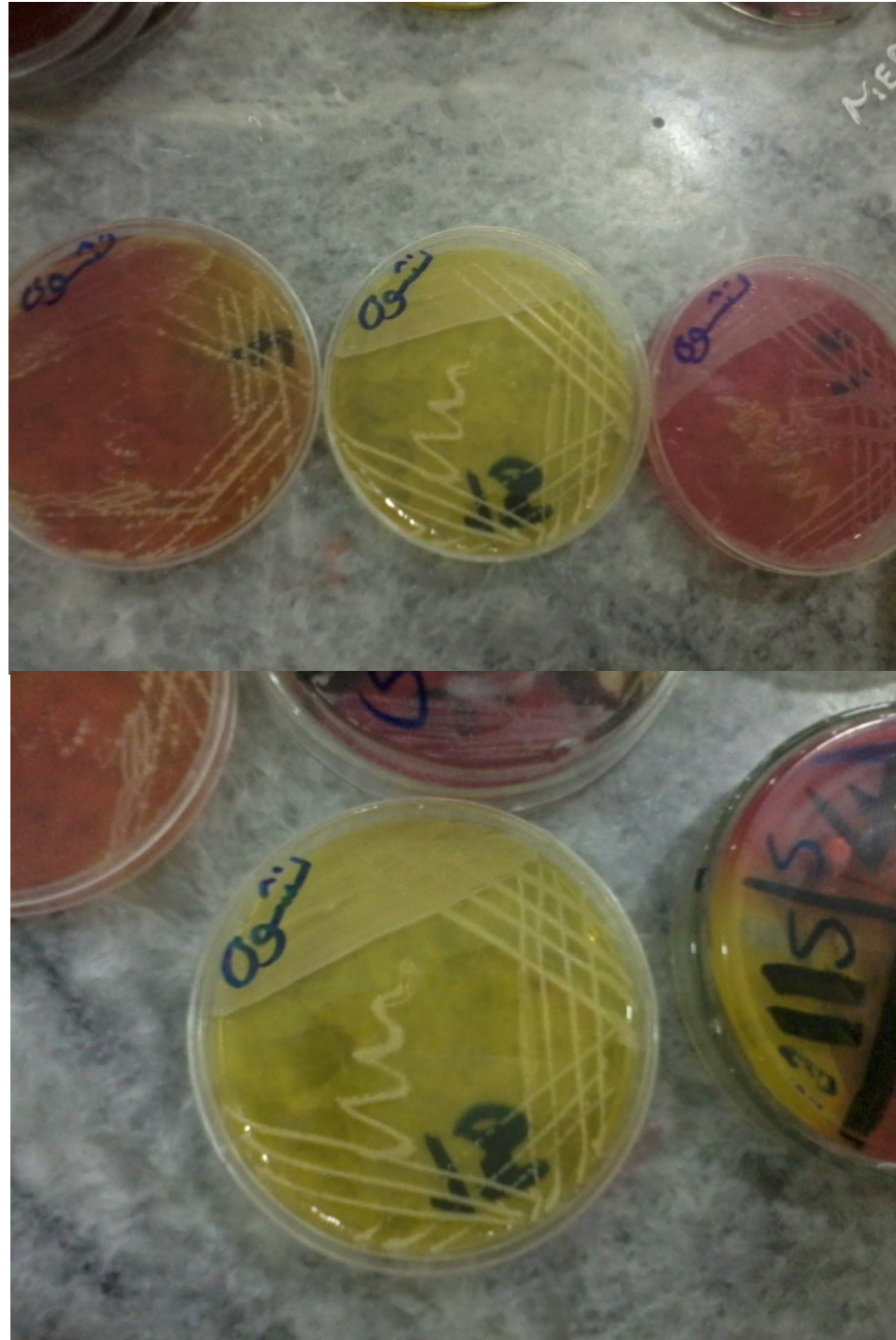


Figure 3: Shows the percentage of Left hand carrier of *S.aureus* among food handlers



Yellow, mannitol fermenting colonies of *S. aureus* on mannitol salt agar (MSA)



Disc diffusion method on Muller Hinton agar and the zone of inhibition was measured by millimeter ruler

Chapter Five

Dissection and Conclusion

Of the 20 food handlers works in cafaterias of Sudan university of Science and Technology, examined, 25 (36.4%) were found to be harboring *S. aureus* in their nostrils, this result is similar to the findings reported in Brazil (Acco *et al.*, 2003) and Botswana (Loeto *et al.*, 2007) as 30%, and 44.6%; respectively. However, our finding was found to be higher than that reported in two studies conducted in Sudan. The first reported 56(21.6%) out of 259 food workers (Saeed and Hamid, 2010), While the second study reported (13.2%) of hospital workers (Abdalla *et al.*, 1998) were nasal carriers of *S. aureus*. But higher than the study conducted at Gondar University in Northwest Ethiopia(Dagneu *et al.*, 2012) and in Fayoum University in Egypt(Bassyouni *et al.*, 2012) as (20.5%)and (17.1%) respectively. Nasal carriage rates reported in these several studies vary and the variation has been attributed to the differences in the environmental and personnel hygiene of the study population.

