

1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

A food handler is anyone who works in food business and who either handles food or surfaces that are likely to be in contact with food such as cutlery plates and bowls (Becker *et al.*, 2004). Food handlers may do many different things for a food business. Examples include making, cooking, preparing, serving, packing, displaying and storing food. They can also be involved in manufacturing, producing, collecting, extracting, processing transporting, delivering, thawing, or preserving food (Smith, 2013).

Food handlers with poor personal hygiene could be potential source of infections of many intestinal microorganisms. Compared to other parts of the hand, the area beneath finger nails harbors microorganisms and is most difficult to clean. The spread of diseases via food handlers is common and persistent problem worldwide (Zaglool, 2011).

Sir Alexander Ogston, a Scottish surgeon, first showed in 1880 that a number of human pyogenic diseases were associated with a cluster-forming microorganism. He introduced the name “staphylococcus” (David *et al.*, 2007). *S. aureus* is resistant to some antibiotics and is responsible for many diseases in warm blooded animals. Illness is caused by the production of toxin in the food as a result of bacterial growth. *S. aureus* remain one of the most frequently reported causative agent of food borne illness (Hocking, 2003).

Food-borne diseases (FBD) are defined by the World Health Organization as “disease of infectious or toxic nature caused by, or thought to be caused by the consumption of food or water. In many countries national health care organizations record FBD outbreaks, defined as; Occurrence of two or more cases of similar illness resulting from ingestion of a common food (Akinden *et al.*, 2001).

According to the Centre of Disease Control and prevention (CDC) staphylococcal food poisoning is gastrointestinal illness. It is caused by eating foods contaminated with toxins produced by *S. aureus*. The most common way for food to be contaminated with *S. aureus* is through contact with food workers who carry the bacteria (CDC, 2004).

S. aureus are Gram-positive, catalase positive cocci belonging to the *Staphylococcaceae* family (Becker *et al.*, 2004 ; Murray *et al.*, 2003). They are approximately 0.5-1.5 μm in diameter, nonmotile, non-spore-forming, facultative anaerobes (with the exception of *S. aureus anaerobius*) that usually form in clusters. Many strains produce staphylococcal enterotoxins, the superantigen toxic shock syndrome toxin (TSST-1), and exfoliative toxins. *S. aureus* are part of human flora, and are primarily found in the nose and skin (Becker *et al.*, 2004).

S. aureus is a type of bacteria commonly carried on the skin and in the noses of healthy people without causing infection. This is known as bacterial colonization. However, when *S. aureus* organisms invade the body, they can cause serious infections. Some *S. aureus* organisms can be treated easily with antibiotics (methicillin-sensitive *S. aureus* [MSSA]) while others are resistant to antibiotics, such as methicillin (methicillin-resistant *S. aureus* [MRSA]) (Graham *et al.*, 2006).

The researchers estimated that 84 million people in the United States were colonized with MSSA and 2 million were colonized with MRSA. The highest rate of colonization with MRSA occurred among people who had lived in a long-term care facility, those with diabetes, women, and people who were 65 years of age or older. Half of those colonized with MRSA had a genetic type of *S. aureus* that has previously been associated with hospital-acquired infections, and half had a type that has previously been associated with

community-acquired infections. Black people and those of Mexican birth were less likely than others to be colonized with *S. aureus*. Those with the community type of MRSA were more likely to respond to antibiotics, such as erythromycin, clindamycin, and ciprofloxacin, all of which can be given by mouth (Graham *et al.*, 2004). *Staphylococcus aureus* is able to grow in a wide range of temperatures (7°C to 48.5°C with an optimum of 30°C to 37°C (Schmid *et al.*, 2007), pH (4.2 to 9.3 with an optimum of 7 to 7.5 (Becker *et al.*, 2008) and sodium chloride concentrations (up to 15% NaCl). These characteristics enable *S. aureus* to grow in a wide variety of foods. This, plus their ecological niche, can easily explain their incidence in foodstuffs that require manipulation during processing, including fermented food products, such as cheeses. Risk assessment in foodstuffs relies on classical microbial detection and quantification of coagulase positive staphylococci on a selective Baird-Parker medium, whose composition is standardized (for France, norms AFNOR V08-057/1 and 2, ISO 6888/1 and 2). Sensitivity of these routine tests is around 10² cfu/g for solid foodstuffs and 10 cfu/g for liquid samples. The different media used for the detection and quantification of *S. aureus* have been reviewed by Baird *et al.*, (1995). In many countries, low degree contaminations by *S. aureus* are tolerated in most foodstuffs (up to 10³ cfu/g in raw milk cheeses, in France), as they are not considered a risk for public health (Le Loir *et al.*, 2003).

S. aureus is an opportunistic pathogen that can cause a variety of self-limiting to life-threatening diseases in humans (Murray *et al.*, 2004). The bacteria are a leading cause of food poisoning, resulting from the consumption of food contaminated with enterotoxins (Le Loir *et al.*, 2003). Staphylococcal food intoxication involves rapid onset of nausea, vomiting, abdominal pain, cramps, and diarrhea. Symptoms usually resolve after 24 hours (Murray *et al.*, 2003; Le loir *et al.*, 2003).

S. aureus is one of the most common causes of skin, soft-tissue, and nosocomial infection (Fridkin *et al.*, 2005). Rates of infection in community settings are increasing (David *et al.*, 2006). Around 20% of individuals are persistent carriers of *S. aureus*, about 60% are intermittent carriers, and approximately 20% rarely carry it (Kluytmans *et al.*, 2000).

Children are more likely to be persistent carriers of the bacteria. Young women are at a higher risk for infection. The preferable hosts for *S. aureus* are; humans, wild and domestic animals, least 100,000 organisms in humans (Schmid-Hempel *et al.*, 2007).

