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ISOLATION AND IDENTIFICATION OF BACTERIA FROM FOOD VENDORS AND SOME VEGETABLE AVAILABLE AT OGBETE MARKET ENUGU.

BY

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CERTIFICATION PAGE

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DEDICATION

This work is dedicated to Almighty God and my mother Mary, for protection and love throughout my four years in school and to my parent (Mr. & Mrs. Simeon Edeh) for considering my education a priority.

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A Marvelous thanks to almighty God for his wisdom, protection, favour, and blessing. And to my incomparable and treasured parent Mr. & Mrs. S.O.N Edeh who made my dream come true, also to my siblings Johnbosoco, Priscilla, Irene, Paul and Cynthia Edeh for their prayers and to my aunty Mrs. Rosemary Israel my friend Akueme Maximillian. I owe you all a lot.

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ABSTRACT

Safety of food is a basic requirement of food quality. A total of 25 street food samples (Jollof rice, egwusi soup, ugu, water leaf and green), were randomly purchased from five different vendors in Ogbete main market Enugu. The samples were transported in ice to the laboratory. The samples were bacteriologically analyzed using pour plate technique and sub-culture. Pour plate techniques was done by carrying out serial dilution of the sample after which the first tube and the last tube were picked and 1ml of each sample was pipette into a Nutrient agar, the plate was then incubated for 24 hours at 37^{o}C after which the plate were examined for growth. Sub culture was done using bacteriological agar. All the screened food samples had varying levels of bacterial growth ranging from 1.0×10^{5} to 3.0×10^{6} cfu/ml. ninety percent of the sampled foods had bacterial counts above the acceptable limits (10^{4} cfu/ml) and 10% of the samples had bacterial counts less than ($<10^{4}$ cfu/ml). Six bacterial species were isolated from the foods sampled. *Staphylococcus arueus, Bacillus cereus, Vibrio spp, Salmonella spp, Escherichia coli and Shigella spp*. More than one pathogenic micro organism were isolated from jollof rice and water leaf. The findings revealed that street foods are potential vehicles for transmitting food borne illnesses thus the need to develop practical strategies geared toward street food safety.

CHAPTER ONE

1.0 INTRODUCTION

Bacterial are group of microorganism all of which lack a distinct nuclear membrane (and hence are considered more primitive than animal and plant cells) and most of which have a cell wall of unique composition. Most bacterial are unicellular; the cells may be spherical (coccu) rod – shaped (bacillus), spiral (spirillum), comma – shaped (vibrio) or corkscrew-shaped (spierocheate). Generally, they range in size between 0.5 and 5um. (Elizabeth and Martin, 2003).

Food is any substance that people or animal eat or drink or that plants absorb to maintain life and growth. Food is any substance consumed for nutritional support for the body; it is usually of plant or animal origin. (Ezeronye, 2007). Food consists of chemical compounds which heterophilic living thing consumes in order to carry out metabolic processed. They are also substances which when introduced to the digestive system under normal circumstances contribute to growth, repair and production of energy.

(Ezeronye, 2007). Foods are classified into six essential nutrients known as protein, carbohydrate, vitamin mineral, fat and oil, water.

PROTEIN: - One of a group of organic compounds of carbon, hydrogen, oxygen and nitrogen (sulphur and phosphorus may also be present). The protein molecule is a complex structure made up of one or more chains of amino acid, which are linked by peptide bonds. Proteins are essential constituents of the body; they form the structural material of muscles, tissues, organs, etc. and are equally important as regulators of function, as enzymes and hormones, proteins are synthesized in the body from their constituent amino acids, which are obtained from the digestion of protein in the diet (Elizabeth and Martin, 2003).

CARBOHYDRATE: - One of a large group of compounds, including the sugar and starch, that contain carbon, hydrogen and oxygen and have the general formular C_X ($H_2$0) Y- Carbohydrates are important as a source of energy: they are manufactured by plants and obtained by animals from the diet, being one of the three main constituent of food. All carbohydrates are eventually broken down in the body to the simple sugar glucose which can

then take part in energy producing metabolic processes. Excess carbohydrate, not immediately required by the body is stored in the liver and muscles in the form of glycogen. In plants carbohydrate are important structural materials (e.g. cellulose and storage products (commonly in the form of starch). (Elizabeth and Martin, 2003).

VITAMIN:- Any of a group of substances that are required in very small amounts, for healthy growth and development: they cannot be synthesized by the body and are therefore essential constituents of the diet. Vitamins are divided into two groups, according to whether they are soluble in water or fat. The water soluble groups include the vitamin C; the fat soluble vitamins are vitamins A, D, E and K. Lack of sufficient quantities of any of the vitamins in the diet results in specific vitamin deficiency diseased (Elizabeth and Martin, 2003).

FAT:- A substance that contains one or more fatty acids (in the form of triglyceride) and is the principal form in which energy is stored by the body (in adipose tissue). It also serves as an insulating material beneath the skin (in the subcutaneous tissue) and around certain organs (including the

kidney). Fat is one of the three main constituents of food; it is necessary in the diet to provide an adequate supply of essential fatty acid and from the efficient absorption of fat –soluble vitamins from the intestine. Excessive deposition of fat in the body leads to obesity. (Elizabeth and Martin, 2003).

