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Examination of Jollof Rice Served in Some Restaurants in Bonny Island for Contamination with *Salmonella Typhii* and *Staphylococcus Aureus*

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ABSTRACT

Jollof-rice sold in some restaurants in Bonny Island, Rivers State was examined for contamination by *Salmonella typhii* and *Staphylococcus aureus*. The microbial load (Total Heterotrophic Bacteria) in the rice was determined using the plate-count method. *Salmonella typhii* was isolated and characterized using Salmonella/Shigella Agar (SSA). *Staphylococcus aureus* was isolated and characterized using manitol salt agar. The microbial load of the Jollof- rice samples studied ranged from1.3 x 10^4 cfu/g to 1.0 x 10^3 cfu/g. Twenty-seven (27) isolates were recovered in the study, composed of 12 and 15 each of *Salmonella typhii* and *Staphylococcus aureus*. Due to the presence of these organisms in Jollof rice sold in restaurant in Bonny Island. The Jollof- rice is unsafe for consumption. Hence, for safety of consumers, a good hygienic condition should be maintained at the restaurants and food handlers who are carriers, should be trained in the area of good hygiene practices.

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CHAPTER ONE
INTRODUCTION

➢ *Background to the Study*

- *Rice*

Rice is the seed of the monocot plant of the genus *Oryza* and of the grass family Poaceae (formally Graminae) which includes twenty wild species and two cultivated ones, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (Ajala and Gana, 2015). Rice is grown in all the ecological dietary zones and tropical regions of Nigeria with different varieties possessing adaptative traits for each ecology (Oluwafemi and Simisaye, 2005). The two commonly cultivated varieties of rice in Nigeria are *Oryza saliva* and *Oryza glaberrima* (Ajala and Gana, 2015) mainly cultivated in Abakiliki, South-East, Nigeria (African rice).

Rice is one of the most important cereals in human nutrition, consumed by about 75% of the global human population including the Bonny Kingdom in Rives State, Nigeria. .Among the cereals, rice and wheat share equal importance as leading food sources for human (Oluwafemi and Simisaye, 2005). Rice food is consumed by nearly one-third of the world's population (Oluwafemi and Simisaye, 2005). Thus, Rice meal has been considered the best staple food among all cereals for over 3 billion people constituting over half of the world's population (Oluwafemi and Simisaye, 2005). Rice meal is the most common served meal in most family dining tables and eateries (Ihekononye and Ngoddy (1979).

Rice constitutes minerals such as calcium, magnesium, phosphorus are present along with some traces of iron, copper, zinc and manganese (Davidson, 2014).

Basically, the ingestion of food is very important to the body (Mottram, 1991) so also eating daily is vital to the human well being (Mottram, 1991) however most persons subject themselves; not eating during religious activities (Fasting and Praying Programs) where there is need for food in their system. The intake of food helps the body system to speed up metabolic rate, reduce acquired diseases and even fight diseases (Mottram, 1991). Mottram (1991) further stated that food intake helps regulate body temperature and relieves pain. Moreover, eating the right food thereby boost the immune system thins the blood vessel and check health affect.

The food eaten is sourced from green plants, this green plant with the aid of sunlight, water and the right amount of minerals/element in the soil makes their own food (Osseo-Asara, 2005). In the soil, the food is liable to contamination by microbes (pest), due to plant/microbial feeding interphase in the soil; as a result of this, sometimes the food may be wasted before and after harvest or eating (Thomas, 1990). In some other case, plants harvested as raw food are taken to an open market, where they are sold to the public. The food products bought are then taken home and prepared into several delicacies as desired (Christison et al.,2008). Sometimes these food sourced from the market are prepared for consumption in the home and outside the home. The importance of food to living things specifically humans, can never be over emphasized. All living organisms must have a constant supply of food (energy), this energy may be supplied to cells or organs which are stored internally (e.g fats or carbohydrate) or externally in the environment (Bor et al., 1972).

Energy is required for the various enzymatic reactions that the body need for movement of the muscles, digestion, respiration, excretion, reproduction etc (Sanni et al., 2005). Food also supplies the structural material required for living organisms to make new macromolecules for repair of damaged structure (Alverlino et al.1970). Apart from its vital function of sustaining life, food is referred to as a harbor of pathogenic organisms, if not prepared in clean environment (Ihekononye et al.,1979).

- *Jollof- Rice*

Jollof-rice as a food, is also one of the most common dishes in West Africa. Based on its name, the origins of Jollof rice can be traced to the Senegambian region, ruled by the Jollof Empire. However, there are several regional variations in name of Jollof-rice (Ayto, 2012); in Mali, it is called Bamanankan, while in Senegal and Gambia, referred to Wolof. In french-speaking areas, called riz-au-gras. Across the regions, Jellof-rice has become the best-known African dish outside the continent (McCann et al., 2014).

The preparation of rice is noted to take diverse form. Hence, variations of cooked rice ready to eat exist for human menu, these includes; coconut Jollof-rice, fisherman Jollof-rice (made with prawns, periwinkles, crayfish), vegetable Jollof-rice, Jellof-rice with beans and the “concoction rice The rice is cooked in the homes and in eateries, specifically restaurants (Ayto, 2012).