Transmission of *S. aureus* occurs through ingestion of food containing enterotoxins (Le Loir *et al.*, 2003). Vertical transmission during vaginal delivery is uncommon (Reusch *et al.*, 2008). Person-to-person transmission occurs through contact with a purulent lesion or with a carrier (Stevens *et al.*, 2010). Unsanitary conditions and crowded community settings increase exposure to *S. aureus* (Stevens *et al.*, 2010). Infection may be spread from person-to-person through health care workers or patients (Kluytmans *et al.*, 2000).

Nasal colonization can lead to auto-infection (Van Belkum *et al.*, 2007).

S. aureus grows harmlessly on the moist skin of the nostrils in about 30% of healthy persons, and the premium is also commonly colonized. Organism is spread from these sites into environment by the hands, handkerchiefs, clothing, and dust consisting of skin squames and cloth fibers. Some carriers, called shedders, disseminate exceptionally large numbers of staphylococci (David *et al.*, 2007).

Acquisition of infection may be exogenous (from an external source) or endogenous (from a carriage site, or minor lesion elsewhere in the patient own body). It is important to remember that the body surfaces of human beings and animals are the main reservoir.

Although not spore forming, staphylococci may remain alive in a dormant state for several months when dried in pus, sputum, bed clothes or dust, or inanimate surfaces such

as floors. They are fairly readily killed by heat, by exposure to light and by common disinfectants (Ala Adeen *et al.*, 2004).

Incubation period after consuming contaminated food with *S. aureus* is usually 30 minutes to 8 hours (Le Loir *et al.*, 2003). Colonies of *S. aureus* can be carried for an undetermined amount of time; some individuals may carry it chronically, and some may carry it intermittently (Klytman *et al.*, 1997).

Staphylococcus aureus is found in humans in the nose, groin, axillae, perineal area (males), mucous membranes, the mouth, mammary glands, hair, and the intestinal, genitourinary and upper respiratory tracts. (Le Loir *et al.*, 2003). Many animals act as reservoirs, particularly cows with infected udders (Fitzgerald *et al.*, 2001).

1.2. Rationale

S. aureus colonizes in 30% to 50% of healthy human population (Lowy, 1998), and the anterior nares of the nose are the most frequent carriage site for the bacteria (Wertheim *et al.*, 2005).

Prevention of staphylococcal food poisoning from the infected food handlers may be difficult as carriers are asymptomatic (Schmid *et al.*, 2007). However, infected food handlers remain the source of most reported food borne outbreak, a review of food borne outbreaks in United States found that infected food handler who handled uncooked food or food after it had been cooked and during that infectious period were the most common source of food borne outbreaks (Fiore, 2004). Previous outbreak of food poisoning in some cafeterias of Sudan University of Science and Technology caused problem to their students may lead to asking some questions about safety and hygiene level of food handler who working in this university.

Though there are no documented data or few indicative studies have been taken about prevalence of Staphylococcal hand carriers in Sudan among food handler according to last a 7 systematic review of world health organization (WHO). There is no doubt food borne illnesses resulted from improper food handling – therefore; this study was conducted to identify prevalence hand carriers among food handler workers in cafeterias who carry *S. aureus* in Khartoum province.

1.3. Objectives

1.3.1. General objective

To detect phenotypical hand carriage of *S. aureus* among food handlers working in cafeterias.

1.3.2. Specific objectives

1. To determine the carriers of *S. aureus* among food handlers workers in cafeterias in Khartoum province.
2. To detect *S. aureus* in hand swab collected from food handlers working in Sudan University cafeterias by using ordinary culturing techniques.
3. To determine the risk factors associated with *S. aureus* hand carriers among food handlers.

2. LITERATURE REVIEW

2.1 Food borne disease

The food borne diseases are major health problems in developed and developing countries. 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). The spread of food borne diseases via food handlers are a common and persistent problem worldwide (Zain *et al.*, 2002). Many diseases are communicable and caused by micro-organisms that enter into the body via food (Essex-caster, 1987). Numerous outbreaks of gastroenteritis have been associated with ingestion of raw foods, foods incorporating raw ingredients or foods obtained from unsafe sources.

Food poisoning has been reported to be a result of infection with enterotoxigenic strains of (*S. aureus*). It accounts for 14–20% of outbreaks involving contaminated food in the USA, and in the United Kingdom restaurants is the second most important place for Acquired staphylococcal food poisoning. This organism may exist on food handler's nose or skin, from which it may be transmitted to cooked moist protein-rich foods, and become intoxication agent, if these foods are then kept for several hours without refrigeration or stored in containers (Mulat *et al.*, 2012).

Approximately 1.8 million children in developing countries (excluding China) died from diarrhoeal disease in 1998, caused by microbiological agents, mostly originating from food and water. One person in three in industrialized countries may be affected by food-borne illness each year. In the USA, some 76 million cases of food-borne illness, resulting

in 325 000 hospitalizations and 5000 deaths, are estimated to occur each year. There are only limited data on the economic consequences of food contamination and food-borne disease. In studies in the (USA) in 1995, it was estimated that the annual cost of the 3.3-12 million cases of food-borne illness caused by seven pathogens was US\$ 6.5-35 billion. The medical costs and the value of the lives lost during just five food-borne outbreaks in England and Wales in 1996 were estimated at GB£ 300-700 million. The cost of the estimated 11 500 daily cases of food poisoning in Australia was calculated at AU\$ 2.6 billion annually. The increased incidence of food-borne disease due to microbiological hazards is the result of a multiplicity of factors, all associated with our fast-changing world. Demographic profiles are being altered, with increasing proportions of people who are more susceptible to microorganisms in food. Changes in farm practices, more extensive food distribution systems and the increasing preference for meat and poultry in developing countries all have the potential to increase the incidence of food-borne illness. Extensive food distribution systems raise the potential for rapid, widespread distribution of contaminated food products. Changes in food production result in new types of food that may harbor less common pathogens. Intensive animal husbandry technologies, introduced to minimize production costs, have led to the emergence of new zoonotic diseases, which affect humans. Safe disposal of manure from large-scale animal and poultry production facilities is a growing food safety problem in much of the world, as manure frequently contains pathogens (WHO, 2014).