A vendor is a person selling something (en.Wikipedia org/Wiki/vendor). The world Health Organization (WHO) Indicated that food-borne diseases most of which are of microbial origin are perhaps the most widespread problems in the contemporary world and this is responsible for about one third of death world wide, through infectious conditions with adverse effects can reduce economic productivity. Poor sanitary condition in most of the local markets and the environment being highly polluted and charged with spoilage and pathogenic flora is likely the source of contamination of food items sold by such vendors. (Oweghe *et al.,* 2001). It is known that poor hygienic conditions in a food environment may encourage the multiplication of pathogenic organisms in food (Egeonu, 2002). It has been observed that *Bacillus cereus* and *Staphylococcus aureus* grow to oxygenic levels in food at 30^0c (Egeonu, 2003). Therefore microbiological examination of foods and food contact surfaces may provide

information concerning the quality of the raw food, and the sanitary conditions under which the food is processed (Michael *et al;* 2004). Microorganisms live throughout the kitchen and can easily move around by attaching themselves to people easily move around by attaching themselves to people, food and equipment. Bacteria may pass from equipment to food which has not been properly cleaned and sanitized before being used to prepare another food. Examination of food consumed and also wholesomeness. This implies that the food to be consumed by humans should be pure and free from contamination especially by pathogenic and spoilage micro organisms. Failure to ensure the safety and wholesomeness of the food consumed by the public might lead to some illness. To reduce contamination by microorganisms to a minimum level, and obtain good keeping quality of the products, the raw materials should regularly be monitored and examined.

Food contacts surfaces are a major concern for food service facilities in controlling the spread of food-borne pathogens, surfaces such as bench tops, table, etc. may have bacterial on them from contact with people, raw foods, dirty equipments or other things such as cartons that have been stored

on the floor. If the bench tops are not properly cleaned, any food on them will be contaminated by the bacterial (Kamil, 2005).

1.1 AIM

The overall aim of this work is to access selected foods sold by vendors in Ogbete main market Enugu for bacterial contamination.

OBJECTIVES

1. To isolate and identify bacterial species associated with food contamination.
2. To determine the microbial load of isolated bacteria.
3. To establish the public health implication of consumption of such foods.

CHAPTER TWO

2.1 LITERATURE REVIEW

Food borne illness caused by microbial contamination of foods in an important international public health problem and is known to be a major cause of diarrhea diseased especially in developing countries (Mensah, 1997). In these developing countries a major source of ready - to - eat foods are prepare and or sold at public places such as markets place, schools, canteens and along the streets, all together termed street foods (SFS). The SFS offer food at relatively cheaper cost and at easily accessible places. Furthermore, it offers the traditional meals and preparations of a number of them are quite laborious and time consuming. (Amoah, 1992; chakra Varky and Canet, 2002).

However, a number of observational studies have shown that these foods are sometimes held at improper temperature, excessively handle by food vendors and sold at very dirty surrounding (WHO, 2001, 2003; Ghosh *et al.*, 2007). In addition the vendors practice poor personal hygiene and

reports of food vendor being carriers and therefore could serve as a potential source of transmission of enteric fevers are many. Most of the vendors have had either no formal education or few years of schooling and therefore, lack knowledge on proper food handling and their role in the transmission of pathogens (Mensah *et al*.,1999). At the same time, most of the people who patronize these foods are more interested in its convenience than question of its bacteriological quality and hygiene. The bacteriological quality of food indicates the amount of bacterial contaminants it has; a high level of contamination indicates low quality and more likely to transmit infection. The concerns have been raised by the food and Agricultural organization (FAO) and other about these foods serving as a potential source of food poisoning outbreaks (Chakravarty and Canet, 2002).

In Nigeria, consumption of street food has witnessed a phenomenal growth over the years as rapid population growth over the years as rapid population growth, Urbanization, Unemployment and poverty; occupational pressures and lifestyles changes has created a poll of mobile and transient population who depend almost entirely on these relatively low cost foods for their nutrition. (Martin, 2006).

Although epidemiological data on the incidence of forborne diseases are inadequate, and the outbreak often not investigated, the recurrent episodes of food borne illnesses with symptoms of gastro intestinal distress like diarrhea, vomiting, abdominal cramp and nausea has remained a major cause of mortality and morbidity in Nigeria. (Nweze, 2010).

Chemicals heavily metals, parasites, fungi, viruses and bacteria can cause food – borne illness, bacteria related food poisoning is the most common, but fewer than 20 of the culprits. More than 90% of the cases of food poisoning each year are caused by *Staphylococcus aureus*,

.*Salmonella*, *Clostridium perfringes*, *Clostridium botulinum*, *Campylobacter*, *vibro parahaemolyticus*, *Bacillus cerus* and Entropathogenic *Escherichia coli*. These bacteria are commonly found on many raw foods. Normally a large number of food – poisoning bacteria must be presented to cause illness, therefore illness can be prevented controlling number of bacteria present ,by preventing the small number from growing, destroying the bacteria by proper cooking and avoiding re contamination (De Boer and Beuner,2011).

Poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensil course contamination of raw and cooked foods. Mishandling of raw and cooked foods allows bacteria to grow. The temperature range in which most bacteria grow is between 40 degrees (5^0c) and 140^0f (60^0c). Raw and cooked foods should not be kept in this danger zone any longer than absolutely necessary.

Analyzing foods for the presence of both pathogenic and spoilage bacteria is a standard way of enduring food safety and quality (De Boer and Beuner, 2011). If micro organisms are able to survive and grow on food which are sold and consumed by people, then the risk of food borne – illness is increased in the society. The presence of micro organisms on food can be important, because the essential nutrients of the food are ingested by some organisms stimulate growth, while some organisms are known to be pathogenic to man as long as their growth conditions are favorable (De Boer Beuner, 2011).

