Bacterial contamination of restaurant food in Khartoum state

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DEDICATION

To my Parents

With thankful

To my Brother

With love
PREFACE

This work has been carried out the Department of microbiology, faculty of Veterinary Medicine University of Khartoum, under the supervision of Dr. Suliman Mohammed El Hassan
ABSTRACT

This study was carried out to isolate the bacterial contamination of restaurant food in Khartoum state, during August to December 2005.

Sixty swabs were collected from restaurant food, worker’s hands and dishes as follows:

Twenty swabs from restaurant food, twenty from worker’s hands and twenty from dishes which used to:

1- Isolate the bacteria may contaminated restaurant food.
2- Determine the type of bacteria may contaminate restaurant food.
3- Determine the important source of restaurant food included worker’s hands and utensils.
4- Determine the pathogenic bacteria for human and recommended the best methods to guarantee the hygienic safety of consumer.

The samples cultured onto Blood agar, MacConkey agar and Nutrient agar.

A total number of 155 bacterial isolates were obtained and characterized according to their morphology, Gram stain reaction and biochemical proprieties. The isolated included Gram-positive bacteria were *Bacillus mycoides*, *Bacillus cereus*, *Bacillus thringiensis*, *Bacillus sphaericus*, *Bacillus pantothenticus*, *Bacillus subtilis* and *Staphylococcus capre*.

And Gram-negative bacteria were, *Acinetobacter coloaceticus*, *Moraxella spp*, *Pseudomonas fluorescens*, *Aeromonas salmonicida*, *Flavobacterium thalpophilum*, *Klebsiella pneumonia subsp. ozaenae*, *kluyvera spp*, *Proteusmirabilis*, *Enterbacter cloaceae*, *E. coli*, *Hafnia alvei* and *Enterobacter sakazakii*.

The high contamination rate by Gram-negative and spore forming bacilli (*Bacillus mycoides*, *Bacillus cereus*, *Bacillus thringiensis*, *Bacillus*
spharicus, Bacillus pantothenicus, Bacillus subtilis) which considered a potential food poisoning bacteria.

This study detected a potential source of contaminated restaurant food was worker’s hands. The isolated of Bacillus species and Coliform species indicated the poor personal hygienic.

Worker’s hands and utensils showed contamination by organisms usually found in food such as: Bacillus mycoides, Bacillus cereus, Bacillus thringiensis and Proteus mirabilis, Flavobacterium thalophilum, Klebsiella pneumonia subsp. ozaenae and. This indicates re-contamination by worker’s who come in contact with food through all stages of processing.

Other source of contamination were utensils which my contaminated by water and soil.

From this point we concluded food hygienic depend on the hygienic and awareness food worker’s. There for we advise to raise hygienic awareness food worker’s.
 الملخص الإطروحة

أجريت هذه الدراسة لعزل البكتيريا الملوثة للغذاء في المطاعم بولاية الخرطوم في الفترة

ما بين أغسطس إلى ديسمبر 2005.

حيث جمعت ستون نموذج تحليلي مكروه من الغذاء وأيادي العمال والصحون وذلك بهدف: -

1- عزل البكتيريا الملوثة للغذاء.
2- تكثيف نوع البكتيريا الملوثة للغذاء.
3. تحديد أهم مصادر تلوث الغذاء بالمطاعم متمثلة في أيادي العمال والصحون.
4. تحديد أنواع المرضى للإنسان و الوصول لأفضل الطرق لضمان صحة المستهلك.

وزرعت العينات في أوساط أجار الدم، أجار ماكرونكي و الأجار المغذي. بلغت موجة البكتيريا
المعزولة 155 عزلة وصنفت بناءً على الشكل، تفاعلها مع صبغة جرام و الاختبارات الكيميائية
الحيوية.

اشتملت البكتيريا المزعولة على الأنواع الموجبة الجرام مثل

*Bacillus mycoides, Bacillus cerus, Bacillus thringiensis, Bacillus sphericus, Bacillus pantothenticus, Bacillus subtili and Staphylo coccus capre*

و الأنواع السالبة الجرام مثل

*Acinetobacter coloaceticus, Moraxella spp, Pseudomonas fluorescens, Aeromonas salmonicida, Flavobacterium thalophilum, Klebsiella pneumoniae ozaenae, klyvera, Proteus mirabilis, Enterbacter cloaceae, Hafnia alvei, E. coli and Enterobacter sakazakii*

أعلى معدلات تلوث بكتيري كانت بالبكتيريا السالبة الجرام والعصويات المكونة للبواغ والتي
تعتبر من أهم الأنواع المسببة للتسمم الغذائي.

كشفت الدراسة عن أخطر مصدر تلوث غذاء المطاعم هو أيادي العمال حيث عزلت
البكتيريا العصوية والتولوزية بأنواعها مما يدل على عدم ممارسة الإجراءات الصحية من قبل
العمال، كما وتعتبر الأدوات مصدر ثاني للتلوث.

أيادي العمال والصحون كانت ملوثة ببعض أنواع البكتيريا التي عزلت أيضا من الطعام مثل:
Bacillus mycoides, Bacillus cerus, Bacillus thringiensis, Proteus mirabilis, Flavobacterium thalpophilum, Klebsiella pneumoniae subsp. ozaenae and Actinobacillus suis.

ما يدل على إعادة التلوث أثناء تجهيز وتقديم الطعام وثبوت الأدوات في المطاعم قد ينتج من التلوث بالماء والاتربة.

ومع هذا ننصح أن صحة الغذاء تنتج من صحة ووعي الكادر البشري الذي يقوم على تجهيز الطعام، لذا نوصي برفع الوعي الصحي للعاملين بالمطاعم.
Introduction

Harmful bacteria are the most common causes of food borne illnesses. Some bacteria may be present on foods when purchased. Raw foods are not sterile, raw meat and poultry may become contaminated during slaughter. Seafood may become contaminated during catch or through processing. Contamination can occur during growing, harvesting, processing, storing, shipping, or final preparation in the restaurant or in the person's kitchen. Food handlers who do not wash their hands after using the bathroom or have infections themselves often cause contamination. Indirect contamination using an intermediate vehicle is the most common, e.g. the movement of bacteria from the intestine of a food handler to food via their hands, after using the toilet (Jay, 2000).

Improper cleaning of storage and preparation areas and unclean utensils also cause contamination of raw and cooked foods (Davis and Samuel, 1997).

Sources of contamination are varied, contamination may also occur during food preparation when food is cooked and left out for more than 2 hours at room temperature, bacteria can multiply quickly. Most bacteria grow undetected because they do not produce an "off" odor or change the color or texture of the food. Freezing food slows or stops bacteria's growth but does not destroy the bacteria. The microbes can become reactivated when the food is thawed (Chen et al., 2001).

More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and Entero-pathogenic *Escherichia coli*. These bacteria
are commonly found on many raw foods. Normally a large number of food-poisoning bacteria must be present to cause illness (Jack and Marianne, 1999).

This study was undertaken to detect the level of contamination of food in restaurants of Khartoum state and to know the source of contamination to prevent consumers infection.

Objectives
This study aim to
5- Isolate the bacteria that may contamination restaurant food.
6- To identify the bacteria isolate from restaurant.
7- Determine the source of restaurant food contamination.
1.1 **Food contamination**

The origins of microbial contamination in food service include environment, the food worker, the source of food, food itself (Beuchat, 1995; Jack and Marianne, 1999) kitchens surfaces and faucet spigot may be significant source of cross contamination (Chen *et al.*, 2001)

Contamination may occur during purchase, storage, preparation, serving and handling leftovers (Torok *et al.*, 1997; Buchanan *et al.*, 1997; Kednall, 2003; Miller *et al.*, 1996; Cliver and Kaspar, 1994).

There are two types of contamination. Bacterial contamination which usually happens inside the food premises because of ignorance, inadequate space, poor design or due to food handlers taking short cuts. Contamination of this kind is the most serious and can result in food spoilage, food poisoning and food intoxication (Daivs and Samuel, 1997). The other type of contamination is the physical contamination by foreign objects which may be dangerous e.g. glass, stones, nails or others like hair, insects or vermin and all of which are unpleasant and offensive (Daivs and Samuel, 1997; Miller *et al.*, 1996).

1.2 **Food spoilage**

Food spoilage may be defined as any change that renders food unfit for human consumption. These changes may be caused by various factors, including contamination by microorganisms, infestation by
insects, or degradation by endogenous enzymes present naturally in the food (MacRae et al., 1993).

Bacteria and fungi (yeasts and molds) are the principal types of microorganisms that cause food spoilage and food-borne illnesses. Foods may be contaminated by microorganisms at any time during harvest, storage, processing, distribution, handling, or preparation (Jay, 2000). The primary sources of microbial contamination are soil, air, animal feed, animal hides and intestines, plant surfaces, sewage, and food processing machinery or utensils (Jay, 2000; Hui, 1992).

Food spoilage bacteria grow best at environmental temperature of 70-100 F and when generation time for bacterium was shorter, the faster food spoilage would occur. Spoilage bacteria in log phase of growth produce the signs of spoilage (Veiga et al., 2003).