2.2. *Staphylococcus aureus* (*S. aureus*)

Staphylococcal food poisoning is a gastrointestinal illness. It is caused by eating foods contaminated with toxins produced by *S. aureus*. The most common way for food to be contaminated with *Staphylococcus* is through contact with food handlers who carry the bacteria or through contaminated milk and cheeses. *Staphylococcus* is salt tolerant and can grow in salty foods like ham. As the germ multiplies in food; it produces toxins that can cause illness. Staphylococcal toxins are resistant to heat and cannot be destroyed by cooking. Foods at highest risk of contamination with *S. aureus* and subsequent toxin production are those that are made by hand and require no cooking. Some examples of foods that have caused staphylococcal food poisoning are sliced meat, puddings, some pastries and sandwiches (CDC, 2004).

2.2.1 Staphylococcal food contamination

The frequently incriminated foods include meat and meat products; poultry and egg products; salads; cream-filled pastries; sandwich fillings; and milk and dairy products. However, the foods most often involved in outbreaks differ widely from one country to Another because of the variation in food consumption and habits. For example, in England and Wales, 60% of the staphylococcal food poisoning outbreak reports recorded Between 1992 and 2009 were due to poultry meat and red meat. On the contrary, in Japan, 36% of the staphylococcal food poisoning outbreaks reported between 1995 and 1999 were due to grains like rice balls and composite ready-to-eat food, 5.6% of the incidents were due to fish and shellfish, and less than 1% was due to milk products (IDSC, 2010).

2.2.2 Toxins of *S. aureus*

S. aureus produces a wide variety of toxins including staphylococcal enterotoxins (SEs; SEA to SEE, SEG to SEI, SER to SET) with demonstrated emetic activity, and staphylococcal-like (SEI) proteins, which are not emetic in a primate model (SE/L and SE/Q) or have yet to be tested (SE/J, SE/K, SE/M to SE/P, SE/U, SE/U2 and SE/V). SEs and SEIs have been traditionally subdivided into classical (SEA to SEE) and new (SEG to SE/U2) types. All possess superantigenic activity and are encoded by accessory genetic elements, including plasmids, prophages, pathogenicity islands, *vSa* genomic islands, or by genes located next to the staphylococcal cassette chromosome (SCC) implicated in methicillin resistance. SEs are a major cause of food poisoning which typically occurs after ingestion of different foods, particularly processed meat and dairy product, contaminated with *S. aureus* by improper handling and subsequent storage at elevated temperatures. Symptoms are of rapid onset and include nausea and violent vomiting, with or without diarrhea. The illness is usually self-limiting and only occasionally it is severe enough to warrant hospitalization. SEA is the most common cause of staphylococcal food poisoning worldwide, but the involvement of other classical SEs has been also demonstrated. Of the new SE/SEIs, only SHE have clearly been associated with food poisoning. However, genes encoding novel SEs as well as SEIs with untested emetic activity are widely represented in *S. aureus*, and their role in pathogenesis may be underestimated (Maria *et al.*, 2010).

2.2.3 Verulence factors

S. aureus can cause localized and invasive infections in humans. This is attributed to its ability to produce a variety of virulence factors such as capsular polysaccharides, staphylococcal enterotoxins (SEs), toxic shock syndrome toxin 1 (TSST-

1), panton – valentine leukocidin (PVL) and accessory gene regulators (agr). Although *S. aureus* isolates produce one of 11 serotypes of capsular polysaccharides, most clinical isolates belong to serotypes 5 and 8. Enterotoxin-producing *S. aureus* are common causes of food poisoning in many parts of the world. The ingestion of the preformed toxins in food often leads to the development of food poisoning. The symptoms typically have a rapid onset (2–6 h) and may include nausea, vomiting, diarrhea and abdominal pain. Nasal and hand carriage of enterotoxigenic *S. aureus* by food handlers is an important source of staphylococcal food contamination in restaurants and fast food outlets. Therefore it is important to detect *S. aureus* carriage among food handlers to prevent possible food contamination by them resulting in food poisoning (Edet *et al.*, 2008). 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 (Mainous *et al.*, 2006). Prevention of staphylococcal food poisoning from the infected food handlers may be difficult as carriers are asymptomatic (Schimed *et al.*, 2007). Other studies also reported high prevalence of enterotoxin-producing *S. aureus* in food handlers.

2.2.4 Hand carriage among food handlers

A cross-sectional study conducted among 127 food handlers working in cafeterias in Ethiopia indicated that 16.5% of fingernail contents of the food handlers were cultured positive for *S. aureus* (Andagric *et al.*, 2008). Another study done in Botswana reported that an even higher proportion (57.5% out of 200 food handlers) was tested positive for *S. aureus* (Loeto *et al.*, 2007).

A study conducted in Department for Thoracic and Cardiovascular Surgery at the University Hospital of Uppsala for detecting nasal and hand carriage of *S.*

aureus in staff, *S. aureus* was found on the hands of 16.7% of the men and 9.6% of the women, and in the noses of 33.3% of the men and 17.4% of the women. The risk ratio for *S. aureus* carriage on the hands with nasal carriage was 7.4 (95% confidence interval, 2.7 to 20.2; $P < .001$). Among the 14 healthcare workers carrying *S. aureus* on their hands, strain likeness to the nasal isolate was documented for 7 (50%) (Tamellin *et al.*, 2003).

In Kuwait city, 200 *S. aureus* isolates were recovered from food handlers working in 50 Kuwait City restaurants from 2003 to 2005. They consisted of 133 (102 isolates from nasal and 31 hand swabs) of 500 swabs from 250 adult male workers in 50 restaurants, during screening of food handlers as demanded by the City Council, yielding a hand carriage rate of 53.2% (Edet *et al.*, 2008).