Bacteria may pass from equipment to food when the equipment that has touched the food has not been properly cleaned and sanitized before being

used tom prepare another food (James, 2005). Food eaten has direct influence on health; it is manufactures and food handlers to keep food safe from pathogenic micro organisms, especially when such foods are to be consumed without further processing (Munide and Kuria, 2005) when a food with harmful bacteria is ingested there is a period of time before symptoms of the food – borne illness begin. The amount of times varies with the different bacteria, how many consumed and the individual's physical condition. Many different harmful organisms produce the same symptoms. (FDA, 2004).

2.2 FOOD HYGIENE

Food hygiene is defined as a sanitary science which aims at producing food which is safe for human consumption and of good keeping quality and this includes any sanitation measures designed to prevent bacteria and other micro organisms of human origin from reaching food stuff (Umoh and Odibo, 1999). Food hygiene is a subject of wide scope, it aims at studying methods for production and preparation of food, which is safe and of good quality. It covers not only the proper handling of every variety of food stuff

and drinks, but also food contact surfaces such as utensils, and apparatus used in the preparation, services and consumption of the food and also the care to prevent contamination with food poisoning bacteria which may originate from the animal or part plant host supplying the food (Umoh and Odibo, 1999).

2.3 FACTORS THAT CONTRIBUTE TO FOOD –BORNE ILLNESS

They are, improper cooling of foods, time between preparing and serving, poor personal hygiene, not cooking food properly, Abuse of the time temperature relationship, cross contaminating raw and cooked foods.

Poor Personal Hygiene

Poor personal hygiene can result in food contamination for example when a food personnel, fails to wash hands properly after using the restroom, toilet, is a serious risk of faecal contamination (FDA, 2004). Everyone has bacteria on the skin, mouth, hands and so many other organisms on various parts of the body like hair. Food service personnel can contaminate food and cause food-borne illness. Food workers may transmit pathogens to food from a contaminated surface, from one food to another

food or from hands contaminated with organisms from the gastrointestinal tracts (Munide and Kuria, 2005). Therefore, hand contact with ready – to – eat food i.e. food that is edible with out washing, cooking or additional preparation by the consumer pr by the food establishment and that is expected to be consumed in that manner, represents a potentially important mechanisms by which pathogens may enter the food supply. (Munide and Kuria, 2005).

Abuse Of The Time –Temperature Relationship

Abuse of time temperature relationship is also another factor that can cause food-borne illnesses. To prevent food-borne illness, it is important to control the time that food is in the temperature danger zone. This means hot foods should be kept at 140^0F or above and cold foods at 41^0For below (FDA, 2004). Don't let cooked or refrigerated foods, such as salads, sat at room temperature for more than two hours (FDA, 2004). Time temperature relationship problems occur because

1. Food is not stored, prepared or held at the required temperature;

food is not cooked or reheated to temperature high enough to kill harmful micro organisms

2. Food is prepared in advance of service and proper temperature control is not maintained (FDA, 2004).

Cross-Contaminating Raw And Cooked Food

Cross-contaminating raw and cooked food is transferring of harmful micro organisms from a surface to food or from one food to another food. cross contamination can occur when food contact surfaces is not cleaned or sanitized as necessary for food safety (FDA, 2004). To prevent cross – contamination, it is important to wash hands with soap and warm water before you start preparing food, before you handle a different food (for example, if you just handled raw chicken, wash hands before preparing a salad), and after using the bathroom. Don't sneeze or cough on food. Organisms can "travel" from raw to cooked food, so never let raw food touch cooked food. (FAD, 2004).

Persons At High Risk For Food-Borne Disease Are:

Infants and very young children

The elderly

Pregnant women

Individuals with weakened immune system like HIV, AIDS, liver disease or cancer. (FDA, 2004).

2.4 FEATURES OF COMMON FOOD –BORNE BACTERIA PATHOGENS

Salmonella species

***Salmonella*: -** is a generic name applied to a group of nearly 2,000 biochemical related serotypes responsible for food –borne illness. The disease is grossly underreported because it is generally self- limiting gastroenteritis which may be misdiagnosed as intestinal influenza by patient or the physician. As a consequence, estimates of the true incidence of disease are based as assumptions derived from epidemiological evidence. Clearly, salmonellosis continues to be an important cause of food-borne disease worldwide.

Two clinical manifestations caused by *Salmonella* are recognized:

enteric fever (a severe, life threatening illness) and the more common food-borne illness syndrome: In both cased, the oral route.

Enteric fever, commonly referred to a typhoid fever, is primarily caused by one species, *Salmonella typhi*. But other *Salmonellae* such as *Salmonella paratyphi*_are potentially capable of producing this syndrome. The illness is commonly associated with foreign travel and affects an estimated 800 people annually (Mead et al; 1999). Although the route of entry of the pathogen into the body is primarily oral, the symptoms of enteric fever are generally not elicited through the intestinal tract. However, a short episode of vomiting and diarrhea sometimes occurs in the first day or two in typhoid fever. The onset times vary considerably between typhoid and paratyphoid enteric fevers. Onset time for typhoid is usually 8 – 15 days, seldom as short as five days but sometimes as long as 30 – 35 days; while onset time for paratyphoid fever tends to be shorter, and may be so short as to suggest typical food poisoning. (Parker, 1984). *Salmonella* are destroyed at cooking contamination of cooked foods occurs from contact with utensils that were not properly washed after use with raw products. If *salmonella* is presented in raw or cooked foods, its growth can be controlled by

refrigeration below 40^0F. There are various environmental sources that include water, soil, kitchen surfaces and animal faeces that helps in the transmission, Salmonella are transmitted through the faecal matter of people or animals and are usually transmitted to humans by eating foods that have been contaminated with faecal matter via cross-contaminations. As few as 15 to 20 cells depending on the age and health of the host and strain of bacterial are necessary to cause illness (FDA, 2004). It is estimated that approximately 40,000 cases of *Salmonelosis* are reported each year in the U.S.A. (FDA, 2004).