In addition, physical and chemical changes, such as the tearing of plant or animal tissues or the oxidation of certain constituents of food, may promote food spoilage. Foods obtained from plant or animal sources begin to spoil soon after harvest or slaughter. The enzymes contained in the cells of plant and animal tissues may be released as a result of any mechanical damage inflicted during post harvest handling. These enzymes begin to break down the cellular material (Braun and Fehlhaber, 2001).
The living tissues of plants and animals maintain a balance of enzymatic activity. This balance is disrupted upon harvest or slaughter. In some cases, enzymes that play a useful role in living tissues may catalyze spoilage reactions following harvest or slaughter. For example, the enzyme pepsin is found in the stomach of all animals and is involved in the breakdown of proteins during the normal digestion process. However, soon after the slaughter of an animal, pepsin begins to break down the proteins of the organs, weakening the tissues and making them more susceptible to microbial contamination. After the harvesting of fruits, certain enzymes remain active within the cells of the plant tissues. These enzymes continue to catalyze the biochemical processes of ripening and may eventually lead to rotting, as can be observed in bananas. In addition, oxidative enzymes in fruits continue to carry out cellular respiration (the process of using oxygen to metabolize glucose for energy). This continued respiration decreases the shelf life of fresh fruits and may lead to spoilage. Respiration may be controlled by refrigerated storage or modified-atmosphere packaging (Braun and Fehlhaberk, 2001).

The chemical reactions catalyzed by the enzymes result in the degradation of food quality, such as the development of off-flavors, the deterioration of texture, and the loss of nutrients. (Robin, 1989)

The signs of food spoilage are many (Steel, 1987) and include
1.2.1 Odor

Off odors are smells (like rotten egg) that are produced when bacteria break down food. Taints due to flavor change may also occur.

1.2.2 Sliminess

As the bacteria population grow, the food can became slimy. Mold may also form slimy whiskers.

1.2.3 Discoloration

Some mould have colored spores that give the food distinctive color for example black paint mould on bread, or blue and green mould in citrus fruits and cheese.

1.2.4 Souring

Food go sour when certain bacteria produce acid for example when milk sour from the production of lactic acid caused by growth of bacteria such as Lactobacillus acidophilus

1.2.5 Gas

Bacteria and yeasts often produce gas that can affect food. It is noticed that meat may become spongy or packages and cans swell or having a popping or fizzing sound on opening this may be due to accentuation of gases.

1.3 Bacteria associated with food spoilage:-
1.3.1 Pseudomonas:

*Pseudomonas species* are widely distributed in nature, in soil and water, and because of its wide occurrence it is a frequently contaminated food (Chessbrugh, 2000). *Pseudomonas fluorescens* is common in soil and water. It is associated with the spoilage of egg, cured meats, fish and milk. *P. fluorescens* often produces a diffusible fluorescent pigment that can be noted on decaying meats and fish.

1.3.2 Acinetobacter

These are Gram negative rods they are strict aerobes that do not reduce nitrates. Although rod – shaped cell are formed in young cultures, old culture contain many cocci – shaped cells. They are widely distributed in soils and water and may be found in many food, especially refrigerated fresh products (Jay, 2000)

1.3.3 Proteus

Dolman, (1967) reported that member of this genus are common inhabitants of the intestinal tract of man, animals, and decaying materials. They may be isolated from spoiled meat, and also prevalent in fresh meats which are subjected to too much handling.

1.3.4 Aeromonas
Aeromonas spp can be found in water and soil (Karper et al., 1981). Most are non pathogenic or of low pathogenicity (Chessburgh, 2000). Aeromonas spp have been recovered from the fecal materials of different animals (Gray, 1984; Stern et al., 1987) and found to be common contaminated in foods of animal origin (Myers et al., 1982; Egan, 1984; Gray, 1984).

1.3.5 Hafnia

These enteric rods are important in the spoilage of refrigerated meat and vegetable products; Hafnia alvei is important species in the food spoilage (Jay, 2000).

1.3.6 Flavobacterium

Some are mesotrophs, while others are psychrotrophs, where they participate in the spoilage of refrigerated meats (Jay, 2000). Hansen et al. (1998) isolated Flavobacterium species from hand wound.

1.3.7 Bacillus

Bacillus cereus sometimes causes spoilage in canned foods (Hsieh et al., 1999).

1.4 Food intoxication

Food-poisoning microorganisms can cause health problems by either intoxication or infection. Intoxication occurs when food-poisoning
microorganisms produce a toxin that triggers sickness when ingested. Several different kinds of toxins are produced by the various microorganisms. These toxins usually affect the cells lining the intestinal wall, causing vomiting and diarrhea. Microorganisms capable of causing food-poisoning intoxication include *Clostridium perfringens*, *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus cereus* (Kendall, 2003).

### 1.4.1 Bacteria associated with food intoxication

#### 1.4.1.1 Staphylococcal food intoxication

Staphylococcus is a genus of spherical bacteria of the family Micrococccaceae, the best known species of which are universally present in great numbers on the mucous membranes and skin of human and other warm-blood animals (Kendall, 2003).

The most significant to humans are two variants of the species *S. aureus* and *S. epidermis*. *S. aureus* is an important agent of wound infections, boils, and other human skin infections and one of the most common causes of food intoxication. *S. aureus* also causes udder inflammation, or mastitis in domestic animals and breast infection in women. It is a difficult hospital pathogen because of it's resistance to antibiotics. *S.epidermis* is milder pathogen that is opportunistic in persons with lowered resistance (Evans and Niven, 1950).
Staphylococcal gastroenteritis is caused by ingestion of food that contains one or more enterotoxins, which are produced only by some Staphylococcal species and strains. Although enterotoxin production is believed generally to be associated with \textit{S. aureus} strains that produce coagulase and thermonuclease (TNase), many species of Staphylococcus that produce neither coagulase nor TNase are known to produce enterotoxins (Jay, 2000).

The main reservoir of \textit{S. aureus} is the nasal cavity. From this source, the organisms find their way to the skin and into wound either directly or in directly (Jay, 2000). The most common skin sources are the arms, hand and face (Bergdoll, 1990) and may be found in eyes. The two most important sources to food are nasal carriers and individuals whose hands and arms are infected with boils and carbuncles, who are permitted to handle foods (Jay, 2000).

Mode of transmission is by ingestion of food product containing Staphylococcal enterotoxin. Foods involved are particularly those that come in contact with food handlers hands either without subsequent cooking or with inadequate heating or refrigeration. When these food remain at room temperature for several hours before being eaten, toxin producing Staphylococci multiply and elaborate the heatstable toxin (Evans and Niven, 1950).
Intoxication is abrupt and sometimes violent onset, with severe nausea, cramps, vomiting and prostration often accompanied by diarrhea, and sometimes with subnormal temperature and lowered blood pressure. Deaths are rare, duration of illness is commonly not more than a day or two, but the intensity of symptoms may result in surgical exploration in sporadic cases (Jay, 2000). Diagnosis is easier when a group of cases is seen with characteristic acute, predominantly upper gastroenteritis symptoms and the short interval between eating common food item and the onset of symptoms. Discovery of large numbers of staphylococci (10 organisms or more per gram of food) on routine culture media or detection of enterotoxin is an important fact in diagnosis. Absence of Staphylococci on culture of heated food does not rule out the diagnosis, a Gram stain for food may disclose the organisms that have been heat killed. It may be possible to identify enterotoxin or thermonuclease in the food in the absence of viable organisms.

If we found bacteria of the same phage type from stools or vomits of two or more ill persons this also will confirms the diagnosis, and detection of large numbers of enterotoxin producing Staphylococci from stool or vomits from a single person supports the diagnosis (Bennett, 1986).

1.4.1.2 *Colostridium botulinum*
Poisoning by the toxin, called botulinus or botullin, produced by *Clostridium botulinum* bacteria. These poisoning results most frequently from the eating of improperly sterilized canned foods containing the toxin. *C. botulinum* bacteria cannot survive in the presence of oxygen and normally live in the soil, where they form heat resistant spores that may contaminate fresh food to be canned. The spores survive if the food is not cooked at high enough temperature for sufficient length of time, then inside the sealed can, the spores germinate and release the bacteria and as the bacteria multiply, they secrete their toxin, a protein that is one of the most potent poisons known. Unlike the spores the toxin is readily destroyed by heat but it remains potent if the contaminated food is not heated before it is eaten. Once ingested and absorbed the toxin damages the autonomic nervous system by blocking the transmission of nerve impulses (Kendall, 1989; Alderton and Chen, 1976).

The first symptoms of poisoning, nausea and vomiting, usually appear six hours or less after the contaminated food is eaten, depending upon the amount of toxin ingested. The poisoned person becomes tired and may complain of headache and dizziness. Often his vision is blurred, and he may see double. The mucous membranes of the throat may become dry; the affected person may feel a constriction in the throat, soon associated with difficulty in swallowing and speaking; and a general muscle weakness occurs. The respiratory muscles become involved. Early tracheotomy—the cutting of an emergency air passage into the

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windpipe—and the prompt use of mechanical aids to respiration may be lifesaving; about half the deaths from botulism result from paralysis of the respiratory muscles (Kendall, 1989; Dodds, 1994). Usually a person recovers completely if he survives the paralysis. With early diagnosis, the chance of a person's surviving is greatly enhanced by the prompt administration of botulism antitoxins that neutralize the toxin in the body. However, the severe symptoms often do not develop until the poison has already damaged the nervous system; at this stage, it is ordinarily too late for the antitoxins to be fully effective. Botulism most commonly originates with home-canned foods, rather than with foods that have been canned commercially (Gilbert, 1979).