- *Restaurants / Restaurants in Bonny*

Food ready to eat are sold in facilities called caffetarias, hotels, bar and restaurants. Restaurant according to Mulugeta and Bayeh (2012) is a place where ready to eat foods are sold. These restaurants are mostly provided by individuals to satisfy the need for food outside the home (Mulugeta and Bayeh, 2012). To make life more meaningful most restaurants have some outdoor activities like swimming and indoor activities like GYM and a relaxation center (Greig et al., 2007) According to Oluwafemi and Simisaye (2005), restaurants are known for selling varieties of food. In bonny Rivers State Nigeria, restaurants are situated in

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every nook and crannies of Bonny Kingdom, ranging from open restaurant were foods are sold on a table set up to a closed restaurant were food are sold in a less ventilated or airy environment. The streets in Bonny, (Abalamabie road, Damascus road, Wilbros Pipeline Street, Hospital road, Big market road etc) have at least 10 restaurants open selling food daily (day and night) to inhabitants. Despite the availability of food in the market, food scarcity is still experienced in our time. In essence food scarcity means the lack of access to food. Some reports such as that of Garett (2000) have blamed food scarcity in the Middle East, Asia and some parts of Africa on the observed dislodgement and movement of humans in these areas which in-turn causes social instability. Climatic change is reported to challenge food availability (Shadkam et al.,2016). In response to this, several measures have been put in place by the Nigerian Government. Programs such as the School Feeding Program, the Soingai Bannana Farm Project, the Fish Farm and the Agricultural Development Program (ADP) (Shadkam et al.,2016). Generally, food scarcity and shortage reduce the chances of some homes having good food. In further attempt also to mitigate the shortage of food, restaurants facilities were provided, and these have successfully meet the demand of inhabitants.

The food most at times are contaminated by microbes and altered by physiochemical properties, which in-turn cause's foodborne diseases as reported by (Oluwafemi and Simisaye, 2005).

- *Contamination of Food by Bacteria*

Various sources of food contamination have been identified, amongst which the activities of opportunistic microorganisms in the atmosphere have been implicated to contaminate exposed food; thereby causing foodborne infection when ingested (Trickett 1999). Similarly, Food that are exposed to the atmosphere are equally exposed to the perching of flies which are known to be carries of microorganisms and as a result of this perching they deposit micro-organism and germs on the food. Unclean cooking utensils, when not properly washed after use and then used could contaminate food (Trickett 1999). In the preparation of food, ingredients are used or introduced; the ingredient may have been associated with microbes such as: *Salmonella, Clostridium perfringens, Staphylococcus, Streptococcus* etc. which thus contaminate the food and throws out food poison to consumers of the food (Nicholas, 1998). In other circumstance, food vendors can contaminate food with their unclean hands through the process of delivery (Nicholas, 1998).

Food not properly cooked, after ingestion results to food intoxication, food infection or food poisoning. Food intoxication occur after ingesting toxins produced by microbes that have grown on the food prior to it being eaten while food infection occurs in food as a result of the food being carrier of infectious pathogen (Laurie, 1974). Thus, as a result of food intoxication and food infection, food poisoning emerges. Food poisoning out breaks are recognized by the sudden onset of illness with a short period of time among many individuals who have eaten food which contain the toxins of some pathogenic bacteria which is produced on the food being consumed (Alverlino et al.,1970). Single cases of food poisoning are difficult to identify except in botulism where distinct symptoms are observed. Food pathogenic organism may be one of the most common causes of acute illness. Yet cases of outbreaks are generally under recognized and under-reported (Alverlino et al.,1970).

Consequently, the number of bacteria present in food may be used to determine whether or not the food has been prepared hygienically (Jalapour, 2012). Some diseases are spread by bacteria that enters the body through food where they multiply at an amazing rate provided their growth requirement is in place (temperature and moisture). Nearly all the microorganisms associated with food spoilage have their origin from the soil. According to Santamaria and Toranzos (2003) soil is the single richest source of microorganisms, containing wide varieties and large numbers of microorganisms. The soil has also been identified as a source of food contamination, whereby food spoilage emerges (Santamaria and Toranzos 2003).

The prevention of pathogenic organism in food is important due to food poisoning. In most cases of food poisoning, chains of event take place aimed at reducing the incidence of illness, whereof, the chain must be broken (Thomas,1990). According to Thomas (1990) food pathogenic organisms can be prevented if certain conditions are adhered to;(i) high standard of personal hygiene, (ii)maintenance of equipment (Utensils) in clean condition, (iii)adequate provision of toilet facilities and (iv)correct storage of food stuff at the right temperature. Other measures are; quick cooling of food prior to storage and correct re-heating of food amongst many others (Thomas,1990). However, the relatively high temperature in tropical countries like Nigeria promotes rapid growth of pathogenic bacteria (Jalalpour, 2012). This factor contributes to many outbreaks of food poisoning caused by ingestion of notable food pathogens such as *Salmonella spp., Staphylococcus perfringens, S. aureus, Shigella* spp., *Vibrio parahaemolyticus* and *Bacillus cereus*.

- *Salmonella Species*

The organism *Salmonella* spp. is been considered as the most principal organisms that cause the *Salmonella* infection (Kleven and Yoder, 1998). *Salmonella* considerable alters food without altering the organoleptic property of the food, that is the taste, odour and texture of the food. The source of the organism to food include man, who plays an important role in been a carrier of the organism, without expressing symptoms of ill-health. Man plays an important role in cross contamination of cooked food (Kleven and Yoder, 1998). Salmonellosis are infections of *Salmonella* that result to food-borne disease of humans. Heavy economic losses occur due to morbidity, mortality, reduced egg and meat production in birds (CDC, 2006). Mortality may vary from 10% to 80% or higher in severe outbreaks (Kleven and Yoder, 1998). The transmission of *Salmonella* from bird meat and egg is a great concern for the public health (CDC, 2006).