Staphylococcus aureus

Man's respiratory passage, skins and superficial wounds are common sources of *staphylococcus aureus*. When *Staphylococcus aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although cooking destroys the bacteria, the toxin produce is heat stable and may not be destroyed. Staphylococcal food poisoning occurs most often in foods that require hard preparation. Sometimes these types of foods are left at room temperature for periods of time, allowing the bacteria to grow and produce toxin. Good personal hygiene when handling foods will keep

Staphylococcus aureus out of foods and refrigeration of raw and cooked foods will prevent the growth of these bacteria if any is present (Wagner, 2001)

Shigella

Shigellosis, or bacillary dysentery, as it is commonly known, is caused by bacteria of the genus *shigella*¸ which include *Shigella dysenteriae, shigella flexneri, shigella boydii* and *shigella*. *Sonnei* (Bryan, 1979). The normal habitat for shigella is the intestinal tract of humans and other primates. Primarily mode of transmission appears to be person to-person by the fecal-oral route (Feldman and Riley, 1985). *Shigella* is mostly associated with chicken, raw vegetables, dairy products and poultry. Contamination of these foods is usually through the feacal-Oral route and is most commonly due to faecally contaminated water and unsanitary handling by food handlers (Todar, 2006). As few as 10 cells, depending on the age and body condition of the host are necessary to cause disease. As with *Escherichia*. *coli, Shigella*, are present in the diarrhea stool of infected person and can be transmitted during infection as well as one to two weeks after symptom subsides, most infections that occur are the

result of the bacterium passing from stools or of soiled fingers of one person to the mouth or finger of another person. *Shigella dysenteriae* type cause deadly epidemics in developing countries (CDC – DBM D, 2004). *Shigella* are transmitted through the faecal matter of people or animals and are usually transmitted to humans by eating foods that have been contaminated with faecal matter through cross contamination. As few as 15 – 20 cells, depending on the age and health of the host and strain of bacteria are necessary to cause food-borne illness. Generally, food-borne shigellosis is characterized by a high attack rate, common-source epidemiology, and short incubation periods of 12 – 50 hours (FDA/CFSA N, 2003b).

Enteropathogenic – *Escherichia coli*

A lactose – fermenting species is usually not harmful but some strains cause gastrointestinal infections. Ingestion of the pathogenic serotype *E. coli* 0157 derived from infected meat. Causes colitis with bloody diarrhea, which may give rise to the complications of hemolytic uraemic syndrome (Elizabeth and Martin, 2003). *E. coli* is a significant cause of diarrhea in developing

countries and localities of poor sanitation. Indeed it has been associated with "traveler diarrhea". However, the latest out break in North America occurred in a nursing home in Ontario. There are at least four sub-group of enteropathogenic. *Escherichia coli*, *Enterolnvasive*, *Haemorrhagie* and *Enteropathogenic*. Each strain has different characteristics, the major source of the bacteria in the environment is probably the faces of infected human but there may also be animal reservoirs and untreated water are the most likely sources for contamination of food. *E. coli* 0157: H7 and its link to food become well known to the public as a result of the 1993 *E. coli* 0157: H 7 outbreak caused by contaminated hamburgers. Over 700 people become ill from this outbreak and four children died (Buzby, 2001). *E. coli* 0157: H7 maybe acquired through consumption of meat that has not been sufficiently cooked, and person – to person transmission can occur via the faecal oral route *E. coli* 0157: H7 can be found in the diarrhea stool of infected persons. The pathogens can be spread if personal hygiene and hand washing procedures are inadequate. (Buzby, 2001).

SOME FOOD-BORNE BACTERIA AND AFFILIATED FOODS

TABLE:1

	MICROBE	**AFFLIATED FOOD**	**DISEASE**	**SYMPTOMS**
1	*Bacillus Cereus*	Meats, milk, Rice, potato, And cheese products	*B. cereus* Food poisoning	Diarrhea, Abdominal cramps, Nausea.
2	*Campylobacter Jeini*	Raw Chicken Unpasteurized Milk, Non-Chlorinated Water	*Campylobacteriosis*	Diarrhea, abdominal cramps, nausea and fever, Headache and muscle pain
3	*Clostridium botilinum*	Canned foods including vegetables meats and soups.	Food-borne Botulism	Weakness, double vision, and vertigo, difficulty in speaking, swallowing and breathing, constipation.
4	*Clostridium*	Non-refrigerated prepared foods meats	Perfringens food poisoning	Severe Abdominal

	perfringens	and meat products, Gravy		Cramps Diarrhea
5	*Escherichia coli*	Undercooked meals, Raw Ground Beef	Hemorrhagic colitis	Severe Abdominal pain watery and bloody Diarrhea, Vomiting.
6	*Salmonella species*	Poultry and Eggs milk and Dairy products, Raw meats, fish shrimp, peanut Butter	*salmonellosis*	Diarrhea Abdominal pain, fever, Headache Diarrhea. Vomiting, Blood or mucus in stool.
7	*Shigella* species	Poultry milk and Dairy products, Raw vegetables fecally contaminated water, salads: potato, chicken, Tuna, shrimp.	*Shigellosis*	Diarrhea, Abdomial pain, fever, vomiting, Blood or mucus in stool.
8	*Staphylococcus*	Potato, salads and	Staphyloenterotoxic	Abdominal

	aureus	sandwich, poultry and egg products, meat products, Dairy products.	osis Staphyloenterptoxemia	Cramping, Nausea and vomiting prostration
9	*Vibrio* cholerae	Contaminated water,, shell. Fish.	Cholera	Watery Diarrhea, Abdominal pain, Dehydration, vomiting, shock.