A study in Portugal investigated the prevalence of hand and nose carriage of *S. aureus* among Portuguese higher health school students, results showed 41.7% *S. aureus* colonization among participants, and that the difference between nursing and pharmacy students was statistically significant (Marques *et al.*, 2010).

2.2.4 *S. aureus* food poisoning outbreaks

In May 1990, an outbreak of staphylococcal food poisoning occurred in elementary Schools in a Rhode Island community participating in such a program. In the investigation of the outbreak, students in schools that reported cases were interviewed.

Food preparation, handling, and distribution were reviewed. At School E, 662 lunches were prepared and distributed to 4 additional schools (schools A-D). Schools A and B accounted for nearly all cases of the food poisoning, with rates of 47 percent and 18 percent. Eating ham increased the risk of illness (62 percent of those consuming ham and 3 percent of those who did not, relative risk = 18.0, 95 percent confidence interval = 4.0, 313.4). Large amounts of *S. aureus* were cultured and performed enterotoxin

A was identified in leftover ham. A food handler, who tested positive for the implicated enterotoxigenic strain *S. aureus*, reported having removed the casings from two of nine warm ham rolls 48 hours prior to service. Because of improper refrigeration, prolonged handling, and inadequate reheating, the ham was held at temperatures estimated at 10-49 degrees Celsius (50-120 degrees Fahrenheit) for a minimum of 15 hours. The potential for larger outbreaks prompted a statewide training program in safe food preparation for school lunch personnel, which may have applications for other communities (Richards *et al.*, 1997).

From February through April 1989, four outbreaks of staphylococcal food poisoning in the United States were associated with eating mushrooms canned in the People's Republic of China (PRC). In the four outbreaks, 99 persons who ate at a suspect facility developed gastrointestinal symptoms within 24 h, including 18 who were hospitalized. Illness was associated with eating mushrooms at a university cafeteria (relative risk [RR] = 53.0), a hospital cafeteria (RR = 13.8), a pizzeria (odds ratio [OR] = 0.0), (all $P < 0.0001$).

Staphylococcal enterotoxin A was found by ELISA in mushrooms the sites of two outbreaks and in unopened cans from the three plants thought to have produced mushrooms implicated in outbreaks. These investigations led to multistate recalls and a US Food and Drug Administration order to restrict entry into the United States of all mushrooms produced in the PRC; until this action, the United States imported 50 million pounds yearly (William *et al.*, 1996).

On July 30, 2012, the emergency department at a military hospital was visited by 13 persons seeking care for gastrointestinal illness with onset 2–3 hours after a work lunch party. The hospital responded by opening up temporary evaluation and treatment capacity in primary-care clinics and a progressive-care unit and by diverting one patient to a local

civilian hospital. An immediate outbreak investigation was conducted by local military public health personnel with assistance from CDC. Initial epidemiologic analysis implicated "perlo" (a chicken, sausage, and rice dish) and bacterial intoxication as the outbreak mechanism. This enabled public health personnel to 1) recommend no further consumption of perlo and 2) reassure appropriate authorities that no additional ill persons likely would be seeking care and advise that nothing more than supportive care of ill persons likely would be required. After interviewing party attendees, investigators found nine additional persons who met their case definition. Subsequent CDC laboratory analysis of a sample of perlo detected staphylococcal enterotoxin A, supporting the epidemiologic findings. Improper food handling and preparation measures were identified and addressed by the appropriate authorities, who provided additional detailed education on food preparation safety for the persons who prepared the meal (CDC, 2013).

At the end of 2009, six food poisoning outbreaks caused by staphylococci were reported in France. Soft cheese made from unpasteurized milk was found to be the common source of the outbreaks. Staphylococcal enterotoxin type E was identified and quantified in the cheese using both official and confirmatory methods of the European Union Reference Laboratory (EU-RL). To our knowledge, this is the first report of food poisoning outbreaks caused by staphylococcal enterotoxin type E in France (Ostyn *et al.*, 2010). An outbreak of staphylococcal food poisoning in Europe caused by contaminated lasagne was detected and monitored by both national and international surveillance system. The common source was a pasta-producing factory in Italy and high levels of *S. aureus* were detected in packets of dried lasagne distributed in Luxembourg, the UK, France and Italy. Forty-seven cases were reported in the UK.

Outbreaks of staphylococcal food poisoning attributed to mishandling during the food processing stage are uncommon and pasta as the food vehicle is rare. Prompt recognition of the outbreak and rapid identification of the food vehicle enabled most of the consignment to be withdrawn from the market (Woolaway *et al.*, 1986).

An outbreak of staphylococcal food poisoning due to an egg yolk (EY) reaction-negative strain occurred in Japan. Twenty-one of 53 dam construction workers who ate boxed lunches prepared at their company cafeteria became ill, and eight required hospital treatment. The outbreak showed a typical incubation time (1.5-4 h with a median time of 2.7 h) and symptoms (vomiting and diarrhea) of staphylococcal food poisoning.

S. aureus, which produces staphylococcal enterotoxin (SE) A, was isolated from four fecal specimens of eight patients tested. Scrambled egg in the boxed lunches contained 20-40 ng/g of SEA, and 3.0×10^9 /g of viable *S. aureus* cells that produced this toxin. All isolates from patients and the food were EY reaction-negative, coagulase type II, and showed the same restriction fragment length polymorphism (RFLP) pattern. We concluded that the outbreak was caused by scrambled egg contaminated with EY reaction-negative *S. aureus*. In Japan, outbreaks of staphylococcal food poisoning are mainly caused by EY reaction-positive *S. aureus*, and EY reaction-negative colonies grown on agar plates containing EY are usually not analyzed further for detection of *S. aureus*. The present outbreak suggested that EY reaction-negative isolates should be subjected to further analysis to detect the causative agents of staphylococcal food poisoning (Miwa *et al.*, 2001).