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- *Staphylococcus Aureus*

Staphylococcus aureus is one of the most prevalent and clinically significant pathogens worldwide. They contaminate food and can be made inactive by heating the food at 66-68^{o}C for 35 minutes (Ghosh et al., 2007). However, *Staphylococcus aureus* produces toxins, that are heat resistance. It causes a variety of illnesses ranging from superficial skin eruptions to life-threatening infections with Bacteremia, Endocarditis, Pneumonia and Toxic Shock Syndrome (Ghosh et al., 2007). *Staphylococcus aureus* in its ability to cause superficial skin infection is noted to exist in the skin and nose of the body as normal flora, where they do not cause disease however, caused diseases when the condition of the skin is not favorable (altered), thereby result to eruption of the skin (Ghosh et al., 2007).

➢ *Statement of Problem*

Multiple lines of evidence indicate that food exposed on sale in restaurants may become infected by bacteria. There have been claims that Jollof-rice sold in restaurants in Bonny is responsible for reported cases of ill-health in the area; most consumers complain of ill-health after consuming Jollof- rice. Even at this, restaurant fried rice have shown epidemiological links with illness and this has raised concern in respect to their potential for serious food poisoning outbreaks. Evidently, some of the restaurants in bonny are noted to have low hygiene practices. That is, proper hygiene practice is not well followed or established. Consequent upon this challenge, it becomes necessary to investigate the Jollof-rice sold in these restaurants for contamination with some bacteria associated with food-related illnesses.

➢ *Significance of Study*

- *The study will be beneficial to inhabitants who frequently complain of foodborne illness and thus direct consumers on course of action to Take in militating against illnesses;*
- *The study would help food vendors check-mate/ control the evasion of Staphylococcus aureus and Salmonella typhii in Jollof-rice, as their importance to human health cannot be overemphasized, its thus expedient that their investigation be made due to their implication in food poisoning and hence put a stop to their emergence;*
- *The study would help/assist health workers on proper diagnosis of ill-health on the inhabitance and thus, reveal the level of hygiene adopted by food handlers and vendors in course of service delivery and improvements.*

➢ *Scope of Study*

The research work is limited to Bonny kingdom of Rivers State, Nigeria. The work specifically focused on two distinct bacteria that have often been implicated, severally, as a foodborne pathogen. Again, the work only considered *Staphylococcus aureus* and *Salmonella* spp. in Jollof-rice sold in some restaurants in Bonny Island.

➢ *Aim and Objectives of the Study*

The aim of the study was to examine the Jollof rice sold in restaurants in Bonny Island for possible contamination by *Salmonella typhii* and *Staphylococcus aureus*.

- *The Objectives of the Study were to:*

✓ *Determine the bacterial load (THB) of Jollof-rice sold in some restaurants in Bonny Island;*
✓ *Isolate and characterize Salmonella typhii and Staphylococcus aureus occurring in the Jollof-rice;*
✓ *Determine the prevalence of the bacteria species in the rice sample;*
✓ *Determine the susceptibility of the bacteria (Salmonella typhii and Staphylococcus aureus) isolated from the Jollof-rice.*

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CHAPTER TWO
REVIEW OF RELATED LITERATURE

The word restaurant or hotel refers to a place where people stay or are accommodated for a time been and have their meals. However, according to the Oxford Advanced Learner's Dictionary of Current English, 6th Edition, 2005, hotel may mean a building or "the house or flat/apartment" or a town, region, province and country that one comes from or is currently living and feels belonging. Also, the home may mean a family living together and the way it behaves by which for instance, one family could be described as a clean home while another family could be characterized as a dirty home (Tripadvisor, 2022).

Nevertheless, the restaurant as held and used for the purpose of this work means a place where you can buy and eat meal. Typically, a restaurant has some basic provisions such as spaces preparing food, eating, defecation, and possibly relaxation (Tripadvisor, 2022). Generally, restaurant offer services which are at different hours of the day and night (Tripadvisor, 2022).

In contrast, conventional modern restaurant provides separate spaces such as swimming pools, drinking bar and bedroom (for sleeping). Thus, in terms of space, restaurants differ in terms of their recipe used in preparing and cooking food, thus a signature in the identification of a specific or choice restaurant is demanded or desired. (Tripadvisor, 2022), these of course, may equally affect the economic state of the consumers

All through human history and worldwide, as people are diverse, so are their various kinds of restaurant. Somehow the different kinds of restaurant that consumers patronize is a function of factors such as the culture, geography, socio-economy and perception of the individual (Tripadvisor, 2022).

Particularly, on the basis of economic considerations/factors, restaurants may be differentiated in terms of mobility, that is to say, whether or not the restaurant as built can have its location changed over times (Tripadvisor, 2022). By this criterion, a restaurant is either in a mobile or stationed format (Tripadvisor, 2022). A mobile restaurant is so intended and designed as it can be moved from place to place by means of a vehicular device such as flatbed truck or wheel birrow which can be used for changing the location overtime as at and when desired (Tripadvisor, 2022). Mobile eatery/restaurant as may be called are food hawkers who place the food on their head and at times use truck to convey the prepared food to consumers. In contrast, a stationed restaurant is at a given place or location; not to have it moved to different places at different times (Tripadvisor, 2022).