1.4.1.3 Clostridium perfringens food intoxication

Infectious agents, type A strains of *C. perfringens* (*C. welchii*) cause food poisoning outbreaks, type C strains cause necrotizing enteritis elaborated by the organisms (Duncan, 1968). The source of this bacteria throughout the environment; soil, cooked meat, gravy, sauces soups (Jay, 2000).

The mode of transmission is ingestion of food that was contaminated by soil or feces and then held under conditions that permit multiplication of the organism. Almost all of outbreaks are associated with inadequately heated or reheated meats usually stews, meat pies, and gravies made of
beef, turkey or chicken (Kendall, 1989; Johnson et al., 1983). Spores survive at normal cooking temperatures, germinate and multiply during slow cooling, storage at ambient temperatures, and or inadequate rewarming. Outbreaks are usually traced to food catering firms, restaurants, cafeterias for large scale service. Heavy bacterial contamination (more than $10^5$ organisms per gram of food) is usually required for clinical disease (Duncan, 1973).

An intestinal disorder characterized by sudden onset of colic followed by diarrhea, nausea is common, but vomiting and fever are usually absent. Generally a mid disease of short duration, 1 day or less and rarely fatal in healthy people (McClung, 1945).

In the outbreak setting diagnosis is confirmed by demonstration of *Clostridium perfringens* in semiquantitative anaerobic cultures of food ($10^5$ /g or greater) or patient stool ($10^6$ /g or greater) in addition to clinical and epidemiologic evidence. Detection of enterotoxin in the stool of ill persons also confirms the diagnosis (Johnson et al., 1983).

1.4.1.4 Bacillus

Genus Bacillus, a group of rod-shaped, Gram-positive, aerobic or under some conditions anaerobic bacteria, is widely found in soil and water. The term bacillus in a general sense has been applied to all cylindrical or rods like bacteria. The largest species are about 2 µm across by 7 µm long.
and frequently occur in chains. Bacillus species form dormant spores (endospores) under adverse environmental conditions. These endospores may remain viable for long periods of time. Endospores are resistant to heat, chemicals, and sunlight and are widely distributed in nature, primarily in soil, from which they invade dust particles (Darland and Brock, 1971). Although most are mesophiles, psychrotrophs and thermophils exist (Jay, 2000).

*Bacillus cereus* sometimes causes spoilage in canned foods. *B. subtilis*, also widely disseminated, is a common contaminant of laboratory cultures and is often found on human skin. Most strains of Bacillus are not pathogenic for humans and only infect them incidentally in their role as soil organisms; a notable exception is *B. anthracis*, which causes anthrax in humans and domestic animals. *B. thuringiensis* causes disease in insects; *B. thuringiensis* insecticides are harmless to vertebrates but effective against pests of agricultural products. Medically useful antibiotics are produced by *B. subtilis* (bacitracin) and *B. polymyxa* (polymyxin B) (Hsieh et al., 1999)

1.4.1.5 *Bacillus cereus*:-

It is anaerobic spore forming, rod – normally present in soil, dust, water and spices (Wagner and Jr., 2000). Its optimum temperature for growth is 30°C, with minimum temperature of 10°C and maximum of 49°C.
pH range for growth is 4.9 to 9.3 (Frazier and Westhoff, 1978). It spores possess resistance to heat, typical of other mesophiles (Jay, 2000).

*B. cereus* is a widely spread saprophyte that infecting bovine udder causing gangrenous mastitis (Carter, 1975). Numerous surveys on food and food ingredients have indicated a high percentage of samples containing *B. cereus*. It is widely distributed in nature and food supply (Frazier and Westhoff, 1978). Extremely large numbers of viable cell of *B. cereus* must be ingested to develop signs and symptoms of food borne disease. The mechanism of the pathogenicity involves the lyses of the cell in the intestinal tract and the resulting release of toxin (Frazier and Westhoff, 1978). It is suggested that the food environments influenced enterotoxin production by *B. cereus* (Raevuori et al., 1978). *B cereus* toxin caused food poisoning, and produced the toxin when the bacilli is sporulated (Chessburgh, 2000).

Food poisoning strains of B. cereus produce the following toxins and extra cellular products. Lecithinase, protease, beta- lactamase, emetic enterotoxin. Food borne gastroenteritis syndromes caused by the emetic and the diarrheal enterotoxins. The diarrheagenic toxin is heat labile its production is favored over the pH range 6 – 8.5, and in one study no toxin was found at pH 5. It is sensitive to trypsin and pepsin and induces diarrhea (Jay, 2000).
The emetic enterotoxin (vomiting type) is heat and pH stables and its sensitive to trypsin and pepsin and induced diarrhea (Jay, 2000).

Diarrheal syndrome is rather mild, with symptoms developing within 6 to 12 h the symptom consist of nausea (with vomiting being rare), cramp like abdominal pains, tenesmus and water stool, Fever is generally observed. The similarity between this syndrome and that of *C. perfringens* food poisoning has been noted (Jay, 2000).

In outbreak setting, diagnosis is confirmed by performing quantitative culture with selective media to estimate the number of organisms presenting the suspected food, generally more than $10^5$ organisms per gram of the incriminated food is required. Diagnosis is also confirmed by isolation of organisms from the stool of two or more ill persons and not from stools of controls. Enterotoxin testing is valuable but may not be widely available (Konuma *et al.*, 1998).

Mode of transmission is ingestion of food that has been kept at ambient temperature after cooking, permitting multiplication of the organisms. Outbreaks associated with vomiting have been most commonly associated with cooked rice that has subsequently been held at ambient room temperatures before reheating. Various mishandled food have been implicated in outbreaks associated with diarrhea (Jay, 2000). *Bacillus cereus* was the causative agent of four outbreaks of food
poisoning involving 600 persons which were reported in Oslo, Norway (Hauge, 1955)

1.4.1.6 Vibrio

Vibrio species may cause sporadic case of diarrhea as well as opportunistic infection (Barrow and Felthman, 1993). The name Vibrio usually implies gastrointestinal infection caused by *Vibrio cholerae* or by the marine organisms *Vibrio parahaemolyticus*, which is commonly associated with food-poisoning; but many other *Vibrio cholerae* cause cholera is endemic in about 80 countries. Two serogroups of *Vibrio cholerae* cause epidemic cholera. Several other serogrops of *Vibrio cholerae* cause diarrheal disease but not epidemic cholera (Chessburgh, 2000).

*Vibrio cholerae* serogroups 01 and 0139 and related Vibrios, which produces an enterotoxin that cause cholera, a profuse watery diarrhea that can rapidly lead to dehydration and death in humans, while other Vibrios may cause sepsis or enteritis (Jawetz *et al.*, 2001).

*Vibrio cholerae* is transmitted by the faecal-oral route with most epidemics occurring when water supplies become faecally contaminated. Transmission can also occur by ingesting contaminated food (Chessburgh, 2000).

1.4.1.7 Proteus
Riemann (1969) described Proteus food poisoning four species were among organisms which caused intoxication, *P. vulgaris*, *P. mirabilis*, *P. morganni*, and *P. rettgeri*.

1.5 Food poisoning

Acute gastrointestinal infection resulting from the consumption of foods containing one or more representatives of three main groups of harmful agents: natural poisons present in certain plants and animals, chemical poisons and microorganisms mainly bacteria and their toxic secretion. If bacteria became numerous and the food is eaten, bacteria may continue to grow in intestines and cause infection (Jay, 2000).

The majority of cases of acute food poisoning are caused by bacteria such as Salmonella, Shigella, *Escherichia coli*, and Campylobacter. Cooper (1941) reported an outbreak of food poisoning in Bristol and *Proteus vulgaris* was isolated from the suspected meat and from patients.

1.5.1 Bacteria associated with food poisoning

1.5.1.1 Salmonellosis

Several infections caused by certain species of Salmonella, are important as the cause of a type of food poisoning in humans and of several diseases in domestic animals. The term salmonellosis has been used generally for two main kinds of gastrointestinal diseases in humans:
enteric fevers (including typhoid and paratyphoid fevers) and gastroenteritis (Fraizer and Westhoff, 1978). The latter is caused primarily by *S. typhimurium* and *S. enteritidis*; it occurs following ingestion of the bacteria on or in food or drink or on fingers and other objects (Cheesburgh, 2000). Contamination is mainly from two sources: food products from diseased poultry, hogs, and cattle; and wholesome food subsequently exposed to infected fecal matter during food storage (mice and rats) and during food preparation (human handlers) by hands that are not washed after using the toilet (Davis and Samuel, 1997, Hubbert *et al.*, 1975; Frizer and Westhoff, 1978; Wagner and Jr., 2000; Cheesburgh, 2000). Because of this it is important to make sure hand and working surface are thoroughly washed after contact with raw meat, fish and poultry and before working with that require no further cooking (Davis and Samuel, 1997; Pether and Gillbert, 1971).

The onset of the disease is sudden and sometimes severe, producing nausea, vomiting, diarrhea, prostration, and slight fever occur 12-36 hours after eating infected food. The illness is due to multiplication of the organism within the body. In most cases recovery occurs within a few days and is followed by varying degrees of immunity (Davis and Samuel, 1997, Cheesburgh, 2000).