A mass outbreak of food poisoning caused by the consumption of reconstituted milk occurred in Osaka, Japan, in June 2000, and more than 10,000 cases were reported. A

small amount of SEA and sea gene were detected in the reconstituted milk and the skim milk powder, which was the raw material for the reconstituted milk. In an outbreak of food poisoning in United States caused by SEA present in chocolate milk, 200 ng or less SEA was presumed to be the cause. Although it was considered that the outbreak of food poisoning in Osaka could have been caused by SEA, the quantity of SEA detected, i.e., approximately 80 ng, was insufficient to cause food poisoning on such a large scale. Hence, we investigated the possibility of staphylococcal enterotoxins other than SEA as the cause of the outbreak (Tetsuya *et al.*, 2004).

In Japan, large scale outbreaks of staphylococcal food poisoning had been reported in the past. In 2000, an extensive staphylococcal outbreak occurred in Kansai district affecting as many as 13,420 people (Asao *et al.*, 2003). Investigation reviewed that the incriminated food was the dairy products produced by a factory in Hokkaido which experienced a transient shortage of power supply during the manufacturing process. According to the statistics published by the Ministry of Health, Labour and Welfare, 536 bacterial food poisoning outbreaks were recorded in 2009 and 7.6% of the incidents were caused by *S. aureus*, affecting 690 persons (IDSC, 2010).

Apart from the outbreak in Japan, large scale outbreaks have been reported in other Countries in past decades. In Brazil, a massive staphylococcal food poisoning incident affecting about 4,000 patients was reported in 2004 (Fridkin *et al.*, 2010). The food prepared for the gathering was found to be contaminated by food handlers who were cultured positive for enterotoxigenic *S. aureus* from their nasopharynx and fingernail swabs. In another outbreak of gastroenteritis reported in the United States in 1988, more than 850 students were affected in a school district. Investigation reviewed that the source of the outbreak was chocolate milk containing the SE (Eisenstein, 2008).

In a study done at Kavkas University, 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. Similarly, 13 coagulase positive Staphylococci and 96 coagulase negative staphylococci isolates were cultured from the nasal cavity of the workers. Only one isolate of all the hand isolates was resistant to vancomycin. Nine of all the coagulase negative staphylococci isolate including 4 hand and 5 nasal cavity samples were resistant to Methicillin. Four of 20 coagulase positive staphylococci isolate produced staphylococcal enterotoxins (SE). Only one hand isolate of all the coagulase negative Staphylococci isolates produced staphylococcal enterotoxins E. These results indicate, like before, that the food handlers would have been the main source of the staphylococcal contamination of food. It is important to note that coagulase negative staphylococci can produce staphylococcal enterotoxins and they can also cause to food poisoning (Haluk *et al.*, 2010).

3. MATERIALS AND METHODS

3.1. Type of study

This is cross-sectional study.

3.2. Study area

This study was conducted in Sudan University of Science and Technology (SUST) cafeterias, Khartoum province, Sudan.

3.3. Study duration

The study was carried out during the period June 2013 - Sep. 2013.

3.4. Study population

Food handlers, both males and females with different ages, were enrolled in this study.

3.5. Sampling

Hand swabs were collected from food handlers (from rights and lefts hands of each) working in cafeterias.

3.6. Sampling technique

This study is based on non-probability convenience sampling technique.

3.7. Method of data collection

Data was collected through an interview questionnaire (Appendix I).

3.8. Ethical consideration

Approved to conduct this study was obtained from Research Ethics Committee of the Sudan University of Science and Technology (SUST). After explaining the study and its goal, a verbal consent was taken from the study recruits before proceeding with the study and collecting hand swabs.

3.9. Collection of hand swabs

Hand swabs collected under aseptic condition using sterile swabs from right and left hands from each food handlers (between fingers and under nails), and within less than half an hour cultured on suitable culture media and incubated at 37⁰C.

3.10. Laboratory methods

All swabs collected from food handlers were tested by ordinary culturing techniques, gram staining procedure and biochemical tests to detect *Staphylococcus aureus* bacteria.

3.10.1. Culturing of the swabs

After collection, all swabs were cultured on blood agar, MacConky agar and Mannitol salt agar transferred to incubator at 37⁰ C for 24 hours.

Non-growing Medias excluded and the growing was kept for subsequent identification.

3.10.2. Gram stain

Gram Stain procedure conducted to differentiate growing colonies and gram positive cocci only kept for biochemical testing.

3.10.2.1. Gram stain procedure

The slide flooded with crystal violet solution for one minute and washed off briefly with tap water. Then slide flooded with Gram's Iodine solution, and allowed to act (as a mordant) for about one minute, washed off with tap water. Excess water removed from slide and blotted, so that alcohol used for decolorization is not diluted. The slide flooded with 95% alcohol for 10 seconds and washed off with tap water. (Smears that are excessively thick may require longer decolorization. This is the most sensitive and variable step of the procedure, and requires experience to know just how much to decolorize). The slide drained then flooded with safranin solution and allowed to

counterstain for 30 seconds. Washed off with tap water. Drained and blotted dry with bibulous paper.

3.10.3. Biochemical tests

3.10.3.1. Catalase test

By adding the colonies under test to hydrogen peroxide, staphylococcal colonies (which produce an enzyme called peroxidase) have ability to develop air bubbles when the other bacteria haven't.

3.10.3.2. Mannitol fermentation test

Staphylococcus aureus when cultured on Mannitol Salt Agar grows as yellow colonies due to fermentation of the mannitol and transferring the red colour of the medium to yellow colour due to change in medium PH which detected by indicator phenol red.