Stationed restaurant are fixed to the location where they are built and found (Tripadvisor, 2022). Typical stationed restaurant around the street of Bonny, Port Harcourt are the popular Kilimanjaro outlet, Genesis, Chiken Republic etc.

The use of truck, wheel barrow or head to convey food to interested consumers is one way food have been made available. The mobile eatery help one meet the need for changes in location as may be dictated or demanded by consumers. As a food eatery, it has access to proper and good ventilations. A disadvantaged aspect of mobile eatery service is the absence of convenience which the stationed restaurants have (Tripadvisor, 2022). Thus, water storage and electricity supply facilities are also missing (Jolaosho, 2016).

The stationed restaurants have spaces for food preparation, bathroom/toilet, relaxation spots and kitchen etc (Jolaosho, 2016). They are well ventilated and some have gardens with or without lawn, and have provisions for electricity, water supply and hygienic surrounding or environment. Most restaurants are usually roomy, complex, fixed structures and normally have well spaced-out neighbouring buildings (Jolaosho, 2016). According to Tripadvisor (2022) most mobile eateries are patronized in the absence of a stationed restaurant. For this fact, convenience and cost of food are determined, to play a major role. The cost of a plate of food in the mobile eatery is relatively cheaper than that of the stationed restaurant. (Nsing, 2016). The mobile eatery serves cheap food for people who have not got much money to pay for food in the stationed eatery. (Nsing, 2016). Both eateries (the mobile and stationed) are found in parts of Bonny, Port Harcourt and its environs. They are common in newly developed and developing areas of Port Harcourt and there exist pockets of these eateries in some old developed areas of the city.

Generally, restaurants are assessed in terms of the immediate surroundings, air, ground, space and sanitary condition and the vendors physical appearance. All these factor greats either positive or negative perception on the part of the consumer (Thakadu et al., 2018). These factors brought together, invariably interplay to affect the sensitivity and ability of the consumer to continue the business of eating outside the home (WDTR, 2019).

However, in all these factors for assessing restaurant, the mobile restaurant could be scored very high while the Stationed eatery would score significantly low in all the three criteria for assessing the restaurant. Thus, this rather hypothetical assessment at this stage explains the interest of this study.

Incontestably, the importance of mobile eateries cannot be overemphasized; because they are the most affordable food vendors and of course, the only alternative for low-income earners (Wokekoro and Opuenebo, 2014). Moreover, in the face of obvious socio-economic challenges all over the globe, the only alternative type of eatery to most people are the mobile eatery.

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Fact remains that mobile eateries keep increasing numerically overtime in Port Harcourt and its environs. Their emergence and growth is owing partly to urban economic pressures of poverty or lack as well as social forces of growing rural-to-urban movement or migration of people occasioned by rural area insecurity and cult activities driven internal displacement of people in the countryside (Wokekoro and Opuenebo, 2014).

The act of cooking food has existed for a very long time ago and sometimes it is very demanding. It involves sourcing for the food ingredients, preparing of the raw food and heating the food finally to make it palatable (Duru et al., 2013). It also entails preserving the food from contamination before consumption. The preservation of food from being contaminated is one way food-borne diseases can be eliminated or reduced (Clasen et al., 2006). Several reasons have been advanced for cooking; food were found to be contaminated with feacal indicator bacteria including feacal coliforms and *Escherichia coli*, which were established to be above the recommended World Health Organization (WHO) standards for coliform presence in food. This is informed by the fact that food in the restaurant of the study area is of critical interest since it is prepared and sold in different dimension. In general, food crisis is in the dimensions of availability. However, shortage of food refers to not enough quantity of food and its raw state. Scarcity of food refers to non-availability of food. On the other hand, food crisis connotes shortage and scarcity as well as the quality of food that is either available or accessible. The unavailability of food has forced the storage of food in reservoirs (Ateki, 2015). Meanwhile in reaction to food shortage, the United Nations reported to have warned on the outbreak of foodborne diseases (Bodzewan, 2014).

Safe food is a basic human right despite many foods are frequently contaminated with naturally occurring pathogenic microorganisms, such pathogens that cannot be detected organoleptically but can cause disease of varying severity, including death specially, if the way they are conserved during display for sale. Thus, provides conditions for those microorganisms to grow and reach considerable levels of contamination (Nicholas, 1998).

Food safety issues are of major importance to world health (Mulugeta & Bayeh, 2012). The global incidence of food borne illnesses is difficult to estimate but it has been reported that in 2000 alone 2.1 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (Mulugeta & Bayeh, 2012). Illnesses resulting from the consumption of contaminated food has become one of the most widespread public health problems in contemporary society (Nicholas, 1998). In Nigeria and many other developing countries, a major source of ready-to-eat foods (street foods) are prepared and sold at public places such as market, school, churches and along the streets. The ready-to-eat food offers food at relatively cheaper rate and at easily accessible places (Mensah et al., 2002). Furthermore, it offers the traditional meals and preparations of a number of them are quite laborious and time consuming (Nicholas, 1998). Thus, with the increase in the number of hours spent at work place by parents and schools, the importance of ready -to-eat food in the human feeding is increasingly becoming very important among all socio-economic groups (Olumuyiwa, 2015). A number of observational studies have shown that these foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings (Mulugeta & Bayeh, 2012).