Salmonella bacteria thrive at temperature between 40 -140 F, they are readily destroy by cooking to 165 F and don’t grow at refrigerator or
freezer temperatures. They do survival refrigeration and freezing, however it will begin to grow again once warmed to room temperature (Davis and Samuel, 1997).

In the Sudan, Horgan (1986) investigated an outbreak of food poisoning at WadMadani and S. dublin was isolated from two persons and meat. S. dublin was incriminated of being the cause of infection.

1.5.1.2 Shigella

Genus Shigella is a rod-shaped bacteria in the family Enterobacteriaceae. They are normal inhabitants of the human intestinal tract and can cause dysentery. Shigella is microbiologically characterized as Gram negative, non spore forming, and non motile bacteria. Their cells are 0.4 to 0.6 micrometer across by 1 to 3 micrometers long. (Jawetz et al., 2001).

Shigella species cause bacillary dysentery, or Shigellosis, with Shigella dysentery serotype 1, also known as Shiga bacillus, being the most virulent (Chessburg, 2000). Shigella infect only humans, transmission is mainly by the fecal oral route with poor sanitation, unhygienic condition, and overcrowding which facilitate the rapid spread of infection. Housefly help to transfer shigella from feces to food, the organism is rapidly killed by drying (Thornton, 1968; Cheesburg, 2000).
*S. dysentriae* spread by contaminated water and food, causes the most severe disease because of its potent exotoxin but *S. sonnei* and *S. flexneri* are also implicated as dysentery agents (Parish, 1998). Shigellosis symptoms are diarrhea, cramps, chills and fever that occurs between 1 to 7 days after eating (Jay, 2000).

### 1.5.1.3 Campylobacter

Campylobactriosis or Campylobacter enteritis is caused by consuming food or water contaminated with the bacteria *C. jejuni* (Daivs and Samuel, 1997; Skirrow, 1977; Wokatsch and Bochemuhl, 1980; Blaser *et al.*, 1983; Morris and Pattons, 1985). The organisms are able to produce enterotoxins and cytotoxins (Cheesburgh, 2000). Raw and inadequately cooked food of animal origin and non chlorinated water are the most common source of human infection (Daivs and Samuel, 1997).

Campylobacter transmission to hand may take place through cross contamination of food and utensils when food worker process raw food (Gates *et al.*, 1987). These organisms grow best in reduced oxygen environment, easily killed by heat 120F. It is inhibited by acid salt and drying and not multiply at temperature below 85F (Daivs and Samuel, 1997).

The symptoms of Campylobacter infection (campylobacteriosis) include acute abdominal pain, diarrhea (which can be watery and contain
blood), nausea, headache, muscle pain and fever. Symptoms can begin 2 to 5 day after eating contaminated food and generally last 7 to 10 day (Daivs and Samuel., 1997).

*Campylobacter spp* were effectively removed by hand washing with either soap and water or water alone, followed by drying with paper towels, yet the organisms existed on wet hands (Goatest *et al*., 1987). Preventive measures of campylobacter infection include pasteurizing milk, avoiding post pasteurization contamination, cooking raw meat, poultry and fish and preventing cross contamination between raw and cooked or ready to eat food (Daivs and Samuel, 1997). Campylobacter bacteria are commonly found in raw or undercooked poultry, meat, unpasteurized milk and non chlorinated water (Daivs and Samuel, 1997). *C. jejuni* was first isolated from human diarrhea and in 1971 since then it has gained recognition as disease causing organism in humans (Wagner and Jr, 2000).

1.5.1.4 *Escherichia*

*E. coli* is belonging to family of microorganisms called coliforms (Daivs and Samuel, 1997) and it is commonly found in the intestinal tract of warmed blood animals. Approximately 10% of bacteria found in the human intestine are made up of *E. coli* for that the organism can be associated with any food that may potentially come in contact with
animal or human fecal material (Sussman, 1997; Dupont and Mathewson, 1998; Nataro and Kaper, 1998; Davis and Samuel, 1997).

Most *E. coli* bacteria are harmless and help in the wellness of human health by helping keep the growth of more harmful microorganisms in the check. Fortunately only a few types of *E. coli* are harmful including *E.coli* O157:H7, the O and H represent cellular and flagella antigens, respectively (Daivs and Samuel, 1997).

The human disease syndromes resulting from ingestion of enteropathogenic *E. coli* have been divided into two main groups, the first enterotoxigenic groups responsible for infantile diarrhoeal disease and traveller’s diarrhea, and the second invasive serotype responsible for invasive illness (Ewing *et al.*, 1963; Frazier and Westhoff, 1978). Symptom of *E. coli* O157:H7 begin with non bloody diarrhea one to five days after eating contaminated food and progress to bloody diarrhea, severe abdominal pain, in young children hemolytic uremic syndrome (HUS) is a serious complication that can lead to renal failure and death. In adult the complication sometime lead to thrombocytopenic purpura (TPP), characterized by cerebral nervous system deterioration, seizures and strokes (Restaino and Wind, 1990).

*E. coli* can be transferred between hand, raw food and cooked or processed foods (Pether and Gilbert, 1971; Paulson, 1994; Restaino and Wind, 1990; Snyder, 1997).
1.5.1.5 Listeria

Listeria is widely distributed in nature and can be found on decaying vegetation and in soils, animal’s feces, silage and water. As result of its wide spread in the environment, it is able to survive long periods of time under adverse conditions, and has ability to grow at refrigeration temperature. Listeria is now recognized as an important food borne pathogen (Wagner and Jr, 2000; Davis and Samuel, 1997).

*Listeria spp* have been found to survive on the fingertips for over 11 h thus food may be contaminated with Listeria via the finger of food employer (Snelling *et al.*, 1991). The organisms grow in the pH range of 5.5 to 9.5, it is salt tolerant and relatively resistant to drying but easily destroy by heat (Daivs and Samuel, 1997). Foods found to contain Listeria are unpasteruized milk and cheese, raw or undercooked meat, poultry and fish (Davis and Samuel, 1997).

Listeria symptoms are headache and fever, sometimes accompanied by nausea and vomiting (Davis and Samuel, 1997). Preventive measure for Listeriosis include maintaining good sanitation, avoiding post pasteurization contamination and cooking food thoroughly (Daivs and Samuel, 1997) and when use chlorhexidine gluconate in alcohol was found to be effective in reducing Listeria from fingertips (Snelling *et al.*, 1991).
1.5.1.6 Pseudomonas

Riemann (1969) reported that *P. eruginosa* cause food poisoning.

1.5.1.7 Proteus

Cooper, (1941) reported an outbreak of food poisoning in Bristol and *Proteus vulgaris* was isolated from the suspected meat and from patients.

1.6 Source of food contamination

The origins of microbial contamination in food service include environment, the food worker, the source of food and food itself (Beuchat, 1995; Jack and Marianne, 1999). Kitchens surfaces and faucet spigot may be significant source of cross contamination (Chen *et al.*, 2001).

1.6.1 Food worker

Food worker particularly ill food worker can serve as the source of food contamination (Jack and Marianne, 1999; Torok *et al.*, 1997; Paulson, 1994; 1996; Coates, *et al.*, 1987). Food worker may transmit pathogen to food from contaminated surface, from another food or from hand contaminated with organisms from gastrointestinal tract (Lebaron *et al.*, 1990). Contamination by food worker can occur due to lack of adequate hand washing during processing because the organisms are loosely attached to the surface of the skin and can easily contaminated
food product (Miller, 1994; Paulson, 1994; Coates et al., 1987). According to a report by the Centers for Disease Control and Prevention (CDC) the hand may be the most important source of contamination (Lebaron et al., 1990; Bryan, 1995).

The microflora on the hands and outer garments of handlers generally reflects the environment and habits of individuals and the organisms may be those from soils, water, dust and other environmental sources. Additional important sources are those from the gastrointestinal tract that may enter food through poor personal hygienic practices (Jay, 2000).

During food handling and preparation microorganisms on raw food can be transferred to various surfaces such as cutting boards and water faucet spigots (Miller et al., 1996; Zhao et al., 1998).

Pathogen such as Yersinia, Protus, Campylobacter and Klebsiella, originating from raw animal products can contaminate hand and then be transferred to foods, equipment and other worker (Restaino and Wind, 1990; Paulson, 1994; Snyder, 1997).

Microfloras of skin are categorized in to two types: resident and transient. Resident bacteria are those organisms that normally reside on the skin, in this case the skin of hands. 90% of resident flora of hands are coryneform and coagulase negative Staphylococci (Miller, 1994) among the resident microflora Saphylococcus aures is the only true pathogenic organism of food safety concern (Lowbury et al., 1964; Miller, 1994).
Resident flora are not easily removed by mechanical fraction (Larson, 1995) since they buried deep within the pores where they are protected by sebaceous gland secretions (Restaino and Wind, 1990; Miller, 1994). The variety of resident organisms is significantly less than the kind of microorganisms that can serve as transient organisms (Ristaino and Wind, 1990).

Transient organisms are of concern because they are readily transmitted by hands unless removed by mechanical fraction of washing with soap and water or destroyed by the use of antiseptic solution (Larson, 1995). Transient organisms can be considered skin contamination that are acquired from environmental source and become attached to the outer epidermal skin layer (Restaino and Wind, 1990).