3.10.3.3. Coagulase test

By adding the colonies under test to freshly obtained human plasma, *Staphylococcus aureus* colonies (which produces an enzyme coagulase) has the ability to develop a clotting features in few seconds.

3.10.3.4. Deoxyribonuclease test

Staphylococcus aureus has ability to use DNA as a source of carbon and energy for growth by producing enzyme Deoxyribonuclease. An inoculum from a pure culture is streaked on a sterile plate of DNase agar. The inoculated plate is incubated at 25⁰C for 24 hours. Presence of clear halos surrounding colonies is positive for their ability to digest the DNA and thus indicates presence of DNase.

3.11. Quality control

Reagents, powders were checked for storage, stability and preparation before starting work.

3.12. Data analysis

Data was computed and analyzed by SPSS version 11.00 software programs for interpretation of the results; significance of differences was determined using chi-square test was employed to assess the association between variables. Statistical significance was set at $P > 0.05$. Frequencies, proportion were drawn for numerical data and recurrent response was done for quantitative data.

4. RESULTS

Total of (110) samples of hand (right and left) swabs were collected from (55) food handlers working in cafeterias of Sudan University of Science and Technology (**Table: 1**). Among these food handlers the frequency of males were 51(93%) while 4(7%) were females (**Figure: 1**). All food handlers were classified into 4 groups, age group one 18(33%), age group two 22(40%), age group three 11(20%) and age group four 4 (7%) (**Table: 2**).The data in this study confirmed clearly the existence of *S. aureus* in right hand 17/55(30.9%) (**Figure: 2**) and in left hand 10/55(18.1%) (**Figure: 3**). The prevalence rate of hand carriage was 27(55) 49%. There is no significant value between sociodemographic characteristics like education, sex and age in relation to *S. aureus* detected in right hand and left hand of food handlers (**Table: 3**).

Table 1: Frequency of each sample enrolled in this study:

Type of sample	Frequency	Percent %
Right hand swab	55	50%
Left hand swab	55	50%
Total	110	100%

Table 2: Distribution of age groups among the study population:

Age group(years)	frequency	Percent %
Lowest through 20	18	32.7%
21-31	22	40.0%
32-41	11	20.0%
42 Trough highest	4	7.30%
Total	55	100%

Table 3: Sociodemographic characteristics in relation to *S. aureus* detected in right hand and left hand of food handler:

X2 = chi-square

Characteristic	% of <i>S.aureus</i> in right hand		Associatio nX2 and p value	% of <i>S.aureus</i> in left hand		Associatio nX2 and p value
	positive	negative		positive	Negative	
Education						
Illiterate	2(28.6%)	5(71.4%)	X2 =0.324 p value=0.995	2(28.6%)	2(28.6%)	X2 =1.676 p value=0.642
Primary school	8(34.8%)	15(65.2%)		5(21.7%)	18(78.3)	
Secondary school	6(27.3%)	16(72.7%)		3(13.6%)	19(86.4)	
Certificate	1(33.3%)	2(66.7%)		0(0%)	3(100%)	
Sex						
male	16(31.4%)	35(68.6%)	X2 =.071 p value=0.791	9(17.6%)	42(82.4%)	X2 =.135 p value=0.714
Females	1(25%)	3(75%)		1(25%)	3(75%)	
Age in years						
≤20	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%)		2(9%)	20(91%)	
32-41	4(36.4%)	7(63.6%)		1(9%)	10(91%)	
≥42	0(0%)	4(100%)		2(50%)	2(50%)	