In addition, the vendors practice poor personal hygiene. Reports have it that food vendors being carriers of microbes could serve as a potential source of transmission of enteric fevers. Most of the vendors have 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 unquestionable (Olumuyiwa, 2015). At the same time, most people who consume these food services are more interested in its convenience than the question of microbiological quality and hygiene (Olumuyiwa, 2015). The microbiological quality of food indicates the number of microbial contaminants it has, a high level of contamination indicates low quality of food storage and its handling and more likely to transmit infection and the reverse is true. Thus, concerns have been raised by the food and Agricultural Organization and others about theses foods serving as a potential source of food poisoning outbreaks (Christison et al., 2008).

Conclusively, ready-to-eat food are foods that do not require further significant preparation other than reheating or completion of a cooking process. It has been reported that ready -to-eat food accounts for a large volume of sales of the food service sector, representing more than a third of the food service volume outputs (Olumuyiwa, 2015). Food-borne disease outbreaks linked with ready -to-eat food have been associated with various foodborne pathogens. The initial microbiological load on ready -to-eat food ingredients is important, however, factor such as handling, processing, storage and display may influence the microbiological load of ready -to-eat food at the point of sale (Nwachukwu and Akpata, 2003). Ready -to-eat foods such as salads and sandwiches from food canteens have also been implicated in food-borne illness outbreaks. These foods are often prepared by hand and this direct contact may lead to an increased incidence of contamination with potential food -borne pathogens, such as *Staphylococcus* spp.

The microbiology of ready-to-eat food during preparation in factories, in domestic kitchens, canteens and on street corners by street vendors has previously been investigated (Olumuyiwa, 2015). Ready-to-eat food items can potentially serve as a reservoir of pathogenic microorganism that have got the ability of transmitting diseases. It has been reported by Itoadon et al. (2011), that the presence of mesophilic microorganisms in food items is a suggestion that pathogenic microorganisms are probably present in such food items. Oranusi et al. (2006) have reported a number of food items locally sold in Nigeria as vastly contaminated with *Staphylococcus aureus* has been reported over the years as having the ability of causing food poisoning. This

submission has been supported by the report of Nwachukwu and Akpata (2003) that the bacterium can cause food poisoning and other food-borne diseases. In any community, *Staphylococcus aureus* has been considered as the major pathogen that has got the ability of colonizing and infecting both hospitalized patients exhibiting decreased immunity and healthy immune competent persons. According to CDC (2006) this bacterium produces toxins and is found commonly on the skin, in the nose and throats of up to 25% of healthy people as a normal flora. Cases of most *Staphylococcus aureus* food borne illnesses reported in the literature are mostly, triggered by poor hygiene exhibited by food handlers and grossly improper food handling practices

The human body is basically a machine, which by definition is a device for doing work. When that work is done, the body sweats it out (Mitchel et al. 2005). According to Mitchel et al. (2005), the human body is a system of levers, the long bones in the arm and the fore arm, thigh and leg are rigid lines and each move at a point which is the fulcrum. In otherwords, Aguilar and Gabes (2000) stated that the human body (machine) must be maintained to do work. The body needs premium fuel (food) to do this work (Mitchel et al. 2005). It would be wrong to overestimate the importance of food (diet) in the contribution to man's daily activity; hence choosing good food is a major part of choosing good health (Mitchel et al. 2005). The principle therefore is to eat enough healthy food to feel satisfied and to exercise enough to raise metabolic activity to burn the food off. Mitchel et al. (2005) further explained that the activities the body engages itself with aid in burning off the fat deposited in the body by a singular weight reduction program. For good health and weight control, the right amount of the right kind of food at the right time in the right frame of mind is necessary and important. Eating very quickly leads to overeating which in turn causes obesity (Mitchel at al. 2005). Obesity or overweight is the condition where excessive fat is stored in the body tissue. Obesity is becoming a worldwide problem associated with high level of sweating. It is a serious problem because it predisposes to a vast range of diseases (Mitchel et al. 2005).

The extent of microbial food contamination is different in case by case basis, with regards vegetables and fruits which are used as food for man as reported by Oranusi and Braid (2012), thus depends on the state of the water (weather clean or not), harvesting, transportation and storage temperature of the fruits and vegetables. In these circumstances, the presence of high load of bacteria is an indication that care should be taken in handling vegetable and fruits (food).

In a similar circumstance, according to Jemikalajah (2018) fried rice can be easily contaminated with heavy load of pathogen during preparation and handling, specifically when allowed for more than two hours at room temperature, where the bacteria multiply rapidly.

Changes in food quality are becoming alarming in resent time. The changes vary depending on the method of preparation and preservation. Thus, changes in food quality reflect on the physical, chemical, biological and radiological characteristics of the food. Basically, the biology quality of a food is determined from its method of preservation. Changes in food is depended on the state of the food whether it is safe or unsafe, fit or unfit for consumption. Food can be susceptible to contamination during preparation, cooking, serving, preservation and storage thereby altering the quality of the food (Ovanusi & Braide 2012). Changes in food quality can be a serious problem. Even though there is no immediate problem at the time of eating, but after sometime the body may begin to emit signals of negative side effect (Ovanusi & Braide 2012). Flies are reported to contaminate food, resulting to food wastage. Other contaminative report on food includes solid substances in the atmosphere, carried by breeze or winds are deposited afterwards on surfaces of food (Rose & Osunnaiye, 2003). Meanwhile, nature is said to have self regulatory ability using ultraviolet light (sunlight) to militate against some of this contaminant in food.