The fingers are thought to be the most important part of the hand in terms of the transfer and spread of pathogenic microflora (Ansari et al., 1991). The transfer of organisms and viruses facilitated by wet hand as compared to dry hands (Larson, 1985; Ansari et al., 1988; Spring Thorpe and Satter, 1988).

1.6.2 Raw food

Microorganisms on raw food such as Yersinia, Proteus, Campylobacter and klebsiella which can be transferred to various surfaces such as cutting boards, hands, equipment and workers (Paulson, 1994; Restaino and Wind, 1990; Snyder, 1997; Niskanen and Pohja, 1977; Chen et al., 2001).
1.6.3 Utensils

The phenomenon of bacteria attachment to processing plant surface such as metal, rubber and plastic presents a formidable obstacle for sanitizing and cleaning treatment (Jay, 2000)

1.7 Control

The food safety means prevention of food poisoning, (the transmission of disease through food) and to maintain the wholesomeness of the food product through all stage of processing until it’s finally eaten.

The prevention or minimizing of contamination could be through interventions in certain areas. The reduction of microbial pathogen in food product is the most pressing food safety problem today. They are physical, chemical, and alternative intervention strategies for control bacteria contamination. (Jay, 2000)

1.7.1 Food worker

Firstly exclusion of ill food workers from the working place (Jack and Marianne, 1999). Secondly removal of pathogens from the hands of the food worker by washing to obtain hand hygienic (Ansari et al., 1991; Jack and Marinne, 1999). Hand washing have been recognized as one of the most effective to prevent cross-contamination and minimize transfer of microorganism (Dosterom, 1998; Fendler et al., 1998). The use of germicidal agent or antiseptic detergent result in removal or destruction
of transient microorganisms (Sheena and Stiles, 1982; Ayliffe et al., 1987; Nicolett et al., 1990; Jack and Marinne, 1999) and when increase fraction by rubbing hands together or using a scrub brush that allow for greater reduction of transient bacteria even with the use of plain soap or detergents (Restaino and Wind, 1990).

Washing and rinsing with warmed water bring resident flora from deep skin layer to the surface where they are removed with washing or drying. Since transient microorganisms are not normally local in deep skin layer and are more easily removed by routine hand washing and the water temperature may not play role in the removed of these organisms from the skin. (Ansari et al., 1991).

Thirdly use of barriers to prevent bare hand contact with ready to eat food (Olsen et al, 1993; Ehrenkran, 1992; Fendler et al., 1998; Jack and Marianne, 1999)

American Society for Testing and Materials (ASTM) recommended to follows this protocol  wet hand under warm water (100-108 F); apply 3ml hand wash product ; rub vigorously over hand surfaces ,concentrating on interdigital spaces and nail beds ; apply small amount of water and lather for 15 seconds ; rinse for 30 seconds ; dry with a clean paper towel ( Jack and Marianne,1999 ).

1.7.2 Cooking food
Adequately cooking food and protecting it from outside contamination through cooking is important to destroy harmful bacteria (Davis and Samuel, 1997).

CHAPTER TWO

2- MATERIALS AND METHODS

2.1 Sterilization
a. Flaming

It was used to sterilize glass slides, cover slips, needles and scalpels.

b. Red heat

It was used to sterilize loop wires, points and searing spatulas by holding them over Busen burner flame until became red-hot.

c. Hotair oven

It was used to sterilize glasswares such as test tubes, graduated pipettes, flasks, and forceps and cotton swabs. The holding period was one hour and oven temperature was 180°C.

d. Steaming at 100°C

Repeated steaming (tyndallization) was used for sterilization of sugars and media that could not be autoclaved without detriment effect to their constituents. It was carried out as described by Cruckshank et al. (1975)

e. Moist heat (Autoclave):

Autoclaving at 121°C (15 lb/Inch²) for 15 minutes was used for sterilization of media and plasticwares. Autoclaving at 115°C (10 lb/inch²) for 10 minutes was used for sterilization of some media.

2.2. Reagents

2.2.1. Voges proskauer test reagent

Consisted of two solution:- A) Alpha-naphthol solution consist of 5% alpha-naphthol in ethanol. And B) 40% OF KOH solution.

2.2.2. Hydrogen peroxide

The reagent was obtained from Agropharm limited, Buckingham. It was prepared as 3% aqueous solution and it was used for catalase test.
2.2.3. Methyl red

It was prepared by dissolving methyl red (0.04g) in ethanol (40ml). The volume was made to 100ml with distilled water. It was used for methyl red test.

2.2.4 Tetramethyl-p-phenylene diamine dihydrochloride

This was prepared in a concentration of 3% aqueous solution and was used for oxidase test.

2.2.5. Nitrate test reagent

Nitrate test reagent was consist of two solutions and they were prepared according to Barrow and Feltham (1993). Solution A was composed of 0.33% sulphanilic acid dissolved by gently heating in 5N-acetic acid. Solution B was composed of 0.6% dimethy-alpha-naphthyl amine dissolved by gentle heating in 5N-acetic acid.

2.2.6. Kovac's reagent

This reagent composed of 5g para-dimethyl amino benza aldehyde, 75ml amyl alcohol and 25ml concentrated hydrochloric acid. It was prepared as described by Barrow and Feltham (1993) by dissolving the aldehyde in the alcohol by heating in water bath. It was then cooled and the acid was added. The reagent was stored at 4°C for later use in indole test.

2.3. Collection of blood for enriched media

Blood for enriched media was collected aseptically into sterile flask containing glass beads by venipuncture of the jugular vein of a healthy sheep kept for this purpose. The blood was defibrinated by shaking the sterile flask containing glass bead

2.4. Preparation of Media

2.4.1 Nutrient broth
Thirteen grams of nutrient broth (Oxoid) were added to one liter of distilled water, mixed well and distributed in 3ml amount into clean test tubes, then sterilized by autoclaving at 121°C for 15 minutes

2.4.2. Peptone water
Fifty grams of peptone water powder (Oxoid) were added to one liter of distilled water, mixed well, distributed in 3ml amount into clean test tube and sterilized by autoclaving at 121°C for 15 minutes.

2.4.3. Peptone water sugars
Peptone water sugar medium was prepared as described by Barrow and Feltham (1993). It contained peptone water 900ml, Andrades indicator 10ml, sugar solution 10ml and distilled water 90ml. The pH of peptone water was adjusted to 7.1-7.3 before the addition of Andredes indicator. The complete medium was mixed well, then distributed into portions of 2ml into clean test tubes containing inverted Durham’s tube and sterilized by autoclaving at 115°C (10 lb/inch²) for 10 minutes.

2.4.4. Nitrate broth
The medium used was prepared as described by Barrow and Feltham (1993). Potassium nitrate (1g) was dissolved in one liter of nutrient broth, distributed in 5ml amount into test tubes and sterilized by autoclaving at 115°C for 20 minutes.

2.4.5. Glucose-phosphate medium (MR-VP test medium):
This medium was prepared according to Barrow and Fletham (1993). Peptone (5g) and 5g of phosphate buffer (K HPO) were added to one liter of distilled water, dissolved by steaming, filtered and pH was adjusted to 7.5. Then five grams of glucose were added, mixed well, and distributed into clean test tubes then sterilized by autoclaving at 115°C for 15 minutes.

2.4.6. Nutrient agar
To one liter of nutrient broth (Oxoid), 15g of agar were added, dissolved by boiling, sterilized by autoclaving at 121˚C for minutes. Then cooled to about 50˚C and distributed in 15ml amount per plate. The poured plates were left to solidify at room temperature on leveled surface.

2.4.7. Blood agar

Forty grams of blood agar base NO.2 (Oxoid) were suspended in one liter of distilled water, dissolved by boiling, mixed and sterilized by autoclaving at 121˚C for 15 minutes. Then cooled to about 50˚C and defibrinated sheep blood was added aseptically to give final concentration 10%, mixed gently and 15ml of complete medium were poured into each sterile Petri dish. The poured plates were allowed to solidify at room temperature on flat surface.

2.4.8. MacConkey agar

Fifty two grams of MacConkey agar (Oxoid) were suspended in one liter of distilled water, brought to boil to dissolve the ingredients completely, then sterilized by autoclaving at 121˚C for 15 minutes and poured into sterile Petri dishes in 15ml amount. The poured plates were left to solidify at room temperature on flat surface.

2.4.9. Motility medium-Cragie tube medium

Thirteen grams of dehydrated nutrient broth (Oxoid) were added to 4 grams of Oxoid agar No-3 and dissolved in one liter of distilled water. The pH was adjusted to 7.4. This medium was dispensed in volumes of 5ml into 20ml test tubes containing Cragie tube, and then the medium in the test tubes were sterilized by autoclaving at 121˚C for 15 minutes.

2.4.10. Hugh and Leifsons (O/F) medium

This medium was prepared as described by Barrow and Faltham (1993). Two grams of peptone powder, five grams of sodium chloride, 0.3g of potassium hypophosphate and three grams of agar were added to
one liter of distilled water. Then heated in water bath at 55°C to dissolve the solids. The pH was adjusted to 7.1 and filtered. Then the indicator bromothymol blue (0.2% aqueous solutions) was added and the mixture was sterilized at 115°C for 10 minutes. Filtered sterile glucose solution was added aseptically to give final concentration of 1%. Then the medium was mixed and distributed aseptically in 10ml amount into sterile test tubes of not more than 16mm diameter.