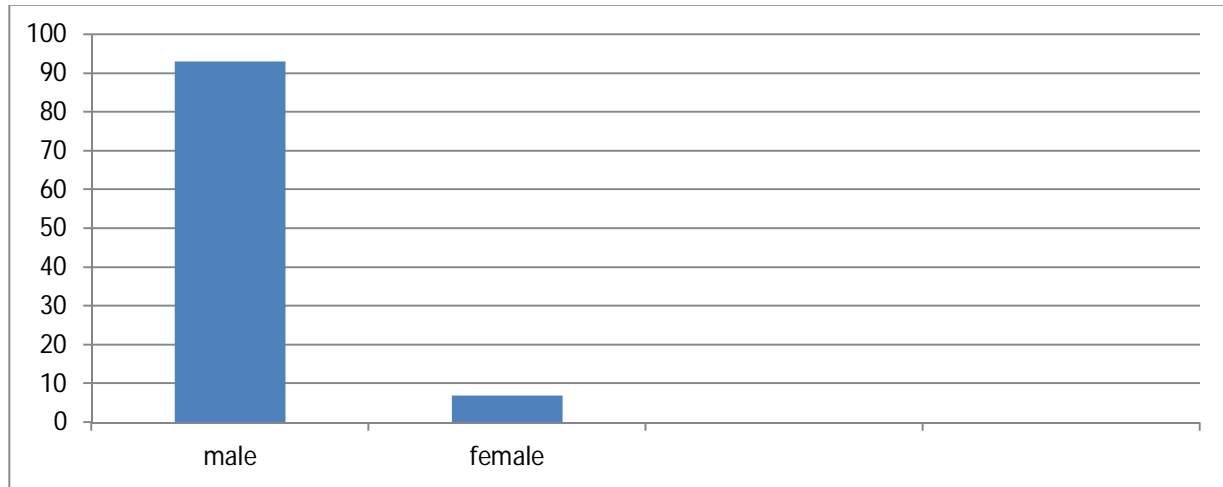


Figure 1: Percentage of males and females among the study population

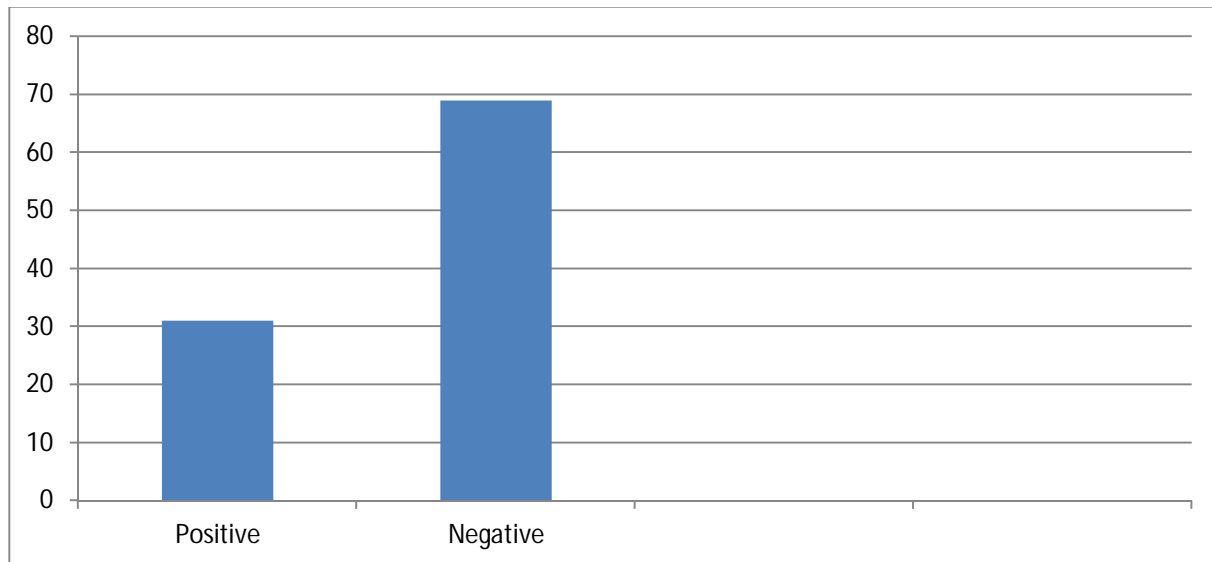


Figure 2: Percentage of Right hand carriers of *S.aureus* among food handlers.

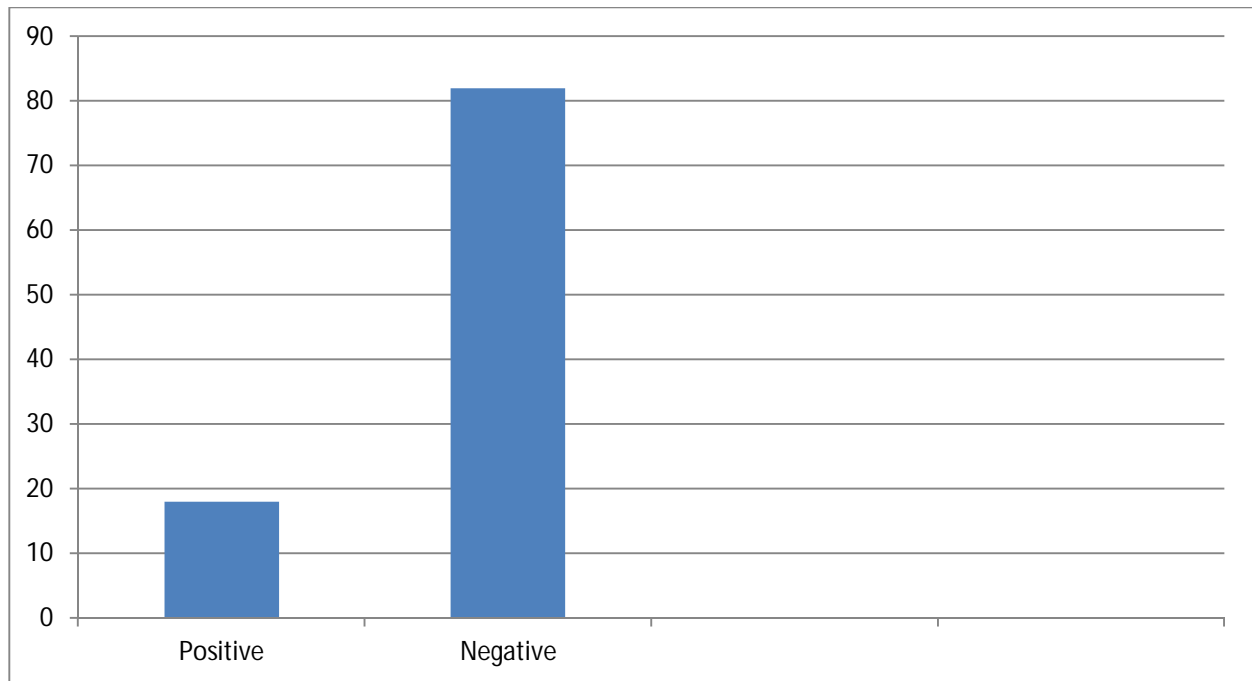


Figure 3: Percentage of Left hand carriers of *S.aureus* among food handlers.

5. DISCUSSION

S. aureus hand carriage among food handlers is an important cause of food poisoning that caused by *S. aureus* and is responsible of many food-borne outbreaks. *S. aureus* carrier food handlers, especially those with poor hygienic practices, can contaminate the food which they handle. Consumption of such food without further processing has been known to result in staphylococcal food poisoning.

This study investigated *S. aureus* hand carriage among food handlers in Khartoum Province. Fifty-five food handlers were selected randomly for the present study. 51(93%) of them were males, and 4(7%) were females. The study detected the existence of *S. aureus* on right hand 17/55(30.9%) and in left hand 10/55(18.1%) i.e the prevalence rate of hand carriage is 27/55(49%). There is no significant value between sociodemographic characteristics like education, sex and age in relation to *S. aureus* detected in right hand and left hand of food handlers. The difference in prevalence between right and left hands is due to the behavior of population because most of them are right-handed.