Source: bad bug book.

2.5 FOODBORNE DISEASE

The centers for Disease control and prevention (CDC) estimates that approximately 76 million people within the united states contact food-borne illness each year (CDC, 2004). Of those 76 million cases, 325,000 hospitalization and 5,000 deaths results. The CDC preliminary food net data reports that in 2005, there were 15,600 diagnosed cases of food-borne illness caused by bacteria pathogens and of these total 6,017 cases were attributed to *salmonella,* 5,215 *to campylobacter,* 3,021 *to shigella* 443 to *Escherichia coli*. (CDC, 2004). Most food-borne illness are classified as “acute” they are

usually self-limiting and of short duration with symptoms including wild gastro-enteritis. However some illness progress to life threatening neurological or renal syndromes called sequelae. Harmful micro organisms may contaminate food during receiving, during preparation and serving, during preparation techniques such as cooking and cooling, by cross-contamination of raw meat poultry or eggs with other foods from employees to food by unwashed hands, coughing or sneezing, from unsanized facilities and equipment, from disease spreading pest such as cockroaches, flies and mice (CDC, 2004).

2.6 PRE-DISPOSING FACTORS TO FOOD-BORNE ILLNESSES

The CDC has identified improper hand washing, sanitizing as some of the major contributing factors to the spread of food-borne illness. Therefore, it is necessary to take the proper steps to ensure that these improper practices are steps to ensure that these improper practices are avoided at all times (NRAEF, 2005).

Improper Hand-Washing

Hand-washing has long been known to be beneficial public health practice for preventing the spread of infectious diseases. According to the CDC, hand-washing is the single most important procedure for preventing the spread of infection. Bacteria, such as the food-borne pathogen *Staphylococcus aureus*, are found naturally on the human body and apparently healthy people may host food-borne pathogens, such as *salmonella*. These people may be "carries" and are capable of infecting others, yet they may not be aware that they are carries because they may not show symptoms or become ill themselves. Therefore, it is necessary to utilized proper hand- washing techniques after coughing sneezing and blowing the nose. Failure to use proper hand techniques increases the risk of transmission of food-borne illness. The Association for professionals in infection control and Epidemiology (APIC) states that hand-washing" causes a significant reduction in the carriage of potential pathogens on the hands and recommends several steps for proper hand-washing to prevent the spread of pathogens. During the hand-washing procedure, failure to cover all surfaces on the hands because of poor techniques or use of insufficient

cleaning agents may lead to subsequent contamination of surfaces. (CDC, 2004).

2.7 PREVENTION OF FOOD-BORNE ILLNESSES

Most cases of food-borne illnesses can be prevented through proper cooking or processing of food, which kills bacteria. In addition, because bacteria multiply rapidly between 40^0F and 140^0F, food must be kept out of this temperature range.

Refrigerate foods promptly. If prepared food stands at room temperature for more than 2 hours, it may not be safe to eat. Set your refrigerator at 40^0F or lower and your freezer at 0^0c

Cook food to the approximate interval temperature 145^0F for roasts, steaks, and chops of beef, veal and lamb, 160^0F for pork, ground veal, and ground beef, 160^0F for ground poultry; and chops of beef, veal and ground beef, 165^0F for ground; and 180^oF for whole poultry. Use a meat thermometer to be sure foods are properly cooked only when they are heated long enough and at a high enough temperature to kill the harmful bacteria that cause illnesses. Handle food properly. Always wash your hands for t least 20 seconds with warm, soapy water before and after handling raw

meat, poultry, fish shellfish, produce or changing diapers, or touching animals.

Wash utensils and surfaces before and after use with hot, soapy water. Better still; sanitize them with diluted bleach-teaspoons of bleach to 1quar of hot water. (CDC, 2004).

CHAPTER THREE

MATERIALS AND METHOD

3.1.1 HARD WARE

The following hardware materials wee used for this research.

i Electric thermostatic incubator (DNP.9022-1A)

ii Autoclave (Yx-280A)

iii Microscope (XSZ-107BN)

iv Refrigerator (FR-330)

v Electronic scale

3.1.2 SOFT WARE

The following software materials were used for this research.

i Nutrient Agar

ii macConkey Agar

iii *Salmonella, shigella* Agar (SSA)

iv Kovac's reagent

v Crystal violet stain

vi Acetone

vii Safranin

viii Hydrogen peroxide

Study Sites

The study was conducted between June – August 5 (five) ready to eat food vending sites in ogbete main market were sampled. These vending sites were chosen because they are very popular among those who patronize such eating places.

Sample Collection

A bacteriological survey was conducted in different vending sites at Ogbete main market Enugu State.

Five jollof rice plates, 5 Egwusi soup plates and five vegetable were purchased from various, vendors in Ogbete main market Enugu and studied to determine their level of bacteria contamination and safety for human consumption.