2.4.11 Starch agar

This media consisted of 10g of potato starch, 50 ml of distill water and 1000 ml of nutrient agar. The starch was triturated with water to a smooth cream, added to the molten nutrient agar and mixed. The medium was sterilized at 115°C for 10 min distributed into sterile Petri-dishes.

2.4.12. Urea agar base

Two point four grams which consisted of 1g peptone, 1g dextrose, 5g NaCl, 1.2g disodium phosphate, 0.8 potassium dihydrogen phosphate, 0.012g phenol red and 15 agar were suspended in 95 ml of distilled water and dissolved. The medium was sterilized by autoclaving at 115°C for 20 min after adjusting the pH to 6.8. The medium was cooled to 50°C before aseptically adding 5ml of sterile 40% urea solution, mixed well, distribute 10 ml amount into sterile bottles and allowed to set in the slope position.

2.4.13. Simmon's citrate agar

Twenty three gram which consisted of 0.2g of magnesium sulphate, 0.2g ammonium dihydrogen phosphate, 0.8 sodium ammonium phosphate, 2g sodium citrate, 5g NaCl, 0.8g bromothymol blue and 15 g agar were suspended in liter of distill water and boiled to dissolve completely. The medium was sterilized by autoclaving at 121°C for 15min after adjusted the pH to 7 then cooled to 50°C and aseptically 5 ml of sterile 40% urea solution were added and mixed well. The medium
was distributed in 10 ml amount of media into sterile bottles and allowed to set in the slope position.

2.4.14. Ammonium salt sugars (ASS)

Consisted of 1g ammonium monohydrogen phosphate, 0.2g potassium chloride, 0.2g magnesium sulphate, 0.2 yeast extract, 20g agar and 4 ml bromocresol purple. The solids were added and the water and dissolved by steaming, the indicator was added and the medium was sterilized at 115 C for 20 min. the basal medium was cooled to about 60 C, then appropriate carbohydrate was added as a sterilized solution to give final concentration of 1% mixed and distributed aseptically into sterile tubes which were inclined so that the medium sets as a slope.

2.5. Collection of samples

A total of 60 samples from hands, dishes and foods were collected randomly from restaurant during August 2004 to December 2004. The area of study was Khartoum state. Sample were collected by a sterile cotton wool swab, then the swab was returned to it is sterile tube and the tube was labeled.

1.5.1 Transportation of collected samples

Samples collected were transferred to the laboratory on ice in container with sufficient speed to avoid unnecessary delay and/ or contamination prior to microbiological examination.

2.6. Culture of specimens

The collected swabs were inoculated onto blood agar and MacConkey agar. The inoculated plates were then incubated for 24-48 hours at 37°C

2.7. Purification

All bacteria isolated were purified by several sub culturing from single well-separated colony on nutrient agar plates. The purity was
checked by examining Gram stained smear. The pure culture was then used for studying cultural and biochemical characteristics.

2.8. Microscopic Examination

Smears were made from each type of colonies on primary culture and from purified colonies, fixed by heating and stained by Gram method (Barrow and Feltham, 1993). The stained smears were examined microscopically by oil immersion lens. The smear was examined for cell morphology, and staining reaction.

2.9. Identification of bacteria

The purified isolates were identified according to the criteria described by Barrow and Fetham (1993) this included staining reaction, organism morphology, growth condition, the colonies characteristics on different media, haemolysis in blood agar, motility and biochemical characteristics.

2.10. Biochemical methods

2.10.1. Catalase test

The test was carried out as described by Barrow and Feltham (1993). A drop of 3% H₂O₂ was placed on clean slide and then colonies of tested culture on nutrient agar was picked by glass rod and added to the drop of 3% H₂O₂. A positive reaction was indicated by production of air bubbles.

2.10.2. Coagulase test

The test was performed as described by Barrow and Feltham (1993). To 0.5ml of 1:10 dilution of human plasma in saline, 0.1ml of 18-24 h old culture of the tested organism was added, then incubated at 37°C and examined after 6-24 h for coagulation. Definite clot formation indicated positive result.
The test was also performed on slide, two colonies of tested culture were placed on a clean glass slide, emulsified in drop of normal saline and then a loopful of human plasma was added to the drop of bacteria suspension. Appearance of coarse visible clump was recorded as positive result.

2.10.3. Oxidase test

The method of Barrow and Feltham (1993) was used. Strip of filter paper was soaked in 1% solution of tetramethy-p-phenylenediamine dihydrochloride and dried in hot air oven and then placed on clean glass slide by sterile forceps. A fresh young tested culture on nutrient agar was picked off with sterile glass rod and rubbed on the filter paper. If a purple color developed within 5-10 seconds, the reaction was considered positive.

2.10.4. Oxidation-fermentation (O/F) test:

The test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated with straight wire into two test tubes of Hugh and Leifeson's medium. To one of the test tube a layer of melted soft paraffin oil was added to the medium to seal it from air. The inoculated tubes were incubated at 37°C and examined daily for fourteen days. Yellow color in open tube indicated oxidation reaction, yellow color in both tubes showed fermentation reaction and blue or green color in open tube and yellow color in the sealed tube indicated production of alkali.

2.10.5. Sugar fermentation test

The test was carried out as described by Barrow and Feltham (1993). The peptone water sugar was inoculated with organism under the test, incubated at 37°C and then examined daily for several days. Acid production was indicated by appearance of reddish color, while gas
production was indicated by presence of empty space in the inverted Durham’s tubes.

2.10.6. Indole production test

Indole production test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into peptone water and incubated at 37°C for 48 h. One milliliter of the Kovacs reagent was run down along side of the test tube. Appearance of pink color in the reagent layer within a minute indicated positive reaction.

2.10.7. Methyl red (MR) test

Methyl red test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into glucose phosphate medium (MR-VP medium) then incubated at 37°C for 48 h. Two drops of methyl red reagent were added, shaken well and examined. Appearance of red color indicated positive reaction, whereas orange or yellow color indicated negative reaction.

2.10.8. Voges-Proskauer (VP) test

The test was preformed as described by Barrow and Feltham (1993). The test culture was inoculated into glucose phosphate medium (MR-VP medium) and incubated at 37°C for 48 h. 0.6 milliliter of 5% alpha-naphthol solution and 0.2 milliliter of 40% potassium hydroxide were added to 1 ml of cultured media, shaken well. When bright pink color developed within 30 minutes, the reaction was regarded as positive.

2.10.9. Nitrate reduction:

The nitrite test was carried out as described by Barrow and Feltham (1993). The test culture was lightly inoculated into nitrate broth and incubated at 37°C for two days. Then 1ml of solution A followed 1ml of solution B of nitrate test reagent were added. Red color indicated positive reaction that showed nitrate had been reduced. If red color did
not develop, powdered zinc was added to see whether there was residual nitrate or not. Red color development indicated that nitrate in medium had been reduced to nitrite by zinc but not by organism, whereas unchanged color indicated nitrite in original medium had been reduced completely and nitrite was further broken down by the organism.

2.10.10. Motility test

The Cragie tube in semi-solid nutrient agar prepared as described by Cruckshank et al. (1975) was inoculated by straight wire. A small piece of colony of the bacterium under test was picked by the end of the straight wire and stabbed in the center of semi-solid agar in the Cragie tube and then incubated at 37°C overnight. The organism was considered motile if there was turbidity in the medium in and outside the Cragie tube.

2.10.11. Urease activity

The test bacteria was streaked on urea agar, and incubated at 37°C for 7 days. Change in color to pink indicated a positive reaction.

2.10.12. Citrate utilization test

The test bacteria was streaked on Simmon's citrate media, and incubated at 37°C for 7 days. An alkalin reaction indicated a positive reaction.

2.10.13. Digestion of casein

Plate of casein agar was streaked, incubation at 37°C and examined for up to 14 days. Clearing zone around the bacterial growth was positive test.

2.10.14. Starch hydrolysis

Starch agar streaked with the test bacteria and incubated at 37°C for 5 days, then flooded with Lugol's iodine solution. Clear zone indicated starch hydrolysis.

2.10.15. Ammonium salt sugar test
Ammonium salt sugar medium was inoculated with the test bacteria and incubated at 37°C for 7 days, growth and acid production indicated a positive reaction.

CHAPTER THREE

3 RESULTS

3.1 Survey
Swab samples were collected at random in Khartoum state from twenty restaurant and three swabs were collected from each restaurant, from food worker, food and dishes. The swabs were cultured onto blood agar, MacConkey agar and nutrient agar. The inoculated plates were incubated aerobically at 37°C. All the inoculated plates showed bacterial growth.

3.2 Characterization and identification of isolates

The bacterial isolates obtained in this investigation were classified on the basis of their cultural characteristic, cell morphology, Gram staining reaction and their biochemical properties as described by Barrow and Feltham (1993).

3.3 Aerobic bacteria isolated from collected samples

Aerobic culture of samples yielded different species of bacteria, the total number of bacteria isolated was 155. Sixty six isolates (110 %) were Gram positive bacteria and 89 isolates (148, 3%) were Gram negative bacteria (Table, 1).

The hand swabs yielded 81 bacterial isolates, 41 (205%) were Gram positive bacteria and 40 (200%) isolates were Gram negative bacteria. The dishes swabs gave 35 bacterial isolates out of which 8 (40%) were Gram positive and 27(135%) were Gram negative. The food samples
yielded a total number of 39 bacterial isolates 17(85%) were Gram positive and 22(110%) were Gram negative bacteria (Table, 1).