Jollof-rice and other food are sometimes associated with microorganisms, that is bacteria have been found to contaminate food surviving in it for weeks (Davidson, 2014, Lerry and Blackburn, 1973). Rice food have been implicated as a vehicle for the transmission of microorganism, where the bacteria in the food cannot be identified by smell, taste or color, but by simple microbiological procedure (Davidson, 2014). Consumption of bacterially contaminated food cause foodborne diseases and thereby constitutes serious public health hazards to consumers (Lerry and Blackburn, 1973). Outbreaks of foodborne disease caused by pathogenic bacteria have been reported in many parts of the world, foodborne outbreaks such as Campylobacteriosis, Salmonelosis, Listeriosis, Botulism have been reported (Lerry and Blackburn, 1973). Some microorganism when consumed in food causes food poisoning. According to Balaban (2000), food poisoning is an illness that gives people diarrhea, an ill-condition resulting to liquid feces. Several studies have attributed diarrhea and associated death to the consumption of rice especially among children (Davidson, 2014), with the reported incidence of diarrhea, microbes specifically bacteria have been implicated. The bacteria *Bacillus cereus* in rice is arguably the most important food stuff associated with *Bacillus cereus* food poisoning. The agent *Bacillus cereus* makes up to 10% of soil microflora in rice (Varnam and Evans, 1991). In rice *Bacillus cereus* produces emetic toxins in a relatively short time on cooked rice and other starchy foods stored at room temperature. According to Varnam and Evans, (1991), once the *Bacillus cereus* have produced the toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. The food when served and consumed then becomes food poisoning (Varnam and Evans, 1991).

Food-bore infections are commonly caused by a carrier engaged in handling and preparing food, but sometimes the basic food-stuff is itself contaminated (Aguilar & Galbes, 2000). Outbreaks due to bacterial toxins have an explosive onset with the majority of persons affected at roughly the same time, usually a few hours after consumption of the affected food. Example 6 to

12 hours with *Clostridium perfringens*. The symptoms may be severe but rarely last more than a day. Prostration is common but pyrexia does not occur, vomiting is unusual (Aguilar and Galbes, 2000).

Milk as a food, is a dangerous vehicle for bacteria transmission, unless refrigerated, it offers an excellent medium for multiplication of bacteria (Mbah & Oselebe, 2004). The pathogen that may be present in milk are: (1) those derived from the cow example *Mycobacterium tuberculosis* and the Streptocopcci of bovine mastitis which are mostly avirulent for man (2) those introduced by farm or dairy workers or by contaminated water or equipment example *Streptococcus pyogenes, Clostridium diphtheriae* and *Shigella*. Other organisms commonly present are *Escherichia coli* and *Lactose bacilli*, the bacteria turn milk sour but are harmless. All the pathogens are killed by pasteurization. Pasteurization at 65º Cenlsius for 30 minutes kills all pathogens present in milk but leaves a few unimportant heat-resistant vegetative organisms and spores. After pasteurization the milk is immediately cooled to discourage growth of these organisms (Mbah & Oselebe, 2004).

Staphylococcus aureus was first identified in 1961, it has become the most common cause of nosocomial and foodborne infections worldwide (Rhodes and Fletcher, 1966). Generally, the Coagulase-positive Staphylococci have been regarded as an opportunistic pathogen. Furthermore, some coagulase positive and negative Staphylococci strains are erroneously identified as Micrococci and are generally regarded as non-pathogens (Rhodes and Fletcher, 1966). Staphylococci are easily spread between animals and under certain conditions to humans through contact with excretions such as saliva or aerosols released during sneezing and coughing. Moreover, Staphylococci spread by animal products such as non-pasteurized milk (Rhodes and Fletcher, 1966). *Staphylococcus aureus* causes problems like septicemia and skeletal infections in commercial broiler chicken (Balaban and Rossoly, 2000). The mechanism of spread of *Staphylococcus aureus* infection through poultry flocks is not fully understood (Balaban and Rossoly, 2000). Serious *Staphylococcus aureus* infections are caused by strains those are methicillin resistant (MRSA) or susceptible that not expresses the pathogenic Panton-Valentine leucocidin (PVL) toxin (Balaban and Rossoly, 2000). *Staphylococcus aureus* have the ability to make toxins. Thus, Staphylococcal food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus* (Ghosh et al., 2007).

Salmonella sp. is a natural inhabitant in the gastrointestinal tract of many animals: reptile, birds and human. The organism is originally, able to adapt to the gastrointestinal tract of animals and once released through defecation on the soil can be transferred through rainfall to surface water run-off, were it survives several challenges such as ultra violet radiation from sunlight, poor nutrient, changes in pH and temperature (Corry et al., 1982) *Salmonella* sp. must persist under these adverse environmental conditions at sufficient concentration to cause human illness (Corry et al., 1982). Source of *Salmonella* sp. may be obtained from the excretion of cows in their carcases and spores on hides, bone meal etc. similarly, hens and ducks often secrete *Salmonella* in their faeces. Duck eggs are particularly liable to contain *Salmonella*, the organism enter the egg before the shell is deposited.

Salmonellosis, a disease condition in human caused by the ingestion of non-typhoidal *Salmonella*, it ranks among the highest in all gastroenteritis cases linked to food consumption (Muleta and Ashanti, 2001). It is estimated that *Salmonella* sp. cause 93.8 million cases of gastroenteritis worldwide annually with 155,000 deaths (CDC, 2006). Several food produces have been associated with *Salmonella* food outbreaks; the food produce could be used as ingredient in the cooking of rice meal. This food produce include: tomatoes, hot peppers, lettuce, cucumbers (Mulugeta and Bayeh, 2012) (Mensah et al, 2001).