METHOD

All media used were weighed appropriate and prepare according to Manufactures instruction. They were autoclaved at 121^0C for 15 minutes. The cooled were poured into Petri dishes and then allowed to cool and solidify (see Appendix 1).

i. A clean sterile covered plates were plates were used to dish the foods.

ii. Sterile polythene bags were used to collect and transport the purchased samples on ice to prevent bacteria multiplication during sample transportation to the department's laboratory were the analysis was done.

iii. 10g portion of each food sample were macerated.

iv. 9ml of sterile distilled water was poured into test tube.

v. 1ml of each macerated sample was added into the test tube containing 9ml of sterile distilled water.

vi. Fourthly fold serial dilutions were made from 10^{-1} to 10^{40} was examined by means of the pour plate method.

Culture Of Sample (Pour Plate)

i. Briefly each plate was carefully labeled on top and one militer (1ml) of each dilution from $10^{-1} – 10^{40}$ were pipetted into nutrient agar plates.

ii. Shaking of these plates were done as soon as the agar were poured, so as to have the micro organisms separated during growth.

iii. The medium was allowed to set on a flat top bench after which plates were incubated aerobically and anaerobically at 37^0c for 24hours.

Sub-Culturing Of The Culture

i. The colonies were sub-culturing the culture, then in fresh nutrient agar, macConkey agar and *salmonella shigella* agar plates.

ii. The plates were incubated aerobically and anaerobically at 37^0c for 24 hours.

IDENTIFICATION OF VARIOUS ISOLATES OBTAINED IN THE CULTURES

The following biochemical test were carried out for the characterization and identification of the organisms.

i. Gram's stain
ii. Catalase test
iii. Coagulase test
iv. Citrate utilization test
v. Indole test
vi. Oxidase test
vii. Voges prokauer test
viii. Methyl red test
ix. Motility test

Gram's Staining

These tests were done according to (Nester et al; 2007). The Gram stain is by far the most widely used procedure for staining bacteria and separating it into two major groups: Gram (+) positive and Gram (-) negative.

Spread thin film of specimen over a clean grease free slide and allow to air dry. Fix it by passing it over a Bunsen flame thrice. Flood the film with crystal violet and leave for 60 seconds. To the slide wash off and flood the stain with lugol's iodine and (mordant) and leave for 60 seconds. Wash off iodine and decolorized the slide with acetone (decolourizer) for a second, wash the slide and train with safranin (counter stain) for 60 seconds and wash off. Then dry the back of the

slide and air dry. Examine with the oil immersion, x 100 lens. A purple colour signifies Gram (+) positive while the colour of the safranin which is red signifies Gram (-) Negative.

Catalase Test

This test was done according to monica cheese Brough. (2005). The test was performed by dropping a loopful of the isolate mix with the hydrogen peroxide on the slide. The production of gas bubbles (0_2) from the mixture which will occur almost immediately is a positive reaction.

$$2H_20_2 \longrightarrow 2H_20 + 0_2$$

Methyl Red Test

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usually used as an aid in the identification and differentiation of the *Enterobacteriaceae* This test was performed according to Monica cheese Brough (2005). Inoculate the suspected organism into a sterile buffered glucose- peptone broth and incubate at 37^0c for 24 hours. After 24 hours add five drops of methyl red indicator and shake the mixture and observed. A bright red colour is a positive result.

Methyl red test indicator consist of

0.1g methyl Red

300ml of 95% ethyl alcohol.

Citrate Utilization Test

This test was done according to Monica cheese Brough (2005). The test was used to identify which of the isolates can utilize citrate as the sole sources of carbon for metabolism. The test is usually used as an aid in the differentiation of organisms in the *Enterobacteriacea* group. Inoculate simmon's citrate medium in sterile test tubes with a loopful of culture. Incubate tube at 37^0c for 24 hours. A colour change from green to blue is a positive result. The absence of any growth as well as no change in the colour indicates a negative reaction.

Oxidase Test

This test was done by dropping 2 – 5 drops of a freshly prepared oxidase (p-aminodimethylanine) reagent on a filter paper, the suspected organisms is picked using a sterile wire loop and mix with the oxidase reagent. A change from the normal colour to deep purple means a positive result, while no change means negative.

Vogas Proskaeur Test

This test was used to detect which of the isolates were able to produce a neutral red end point acetyl methyl carbinol (acetion) from glucose fermentation or its reductive product butylenes glycerol. The test is usually used to differentiate between Gram negative organisms especially members of the *Enterobacteriaceae*. Monica cheese Brough (2005). Inoculate the suspected organism into a test tube containing buffered glucose peptone water and incubate at 37^{o}c for 24 hours. Into the incubated medium, add 0.6% $^{w/}_{v}$ solution of A and 0.2ml of solution B Shake the mixture and live to stand. A red colour is a positive result. While the development of a yellow colour indicates a negative reaction. Solution A Contains 5g of -naphlho100ml absolute ethyl alcohol Solution B contains100ml Distilled water 40g potassium hydroxide.

The alkalis oxidize the acetyl methyl carbonyl (acetone) to diacetyl which gives the pink colour.

Coagulate Test

This test was done according to Monica Cheese brough, (2005) to differentiate *staphylococcus aureus* and other *staphylococcus* species .Add

2 - 3 drops of normal saline on a grease free slide to the normal saline mix the suspected organism and add 1 – 2 drops of plasma and Rock, the presence of agglutination means a positive result while no agglutination means a negative results.

Indole Test.

This test is done according to Ochei and Kolhather (2001) to differentiate members of enterobacteriacea, *Escherichia coli* is indole positive and only some *shigella* strain are indole positive.