3.4 Identification of isolated Gram-positive and Gram-negative bacteria

3.4.1 Gram-positive bacteria

The total numbers of G+ve bacteria isolated was 66 (110%). The most common G+ve organisms isolated in this study were *Bacillus species*.

The bacillus isolated in this investigation was identified according to their morphology and Gram stain. They were Gram positive bacilli and sporulated. They were also identified on the basis of their biochemical properties. They fermented a number of sugar and catalase positive as shown in table 8. The 58 strains of *Bacillus spp* isolated in this study were 23 *Bacillus mycoides*, 21 *Bacillus cereus*, 5 *Bacillus thuringiensis*, 3 *Bacillus sphericus* and 6 *Bacillus subtilis* (Table, 2, 4, 6). Other Gram positive bacteria isolated were 7(11,6%). *Staphylococcus capre* 4(6,6%) and *Staphylococcus lugdunensis* 3(5%) (Table, 2, 4).

3.4.2 Gram-negative bacteria

The total numbers of G-ve bacteria isolated in this study was 89 (148,3%) isolates. The most common G-ve organisms isolated in this study were enterobacteria (105%). The G-negative bacteria isolated in this investigation were 4 *Acinetobacter coloaceticus*, 1 *Moraxella spp*, 5 *Pseudomonas fluorescens*, 2 *Aeromonas salmonicida*, 2 *Flavobacterium*
thalphilum, 10 Klebsiella pneumoniae ozaenae, 5 Kluyvera, 34 Proteus mirabilis, 6 Enterbacter cloacae, 3 Enterobacter sakazakii, 13 E.coli and 4 Hafnia alvei (Table, 3, 5, 7).

These isolates were Gram negative bacilli and on MacConky agar after overnight incubation at 37°C they produced colonies of different shape and colors. They were further identified by their biochemical properties into different species as shown in Table, 9.

**Table1:** Isolation of Gram-positive and Gram-negative bacteria from swabs sample collected at random from restaurants, worker’s hands, dishes and food in Khartoum state.
<table>
<thead>
<tr>
<th>Type of sample</th>
<th>No of sample examined</th>
<th>No of G+ve isolates (percent)</th>
<th>No of G-ve isolates (percent)</th>
<th>G+ve and G-ve isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand sample</td>
<td>20</td>
<td>41 (205%)</td>
<td>40 (200%)</td>
<td>81 (405%)</td>
</tr>
<tr>
<td>Dishes sample</td>
<td>20</td>
<td>8 (40%)</td>
<td>27 (135%)</td>
<td>35 (175%)</td>
</tr>
<tr>
<td>Food sample</td>
<td>20</td>
<td>17 (85%)</td>
<td>22 (110%)</td>
<td>39 (195%)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>66 (110%)</td>
<td>89 (148,3%)</td>
<td>155 (258,3%)</td>
</tr>
</tbody>
</table>

Table2: Gram positive bacteria isolated from worker’s hand swab samples collected at random from restaurant in Khartoum state.
Table 3: Gram negative bacteria isolated from worker’s hand swab samples collected at random from restaurant in Khartoum state.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number of samples examined</th>
<th>Number of isolates</th>
<th>Isolation percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus capre</em></td>
<td>20</td>
<td>4</td>
<td>25%</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>20</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td><em>Bacillus sphericus</em></td>
<td>20</td>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td><em>Bacillus thringiensis</em></td>
<td>20</td>
<td>4</td>
<td>20%</td>
</tr>
<tr>
<td><em>Bacillus mycoides</em></td>
<td>20</td>
<td>18</td>
<td>85%</td>
</tr>
<tr>
<td>Isolates</td>
<td>Number of samples examined</td>
<td>Number of isolates</td>
<td>Isolation percentages</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------------------------</td>
<td>--------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>20</td>
<td>6</td>
<td>30%</td>
</tr>
<tr>
<td>Isolates</td>
<td>Number of samples examined</td>
<td>Number of isolates</td>
<td>Isolation percentages</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---------------------------</td>
<td>--------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>20</td>
<td>4</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Bacillus mycoides</strong></td>
<td>20</td>
<td>4</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Staphylococcus lugdunensis</strong></td>
<td>20</td>
<td>3</td>
<td>15%</td>
</tr>
</tbody>
</table>

**Table 5:** Gram negative bacteria isolated from food samples collected at random from restaurant in Khartoum state.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number of samples examined</th>
<th>Number of isolates</th>
<th>Isolation percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hafnia alvei</strong></td>
<td>20</td>
<td>4</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong></td>
<td>20</td>
<td>10</td>
<td>50%</td>
</tr>
<tr>
<td><strong>Klebseilla pneumoniae ozaene</strong></td>
<td>20</td>
<td>8</td>
<td>40%</td>
</tr>
</tbody>
</table>

**Table 6:** Gram positive bacteria isolated from dishes swab samples collected at random from restaurant in Khartoum state.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number of samples examined</th>
<th>Number of isolates</th>
<th>Isolation percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>20</td>
<td>5</td>
<td>25%</td>
</tr>
<tr>
<td><strong>Bacillus mycoides</strong></td>
<td>20</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Bacillus threngensis</strong></td>
<td>20</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----</td>
<td>---</td>
<td>----</td>
</tr>
</tbody>
</table>

**Table 7:** Gram negative bacteria isolated from dishes swab samples collected at random from restaurant in Khartoum state.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number of samples examined</th>
<th>Number of isolates</th>
<th>Isolation percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.coli</em></td>
<td>20</td>
<td>13</td>
<td>60%</td>
</tr>
<tr>
<td><em>Protues mirbilis</em></td>
<td>20</td>
<td>10</td>
<td>50%</td>
</tr>
<tr>
<td><em>Kluyvera spp</em></td>
<td>20</td>
<td>4</td>
<td>20%</td>
</tr>
</tbody>
</table>

**Table 8:** Characters and biochemical reaction of Gram negative bacteria isolated from swab samples collected from restaurant in Khartoum state.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gram reaction</th>
<th>shape</th>
<th>Motility</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Citrat</th>
<th>Urease</th>
<th>M.R</th>
<th>V.P</th>
<th>Indol</th>
<th>Lactose</th>
<th>Maltos</th>
<th>Salicin</th>
<th>Sacaros</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klepsilla Pneumoniae. Ozaenae</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Ecoli</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Hafnia Alvei</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Gram reaction</td>
<td>shape</td>
<td>Motility</td>
<td>Oxidase</td>
<td>Catalase</td>
<td>Citrat</td>
<td>Urease</td>
<td>M.R</td>
<td>V.P</td>
<td>Indol</td>
<td>Lactos</td>
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<td>Salicin</td>
<td>Sucaros</td>
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</tr>
<tr>
<td><em>Kluyvera</em></td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>+</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td><em>Proteus Mirabilis</em></td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>-</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td><em>Enterobacter sakazakii</em></td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td><em>Acinetobacter coloaceticus</em></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Moraxells spp</em></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas fluorscens</em></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Aeromonas salminicida</em></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Flavobacterium thalpophilum</em></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

**Table 9:** Characters and biochemical reaction of Gram positive bacteria isolated from swab samples collected from restaurant in Khartoum state
CHAPTER FOUR

4 DISCUSSION

In this study swab samples were collected from restaurant in Khartoum state. The swabs samples were collected from food, workers hand and dishes. The collected samples were examined for the presence of bacterial contamination. Sixty swabs were collected, cultured onto blood agar and McConky agar and incubated aerobically at 37C. All the

| Bacillus subtili | + | R | + | + | + |
| Staphylococcus capre |   |   |   |   |
| Staphylococcus lugdunensis |   |   |   |   |
swabs samples collected showed bacterial growth. The examination of restaurant samples in this study revealed the presence of Gram-positive and Gram-negative aerobes.

The most common Gram-positive organisms isolated in this study were *Bacillus species*. The bacillus species isolated included *Bacillus mycoides, Bacillus cereus, Bacillus thringiensis, Bacillus sphericus*, and *Bacillus subtilis*.

The *Bacillus cereus* is widely distributed in nature and food stuffs (Gracey, 1986; Kanuma, *et al.*, 1988; Frazier and Westhoff, 1978). *Bacillus cereus* was reported to be the main cause of food intoxication (Frazier and Westhoff, 1978). The *Bacillus cereus* toxin is produced when bacilli sporulated (Cheesbrough, 2000). *Bacillus cereus* was most predominant isolate detected in this study it was isolated from 21(17%) samples collected from workers hand, dishes and food. Four isolates (20%) were recovered from food, 12 isolates (60%) were recovered from hand and 5 isolates (25%) were recovered from dishes.

Other Bacillus isolated in this study was *Bacillus mycoides* which was isolated from hand, dishes and food. This agrees with finding of Musa (2004) who isolated this strain from meat and processing machine this indicate raw and uncooked meat products might be possible source of contamination. However water and soil may also be possible source of food contamination (Jay, 2000). *B. mycoide* was isolated from washed
dishes in this study, this water used for washing dishes might be source of contamination.