Despite the familiar implication of diarrhea in food poisoning, others symptoms could include (i) Stomach pain, a pain in the stomach region of the body, (ii) vomiting, a partially digested food that comes out through the mouth from the stomach, (iii)nausea, a feeling of sickness and dizziness and (iv)head ache, a pain in the head (Ajao and Atere, 2009).

The most common effect produced by growth of *Staphylococcus aureus* and *Salmonella* sp. in the body is Skin Scalded Syndrome and fever respectively (Turk 2015). These conditions comes with pain. The infection of these bacteria initiates an immune response from the host body which either ward the bacteria off or keep them to pase (Turk 2015).

Staphylococcus aureus and *Salmonella* sp. are antigenic when they infected human. The human in-turn produces specific antibodies, these antibodies thus have the power to render the bacteria ineffective (bacteria neutralization) (Mbah & Oselebe 2004). The antibodies are important in the recovery from the diseases and development of immunity (Turk 2015). Other effect of *Staphylococcus aureus* and *Salmonella* sp. is their ability to produce toxins. Very high concentration of *Staphylococcus aureus* and *Salmonella* sp. have direct toxic or lethal effect. Basically, toxicity is a property of an intact microorganism particle and can be neutralized by specific anti serum.

The use of antibiotics have been adopted to control some of these foodborne pathogen, however several measures could be adopted in eliminating food poisoning. Hygiene preparation could eliminate food poisoning amongst many other measures (Okonko et al., 2009). Food standards have been set by regulatory bodies to ward-off and control emerging or evasion of microbes in our food. Food standards are laws prorogated to ensure food is protected. This involves keeping the food safely (CDC, 2006). CDC (2006) stated that this law also involves upgrading of non-protected food sales facilities like restaurants and hotels through a well management plan. The acceptable or allowable bacterial organism and their population in a given food sample varies for

country of the world depending on the climatic condition of the said country. The need for frequent testing of food will help to monitor the standard of the food that people eat (CDC, 2006).

The occurrence or otherwise of antibiotics resistant microbes specifically bacteria in the human body is a phenomenom that influences the success or failure of bacterial infections in humans. However, some drug resistant bacteria have become common in the past three decades probably due to human abuse of the drugs intake directly or indirectly as in the control of food animal diseases in livestock farming. Studies have shown that the resistance of *Cambylobacter* to antimicrobial agents or drugs is high, showing above 70% resistivity to antibiotics such as gentamicin, rifampicin, ampiclox, levofloxacin and ciprofloxacin. Disease causing bacteria obtained from Okinalia flooding was reported to have some level of drug resistance according to Morin et al. (1999). In a study carried out by Bridget (2010), high proportion of antibiotics resistant bacteria were observed when microbial indicators obtained from waste water treatment facilities in a location impacted by Confined Animal Feeding Operations were examined (CAFOs). Furthermore, Bolariiwa (2006) established a high level of antibiotics resistance pattern of bacteria flora of fish. Basically, bacterial resistance to antibiotics drug are on the increase and the phenomenon portends grave health challenges to all and sundry.

These microorganisms have led to foodborne outbreaks and several countries have seen dramatic and steady increases in human outbreaks of salmonellosis, caused by infections in ready-to-eat foods. In addition to human health implications, salmonella is a pathogen of significant importance in animal production especially in the context of the emergence of antibiotic resistant strains. Biological contaminants largely bacteria, viruses, and parasites constitute the major cause of food-borne diseases (Tennant *et al.,* 2010). Even though the restaurant industry plays an important role in the safety of the food supply chain, the proportion of illnesses that result from the consumption of food from restaurants is still unknown (Dereu et al., 2018).

Although *Salmonella* being the second highest causes of food-borne illnesses in humans, has shown the varying prevalence rates in ready-to-eat foods (salad, rice, beans, etc.) from many studies and its implication suggests the need for improved and strict food hygiene and safety management system in the food industry especially restaurants in Bonny Island, during transportation, handling, processing and preparation of food. The rapid diagnosis of foodborne illness causing pathogens is crucial for the food industry and public health sector (Sarina et al., 2010).

- *Numerous recent examinations have confirmed the presence of salmonella from various regions of Nigeria. Salmonella contamination of ready-to-eat foods has been observed in Nsukka, Ibadan, Maiduguri, and Lagos. Local restaurants in Jos and Owerri have recorded prevalence rates of 14.1% (Heredia et al., 2018).*

Due to their varied targets in the body and the various symptoms they produce, typhoidal and non-typhoidal serotypes have different mechanisms of infection. The barrier that the intestinal cell wall creates must be crossed by both groups, but once they have done so, they use various methods to spread infection. gut inflammation and diarrhea are brought on by bacterial-mediated endocytosis, which preferentially allows non-typhoidal serotypes to enter M cells on the gut wall. Additionally, they have the capacity to damage the tight junctions that allow the intestinal wall's cells to halt the passage of ions, water, and immune cells into and out of the gut. Tight connections are damaged when bacteria mediate endocytosis, which results in inflammation (Haraga et al., 2008).

Salmonella can also cross the intestinal barrier through immune cells that are CD18-positive and phagocytosis, which may be a crucial mechanism in typhoidal Salmonella infection. This is regarded to be a more covert method of breaching the intestinal barrier, which may explain why less typhoidal Salmonella is needed to cause infection than non-typhoidal Salmonella (Haraga et al., 2008).Micropinocytosis allows Salmonella cells to penetrate macrophages (Kerr et al., 2010). Typhoidal serotypes can exploit this to spread throughout the body via the mononuclear phagocyte system, which is a web of connective tissue that surrounds immune system-related tissues all over the body (Haraga et al., 2008).