The result is close to those obtained by Loeto *et al.*, (2007) in Botswana and Edit *et al.*, (2008) in Kuwait whom reported prevalence of hand carriage among food handlers as 57% and 53% respectively.

Other results obtained by Andagric *et al.*, (2008) in Ethiopia and Tamellin *et al.*, (2003) in Sweden which reported the prevalence of hand carriage as 16.5% and 16.7%, respectively. Obviously, all the above mentioned studies are not fully comparable, since the use of HCWs (Health Care Workers) from specific specialties, and the differences in the study design such as sample size and method of *S. aureus* identification might account

for the disparity in the carriage rate. In additions, carrier rates might be influenced to poor personal hygiene of study participants, poor sanitation of the areas and difference in sampling techniques.

A lot of outbreaks reported every year due to staphylococcal food poisoning and the main source of this organism is hand and nasal carriers among food handlers.

5.2. CONCLUSION

The findings for carrier individuals indicate that the *S. aureus* may be hidden between fingers and under nails area of food handlers, and may be act as a dormant source of food poisoning outbreak, when working in cafeteria with no good hygiene and no proper hand washing who prepared many cook and uncooked foods served. The effectiveness of the recommended criteria for screening hands when *S. aureus* should be provided to exposed members of the public needs to be evaluated.

Finally, addressing the public health problems associated with the high prevalence of hand carriage in developing countries will require implementing stronger measures to prevent bared contaminated hands contact with food and water.

5.3. RECOMENDATIONS

1. *S. aureus* hand carriage among food handlers should be confirmed by PCR, sequencing and ELIZA done to characterize enterotoxigenic strains in Sudan.
2. larger sample size is needed to accurately determine the prevalence rate of *S. aureus* hand carriage among all Sudanese people and especially in food handlers.
3. Food handlers health education and training, and practices such as hand washing and glove use are essential steps towards ensuring food safety.
4. Periodic medical examination along for food handler necessary should be done.

5. Food handlers who detected positive for *S. aureus* hand carriage should be excluded from food handling duties till cure.

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APPENDICES

1. QUESTIONNAIR

Name: _____ No: _____

Age: _____ Gender: ☐ Male ☐ Female

Residence _____

Type of Food handling: _____ Period of work _____

Certified in food preparation and handling Yes () No ()

Medical check up Yes () No ()

Hand washing after using toilet by water Yes () No ()

Hand washing after using toilet with soap and water Yes () No ()

*Case Details

Symptoms:	Onset Date	Resolution Date
<input type="checkbox"/> Jaundice	_____	_____
<input type="checkbox"/> Fever	_____	_____
<input type="checkbox"/> Abdominal Pain	_____	_____
<input type="checkbox"/> Vomiting	_____	_____
<input type="checkbox"/> Nausea	_____	_____
<input type="checkbox"/> Diarrhea	_____	_____ Other: _____

COLUMBIA BLOOD AGAR BASE (7125)

Intended Use

Columbia Blood Agar Base is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Columbia blood agar base media are typically supplemented with 5-10% sheep, rabbit, or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, Columbia Blood Agar Base is used as a general purpose media. Columbia Blood Agar Base was developed after the Columbia Agar formulation described by Ellner et al. from Columbia University.

1-Columbia (Blood Agar Base) BAB is specified in the Compendium of Methods for the microbiological Examination of Foods.

2-Principles of the Procedure

The nitrogen, vitamin, and carbon, sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, and Yeast Enriched Peptone. Corn Starch increases growth of *Neisseria* spp., and enhances the hemolytic reactions of some streptococci. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of α -hemolytic streptococci.

3-Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms, and aids in determining hemolytic reactions.

Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.

4-Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	8 g
Yeast Enriched Peptone	10 g
Corn Starch	1 g
Sodium Chloride.....	5 g
Agar.....	14 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to skin, eyes, and mucous membranes

MacCONKEY AGAR (7102)

Intended Use

MacConkey Agar is used for the isolation and differentiation of Gram-negative enteric bacilli. Conforms to Harmonized USP/EP/JP Requirements.^{1,2,3}

Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.

⁴ The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5%

sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and coliforms. MacConkey Agar is recommended for the detection and isolation of Gram-negative organisms from clinical samples.

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar. Lactose is the fermentable carbohydrate. During Lactose fermentation a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation. Bile Salts Mixture and Crystal Violet are the selective agents, inhibiting Gram-positive cocci and allowing Gram-negative organisms to grow. Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin.....	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Lactose	10 g
Bile Salts Mixture	1.5 g
Sodium Chloride	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.001 g
Agar	13.5 g
Final pH: 7.1 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

MANNITOL SALT AGAR (7143)

Intended Use

Mannitol Salt Agar is used for the isolation of staphylococci. Conforms to Harmonized USP/EP/JP Requirements.1,2,3

Product Summary and Explanation

Chapman formulated Mannitol Salt Agar to isolate staphylococci by inhibiting growth of most other bacteria with a high salt concentration.

4-Chapman added 7.5% Sodium Chloride to Phenol Red Mannitol Agar, and noted Pathogenic strains of staphylococci (coagulase-positive staphylococci) grew luxuriantly and produced yellow colonies with yellow zones. Nonpathogenic staphylococci produced small red colonies with no color change to the surrounding medium. Mannitol Salt Agar is highly selective, and specimens from heavily contaminated sources may be streaked onto this medium without danger of overgrowth.

5-Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from clinical specimens, cosmetics, and microbial limit tests.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Agar. D-Mannitol is the carbohydrate source. In high concentrations, Sodium Chloride inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator. Agar is the solidifying agent. Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products, turning the Phenol Red pH indicator from red to yellow. Typical pathogenic staphylococci ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic staphylococci do not ferment mannitol and form red colonies.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract.....	1 g
D-Mannitol	10 g
Sodium Chloride.....	75 g
Phenol Red.....	0.025 g
Agar.....	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.