In our study Out of 20 food handlers the hands of 14(70%) were contaminated with *S. aureus*, 8 (57%) carried *S. aureus* in their right hand and 6(43%) carried it in their left hand and from those 5(36%)carried in both hands ,there is no significant(p value) differences between the right and left hand, this finding similar to that reported in Egypt as they found *S. aureus* on the hands of (36.1%) of food handlers work at fayoum university (Bassyouni *et al.*, 2012). However our result is lower than the finding reported in South Africa as they found 88% their hand contaminated with *S. aureus* (Lues and Tonder, 2007). This variation in studies has been attributed to the differences in the degree of safety measure and hand washing in different geographic regions.

It is very important to note that although *S.aureus* can cause severe infections it may also be as a member of the normal flora of the nasal cavity (William, 1993). If by chance, a food handler carries, an enterotoxin producing *S. aureus* he/she may contaminate the food and causes staphylococcal food poisoning outbreak in the population. So in this study detecting the *S. aureus* isolated from 20nasal isolates of them 11(18.3)were s. aureus .

In this study *S.aureus* was highly sensitive to vancomycin, erythromycin and gentamicin(100%), in contrast to the study carried out by Osariemen *et al.*, (2012) in Nigeria who found that *S.aureus* was less sensitive to vancomycin and erythromycin(11%).

However (80%)of *S.aureus* isolated from food handelers were resistant to methicillin (Methicillin Resistant *S.aureus*) were similar to those obtained by El-Tahawy(2000) in Kingdom of Saudi Arabia who found that (30%) of *S.aureus* were resistant to methicillin.

Recommendations

1. Sample size must be increased to obtain high-precision results.
2. Sample must be collect in septic room.
3. Raised the Awareness of food handlers to produce a healthy and good food.
4. Raise the Awareness of people about clearing of of their hands before eating.
4. Decrease the use of the methicillin as treatment due to presence of methicillin Resistant S.aureus.

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Appendix

Appendix 1

Manitol Salt Agar MSA

Ingredient

Meat extract	1g
Casein peptone	5g
Sodium chloride	75g
D.manitol	10g
Phenol red	.025g
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

Appendix 2

Reagents

a. Hydrochloric Acid 1mmol/L

HCL concentrate 8.6ml added to 100ml D.W

b. Hydrogen peroxide(3% H₂O₂) reagent

3ml of H₂O₂ in 100ml D.W

c. Normal saline (physiological) saline

Sodium chloride 2.5g in 1litter D.W

d. Gram stain

Reagent of gram stain

• Crystal violet stain:

-Crystal violet	20g
-Distilled water	1L

• Lugol's iodine:

-potassium iodine	2g
-Iodine	10g
-Distilled water	1L

• Aceton alcohol decolorize.

• Saffranin:

-Saffranin	0.5g
-Distilled water	0.5ml

Appendix 3

Nutrient agar

Ingredient

Peptic digest of the tissue	5g
Sodium chloride	5g
Beef extract	1.5g
Yeast extract	1.5g
Agar	15g

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 PH7.3 .

Appendix 4

DNase Agar

Ingredient

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 121°C for 15 minutes then cool and pour in petridishs. Final PH7.3 .

Appendix 5

Blood agar

Nutrient agar	500ml
Sterile defibrinated blood	25ml

Appendix 6

Muller Hinton agar

Beef infusion form	300g
Casein cid hydrolysate	17.5g
Starch	1.5g
Agar	17g
Distilled water	100ml

Preparation

Suspend 35 grams in I litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121° C for 15 minutes.

McFarland turbidity standard

Prepare 1% v/v solution of sulphuric acid by adding one ml of concentrated sulphuric acid to 99 ml of D.W. and mix well.

Prepare 1% w/v solution of barium chloride by dissolving 0.5 g of dihydrate baium chloride (BaCl2. H2O) in 50 ml of D.W.

Add 0.6 ml of barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.

Transfere asmall volume of the turbid solution to a screw-cap bottle of the same type as used for preparing the test and control inocula.