- The test organism was inoculated in a test tube containing 3ml of sterile trytone water.
- Incubation was done at 37^{o}c for 24hrs
- The test for indole was done by adding 0.5ml of kovac's reagent and shaken gently.
- Examination for a red colour in the surface of the layer within 10minutes means positive, while no colour change means negative.

Motility Test

This test is to identify members of vibranaceae and must members of the enterobacteriaceae which are also motile.

- The mobility medium was inoculated using a needle to make 5 stabs of the test organism to the depth of 1-2cm of the bottom of the tube.
- The tube was incubated at 37^{o}c for 24hrs
-The line of incubation was examined for cloudiness showing the organisms is motile (Monica cheese brough. 2005).

CHAPTER FOUR

RESULT

In this investigation, a total of 25street food samples were examined for bacterial contamination. Results showed that all the street food samples were contaminated with varying level of bacterial counts. The results obtained are shown in table II

TABLE II

Morphological features of bacteria isolated from the different food samples.

Media	**samples**	**morphology of bacteria Colonies**
NA	Jollof Rice	Whitish colony with rough edges
	Jollof Rice	Circular, smooth, raised and deep golden yellow colonies
	Egwusi soup	smooth round colonies that are opaque
MAC	Jollof Rice	Non –lactose fermenter
	Jollof Rice	Non- lactose fermenter
	Egwusi Soup	Non –lactose fermenter
SSA	Jollof Rice	No growth
	Jollof Rice	No growth
	Egwusi Soup	No growth

Vegetable

media	sample	morphology of bacterial colonies
NA	ugu	milky colour, circular with smooth colonies and distinct edges.
	Water leaf	round, smooth, raised and deep golden yellow colonies
	Water leaf	milky in colour circular with smooth colonies and distinct edges
	Green (spinach)	creamy in colour circular with smooth colonies and distinct edges
MAC	ugu	lactose fermenter and smooth colonies with distinct edges
	Water Leaf	Non- lactose fermenter
	Water Leaf	Non–lactose fermenter, flat and smooth colonies
	Green (spinach)	Non–Lactose fermenter, flat and smooth colonies
SSA	Ugu	No growth
	Water Leaf	No growth
	Water leaf	circular milky in colour flat, smooth colonies and distinct edges

	Green (spinach)	milky in colour, circular, flat, smooth colonies and distinct edges.

Key:

NA - Nutrient agar

MAC - MacConkey agar

SSA - *salmonella, Shigella* agar

TABLE III

Total bacteria count (CFU/ML) of street vended foods samples.

No. of colonies	Dilution	Bacterial Counts	Food samples
290	10^{-4}	2.9×10^{6}	Jollof Rice
250	10^{-1}	2.5×10^{3}	Jollof Rice
100	10^{-3}	1.0×10^{5}	Egwusi Soup
300	10^{-4}	3.0×10^{6}	Ugu
240	10^{-4}	2.4×10^{6}	Water Leaf
290	10^{-5}	2.9×10^{7}	Water leaf
290	10^{-4}	2.9×10^{6}	Green (Spinach)

TABLE IV

BIOCHEMICAL TEST

Food samples	gram reaction	Cat	Coa	Ind	Cit	Mr	Vp	Ox	Mt
Jollof rice	+ rod in chains	+	NA	-	-	+	-	+	-
Jollof Rice	+ cocci in cluster	+	+	-	-	-	-	-	-
Egwusi Soup	- rod and curved	-	NA	-	-	-	-	+	+
Ugu	- rod	+	NA	+	-	+	-	-	+
Water leaf	+ cocci in clusters	+	+	-	-	-	-	-	-
Water Leaf	- rod	+	NA	-	-	+	-	-	+
Green (Spinach)	-rod	+	NA	-	-	+	-	-	-

Key:

CAT -	Catalase test	MR-	Methyl red test
COA -	Coagulase test	VP -	Vogas Proskaeur test
IND -	Indole test	OX -	Oxidase test
CIT -	Citrate test	MT-	Motility test
+	Positive	NA -	Not Applicable
_	Negative		

TABLE V

Food samples with their associated bacteria

Food samples	**associated bacteria (Contaminants)**
Jollof Rice	*Bacillus, Cereus, staphylococcus aureus*
Egwusi soup	*Vibrio spp*
Ugu	*Escherichia coli*
Water Leaf	*Staphylococcus aureus, Salmonella spp*
Green (Spinach)	*Shigella Spp*

Key:

Spp –Species.

CHAPTER FIVE

5.1 Discussion

Gastroenteritis has remained a major health care problem in Nigeria both in terms of human suffering and food-borne illness. The isolation of bacterial in all the food samples (n-25). Jollof rice, Egwusi soup and vegetable from different vendors in obgbete main market Enugu indicated that the frequency of *Salmonella*_spp was more significant in water Leaf (2.9×10^7 CFU/ML) and not significant in jollof rice (2.5×10^3 CFU/ML). The unacceptable total bacterial count of $>10^4$ CFU/ML of screened food samples implies extreme contamination and potential health risk of these street food samples. The high incidence of bacterial contamination encountered in this study were mainly due to the largely unhygienic nature of the food preparations and services areas of foods are good indicators of the state of environment in which they are prepared or served. Majority of the street food centers are located beside waste disposal points and duty roads. Furthermore lack of running waster, sewage disposal infrastructure, inappropriate storage conditions and the presentation of these food in the open encouraged multiple contaminations. Results showed that isolates gotten from Jollof rice