*Bacillus subtilis* was isolated in this investigation from food samples only, these agree with Krumer and Gilbert (’1989) findings. They also linked this organism to several cases of food poisoning. This bacterium is commonly recovered from water, soil and air (Jay, 2000). The bacteria produce an endospore that allows it to endure extreme conditions of heat and desiccation in the environment (Claus and Berkeley, 1986). This explains the presence of *B. subtilis* in cooked food.

*Bacillus thringiensis* was isolated during this study from dishes, and worker’s hand. This species is harmless to human (Norris *et al*., 1981; Claus and Berkeley; 1986). In this investigation *Bacillus sparicus* was isolated from workers hand samples only. This species were found in air, dust, water and they cause food poisoning (Knoneman *et al*., 1997).

*Staphylococcus species* which is one of the most important bacteria that caused food intoxication (Jay, 2000) and it universally present in great number on mucus membranes and skin of human (Norris *et al*., 1981). Two Staphylococcus species were isolated in this investigation *Staphylococcus capri* and *Staphylococcus lugdunensis*. *Staphylococcus lugdunensis* was isolated from food samples. This spices was isolated from human clinical specimens (Kawamura *et al*, 1998). This indicate possible contamination of food from workers lesions. *Staphylococcus capri* was
isolated from raw milk, cheeses and raw meat (Perret et al., 1998) and has been primarily associated with animal specimens. Only a few researchers have reported *S. capri* isolation from human clinical specimens (Kawamura et al., 1998). In this investigation *S. capri* was isolated from worker hands. Hence, the contamination source may be raw food of animal origin.

In this study among Gram- negative bacteria that were found to be contaminants of restaurant samples, were mainly belonged to the enterobacteria. In this investigation the most frequently isolated enterobacteria was *Proteus mirabilis*. *Proteus mirabilis* is the main *Proteus species* of medical importance. It caused urinary tract infection, it may also cause abdominal and wound infection (Chessebrough, 2000). Dolman 1967 reported that member of this genus are common inhabitants of intestinal tract of man and animals. *Proteus mirabilis* was isolated from (27.6%) of total sample examined. Fourteen isolates (70%) were recovered from worker’s hands and it was also isolated from food 10(50%) and dishes 10 (50%). The source of contamination might be infected personal wound or urinary tract infection. This contamination can occur due to lack of adequate hand washing and poor personal hygienic practices. In Sudan this bacterium was isolated from ground poultry and ruminants by products (Omara, 2005) and was also isolated from urine collected from urinary tract infected patient (Elshekh, 2003).
*Pseudomonas fluorescens* was isolated in this study from 5 samples of worker's hand (25%). Larson *et al.* (1986) isolated it from hand, while Sheena and stiles 1983 isolated from hand as transient bacteria.

Other Gram-negative bacterium isolated in this study was *Actinobacter cloacae* which was isolated from 4 (20%) worker's hands. These agree with Noble, (1980) who isolated *A. cloacae* from hand as resident bacteria in hand. The presence of this bacterium on the workers hands indicates improper hand washing.

*Enterobacter sakazakii* is one of the large coliform bacteria in enterobacteriacea family. *Enterobacter sakazakii* is a microorganism that has come to world wide attention as the occasional cause of food –borne illness in premature or otherwise at-risk infants. It was isolated from powdered formular fed to infant (Nazarowe and Farber; 1997). In this study *Enterobacter sakazakii* was isolated from 3(15%) worker's hands samples and this may result in contamination of restaurant food with this potentially pathogenic bacteria.

In this investigation *Flavobacterium thalpophilum* was isolated from 2 hand samples (10%) this agrees with finding of Hansen *etal.* (1988) who isolated from hand wound. The source of contamination by this species may be infected wounds on hands of restaurants worker.

*Moraxella* species are the normal bacteria flora in human upper respiratory tract particularly the nasal cavity (Henriksen, 1969). These
species are also isolated from urinary tract and other local infections (Elston and Hoffman, 1966., Henriksen, 1973). In this study it was isolated from worker hand. This indicates possible contamination of restaurant food and environment with respiratory tract and urinary tract pathogens.

*Hafnia* species was isolated from food in this study, and it was also isolated by Hansen and Huss (1998) from spoiled food (old-smoked salmon), and from cheese by Perrone *et al.* (1999), and from water by Das and Mukherjee (1999), and from fresh water fishes and minced meat (Linedberg *et al.*, 1998). The possible source of contamination of cooked food by these organisms might be water use for washing dishes or food became contaminated when come in contact with uncooked contaminated food.

*Aeromonas salmonicida* is a fresh water organism that affects cold blood animals such as fish .It was isolated from malmonid fish by Ullmann *et al* (2005) and by Mary *et al* (2001). In this study *A.salmonicida* was isolated from hand of 2(10%) restaurant workers. The hand worker may become contaminated while, they were preparing fish for cooking.

In the present investigation *E. coli* was isolated from 13 dishes (60%). *E. coli* was isolated from raw uncooked food, from vegetable (Jay, 2000), from untreated water (Wagner and Jr., 2000) and from cooked
food. This bacterium was also isolated from stool (Knonman et al., 1997). The isolation of *E. coli* from 60% of dishes examined in this study indicates high faecal contamination and poor sanitation. *Kluyvera* species was isolated from human’s sputum, urine, stool, throat swabs and blood (Knoneman et al., 1997). In this study *Kluyvera* species was isolated from one hand (5%) sample and 4 dishes (20%). This shows that restaurant workers are one of source of contamination in these restaurants and poor health condition of restaurants staff.

*Klebsiella pneumoniae* causes chest infection and occasionally severe bronchopneumonia with lung abscesses (Cheesburgh, 2000). Filho et al (1985) and Paulson (1994) isolated from hand wound. Bailey (2002) isolated this genus among infectious agents of acute gastroenteritis due to contamination of raw food. In this investigation, *K. pneumoniae* was isolated from 4(20%) food samples and from 2(10%) worker’s hand. This indicates the respiratory tract, hand wound and digestive tract infection of restaurant worker may be possible source of contamination in restaurants surveyed in this study.

This high number of bacterial isolates reported in this investigation indicates high level contamination in the restaurant surveyed. In the private home where food it prepared for a small numbers of people food contamination was restricted. However food contamination in a restaurant kitchen for example, can result in large number of people being affected.
Bean et al. (1996) reported that restaurants were the most common places where foods contaminated.

The outbreaks of food borne disease are caused by food that are contaminated intrinsically or that became contaminated during processing, or preparation by many source of contamination, important one is workers with poor personal hygienic who come in contact with food through all stages of processing until food is finally eaten (Davis and Samul, 1997). This study confirms the restaurant workers and washed dishes could be serious source of contamination in restaurants in Khatoum state. In this study many contamination bacteria include *Staphylococcus capre, Bacillus cereus, Bacillus sphericus, Bacillus thringiensis, Bacillus mycoides, Acinetobacter coloaceticus, Moraxella spp, Pseudomonas fluorscens, Aeromonas salmonicida, Flavobacterium thalpophilum, Klebsiella pnenmonias ozaenae, kluyvera spp, Proteus mirabilis, Enterbacter cloacea and Enterobacter sakazakii* were isolated from worker hands. This revealed the worker staff is important source of infection.

The contaminated organisms may be of human origin from purulent discharges of an infected finger or eye, abscesses, acne or facial eruptions, nasopharyngeal secretions, or apparently normal skin (WSDH, 2002). The transmission of bacteria from person to person, from person to surface or from person to food or vice-versa (Jack and Marianne, 1999).
In the present investigation, pathogenic bacteria to human such as Staphylococcus, capre Bacillus cereus, Bacillus sphericus, Bacillus mycoides, Moraxella spp, Pseudomonas fluorscens, Flavobacterium thalpophilum, Klebsiella pnenmonias ozaenae, kluyvera, Proteus mirabilis and Enterobacter sakazakii.

Conclusion

- Bacterial contaminants were isolated from workers hand, food and dishes.
- Important pathogens such as Bacillus cereus and Proteus mirabilis were isolated from all type of sample examined
- The high isolation rate (45%) of *E. coli* from dishes indicates poor hygiene and sanitation

- Working staff is main source of contamination. Many bacterial pathogens such as *Moraxella spp, Flavobacterium thalpophilum, Klebsiella pneumoniae subsp ozaenae, Kluyvera species Enterobacter sakazakii, Staphylococcus lugdunesis, Staphylococcus capri* were isolated from worker hands, food and dishes.

- Washing water may be source of contamination in these restaurants as different important bacterial species such as *E. coli* and *Bacillus cereus* were isolated from washed dishes.

**RECOMMENDATIONS**

To control food contamination in type of restaurant survey in Khartoum state the following should be considered

1- Restaurants workers should be in good healthy condition and should have health certificate from medical authority.
2- Restaurants workers should be educated on proper hygiene and sanitation to be observed in restaurants. Also should be instructed on proper cleaning and washing techniques especially personal hygiene such as hand washing, clothes and over dressing clothes cleanliness so as to minimize contamination by working staff.

3- High risk food should be kept at temperatures low enough to inhibit the growth of microbial contamination.

4- Raw food and high risk food should be prepared in separate areas with separate equipment.

5- Wooden cutting boards should not be used for cutting raw meat, poultry or fish. Plastic boards are recommended for use because it is easier to clean.

6- After cooking the food should be serviced immediately and as soon as possible and should not be displayed after cooking in the windows. Also the cooked food should be kept in proper container protect from contamination.
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