gave the lowest colony forming unit/ml. The significant or unacceptable colony forming unit was from *salmonella*_spp, isolated in water leaf, are major causes of food borne gastroenteritis and typhoid fever, *Bacillus cereus* was isolated in jollof rice, *Vibrio* species were also isolated from soup sample, this may be a result of cross-contamination either from the raw vegetable or water used in the soup preparation. *Shigella spp* was isolated from green (Spinach) this may be as a result of feacal contamination from the manure used. *Escherichia coli.*_Was isolated in ugu is responsible for the high prevalence of diarrhea, fever, nausea, and cramps in children and adult exposed to contaminated food. *Staphylococcus aureus* isolated from Jollof rice and water leaf, is a pointer to largely poor personal hygiene, improper storage facilities, use of low quality raw materials and unhygienic environment. The use of the so called food thermo flask to store food before sales are contributed to the proliferation of the bacteria and consequently the high level of microbial count recorded in the study as these device hold foods at bacterial growth temperatures.

5.2 Conclusion

Street food business has remained largely unregulated in Nigeria, not withstanding the sector contribution to the nation's food security. Wholesome and nutritious street foods have a positive impact on food security, while consumption of street foods of low and below minimum safety standard is injurious to health on an acute or chronic basis. The findings of this study illustrates that bacterial contamination is present in Jollof rice, egwusi soup and vegetable sold in Ogbete main market Enugu. And that the CFU/ML of *Salmonella spp* is high, since it is more significant (2.9 X 10^7Cfu/ml) and can cause food poisoning. Other organisms isolated such as *coli, Shigella, staphylococcus. aureus, Bacillus cereus* and *Vibrio spp* which were also isolated in insignificant number could still cause food borne illness depending on the consumer's health status. *Staphylococcus aureus* was less significant in jollof rice. Therefore, it is very important and necessary for food vendors to always clean and sanitize food contact surfaces, cook and store food properly, so as to reduce the level of food contamination and also to reduce bacterial load to the lowest level, thereby preventing cases of food borne infections. Results also indicated that factors

such as the vendors itself (e.g personal cleanness etc), the type of food, have an effect on the bacterial contamination present in foods.

5.3 Recommendations

Cleaning and preventing cross –contamination are both essential in making sure that the food served is safe to eat. Effective cleaning gets rid of bacteria on hands, equipment and surface, which helps to stop harmful organisms from spreading into food.

Vendors should make sure that

- They clean food surface and equipment, especially after handling raw foods
- They Keep raw and already to eat foods separate
- They cook food properly and store food in a safe place away from insects
- They wash utensils and surfaces before and after use with hot, soapy water. Better still; sanitize them with diluted bleach 1 tea spoon of bleach to 1 quart of hot water.

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APPENDIX 1

Preparation of Media

Nutrient agar

-4.8g was weighed properly and poured into a sterile conical flask

-200ml of water was added and swirled to dissolve.

-the medium was autoclave of 121^0c for 15minutes

-The medium was allowed to solidify on the media on the laboratory bench before use.

MacConkey agar

-5.6g was weighed and poured into a sterile conical flask.

-200ml of water was added and swirled to dissolve

-The medium was autoclave at 121^0c for 15minutes

-The medium was allowed for some seconds before being poured aseptically into a sterile Petri dish.

-The medium was allowed to solidify on the media on the laboratory bench before use.

Salmonella, Shigella agar

-2.4g was weighed properly and poured into a sterile conical flask

-100ml of water was added and swirled to dissolve

-The medium was autoclave at 121^0c for 15minutes

-The medium was allowed for some seconds before being poured aspetically into a sterile Petri dish

-The medium was allowed to solidify on the media on the laboratory bench before use.

APENDIX II

Determination of number of bacteria colony forming unit per cfu/ml. using pour plate technique

For Jollof rice (Nutrient agar)

Bacillus cereus total number of bacteria count =290

The dilution used $= 10^4$

Total number of bacteria count X

The dilution used = 2900000

Approximate to one decimal point $=2.9X10^6$cfu/ml

For Jollof Rice (Nutrient agar)

Staphylococcus aureus = total number of bacteria count =250

The dilution used $=10^1$

Total number of bacteria count X the dilution used = 2500

Approximate to one decimal point $=2.5X10^3$ cfu/ml

For Egwusi soup (Nutrient agar)

Vibro spp = total number of bacteria count =100

The dilution used $= 10^3$

Total number of bacteria count X the dilution used =1000000

Approximate to one decimal point $=1.0X10^5$cfu/ml

For ugu (Nutrient agar)

Escherichia coli =total number of bacteria count = 300

The dilution used $=10^4$

The number of bacteria count X the dilution used =3000000

Approximate to one decimal point $=3.0X10^6$cfu/ml

For water leaf (Nutrient agar)

Staphylococcus aureus = total number of bacteria count =240

The dilution used $=10^4$

Total number of bacteria count X the dilution used =2400000

Approximate to one decimal point $=2.4X10^6$cfu/ml

For water leaf (Nutrient agar)

Salmonella spp =Total number of bacteria count = 90

The dilution used $= 10^5$

Total number of bacteria count X the dilution used =29000000

Approximate to one decimal point =2.9X10cfu/ml

For Green (Spinach) (Nutrient agar)

Shigella spp =Total number of bacteria count = 290

The dilution used $= 10^4$

Total number of bacteria count X the dilution used =2900000

Approximate to one decimal point $=2.9X10^6$cful/ml

Interpretation of result in terms of significant, less significant and not significant

Less than 10^4 cfu/ml = not significant

Equal to 10^4 cfu/ml = less significant

above 10^4 cfu/ml = significant