Salmonella cells are able to enter macrophages via micropinocytosis (Kerr et al., 2010). Typhoidal serotypes can use this to achieve dissemination throughout the body via the mononuclear phagocyte system, a network of connective tissue that contains immune cells, and surrounds tissue associated with the immune system throughout the body (Haraga et al., 2008).

Much of the success of *Salmonella* in causing infection is attributed to two type III secretion systems which function at different times during an infection. One is required for the invasion of non-phagocytic cells, colonization of the intestine, and induction of intestinal inflammatory responses and diarrhea. The other is important for survival in macrophages and establishment of systemic disease (Haraga et al., 2008). These systems contain many genes which must work co-operatively to achieve infection.

The AvrA toxin injected by the SPI1 type III secretion system of S. Typhimurium works to inhibit the innate immune system by virtue of its serine/threonine acetyltransferase activity, and requires binding to eukaryotic target cell phytic acid (IP6) (Mittal et al., 2010). This leaves the host more susceptible to infection.

Salmonellosis is known to be able to cause back pain or spondylosis. It can manifest as five clinical patterns: gastrointestinal tract infection, enteric fever, bacteremia, local infection, and the chronic reservoir state. The initial symptoms are non specific

fever, weakness, and myalgia among others. In the bacteremia state, it can spread to any parts of the body and this induces localized infection or it forms abscesses. The forms of localized *Salmonella* infections are arthritis, urinary tract infection, infection of the central nervous system, bone infection, soft tissue infection, etc. (Choi et al., 2010). Infection may remain as the latent form for a long time, and when the function of reticular endothelial cells is deteriorated, it may become activated and consequently, it may secondarily induce spreading infection in the bone several months or several years after acute salmonellosis (Choi et al., 2010

Salmonella species are facultative intracellular pathogens (Jantsch et al., 2011). A facultative organism uses oxygen to make ATP; when it is not available, it "exercises its option"—the literal meaning of the term— and makes ATP by fermentation, or by substituting one or more of four less efficient electron acceptors as oxygen at the end of the electron transport chain: sulfate, nitrate, sulfur, or fumarate (Jantsch et al., 2011).

Most infections are due to ingestion of food contaminated by animal feces, or by human feces, such as by a food-service worker at a commercial eatery. Salmonella serotypes can be divided into two main groups—typhoidal and nontyphoidal. Nontyphoidal serotypes are more common, and usually cause self-limiting gastrointestinal disease. They can infect a range of animals, and are zoonotic, meaning they can be transferred between humans and other animals. Typhoidal serotypes include *Salmonella* Typhi and *Salmonella* Paratyphi A, which are adapted to humans and do not occur in other animals (Jantsch et al., 2011).

S. enterica, through some of its serotypes such as *Typhimurium* and *Enteriditis*, shows signs of the ability to infect several different mammalian host species, while other serotypes such as Typhi seem to be restricted to only a few hosts (Thomson et al., 2008). Some of the ways that *Salmonella* serotypes have adapted to their hosts include loss of genetic material and mutation. In more complex mammalian species, immune systems, which include pathogen specific immune responses, target serovars of Salmonella through binding of antibodies to structures such as flagella. Through the loss of the genetic material that codes for a flagellum to form, Salmonella can evade a host's immune system (den Bakker et al., 2011). mgtC leader RNA from bacteria virulence gene (mgtCBR operon) decreases flagellin production during infection by directly base pairing with mRNAs of the fljB gene encoding flagellin and promotes degradation (Choi et al., 2017).

In the study by Kisela et al., more pathogenic serovars of S. enterica were found to have certain adhesions in common that have developed out of convergent evolution (Kisiela et al., 2012). This means that, as these strains of *Salmonella* have been exposed to similar conditions such as immune systems, similar structures evolved separately to negate these similar, more advanced defenses in hosts. Still, many questions remain about the way that *Salmonella* has evolved into so many different types, but *Salmonella* may have evolved through several phases. As (Baumler *et al*. 1998) have suggested, *Salmonella* most likely evolved through horizontal gene transfer, formation of new serovars due to additional pathogenicity islands. an approximation of its ancestry (Bäumler *et al.*, 1998). So, *Salmonella* could have evolved into its many different serotypes through gaining genetic information from different pathogenic bacteria. The presence of several pathogenicity islands in the genome of different serotypes has lent credence to this theory (Bäumler et al., 1998).

Salmonella sv. Newport has signs of adaptation to a plant colonization lifestyle, which may play a role in its disproportionate association with food borne illness linked to produce. A variety of functions selected for during sv. Newport persistence in tomatoes have been reported to be similar to those selected for in sv. Typhimurium from animal hosts (de Moraes et al., 2018). The papA gene, which is unique to sv. Newport, contributes to the strain's fitness in tomatoes, and has homologs in genomes of other Enterobacteriaceae that are able to colonize plant and animal hosts (de Moraes et al., 2018).

Treatment for food poisoning is controversial some physicians recommend no antibiotics since *Salmonella* is self-limited, while others suggest using antibiotics such as ciprofloxacin (Cipro, Cipro XR, Proquin XR) for 10-14 days. Some medical practitioners believe antibiotics prolong the carrier state. However, patients identified as immune suppressed (for example, patients with AIDs or undergoing cancer chemotherapy, infants under 2 months of age, or the elderly ones) should receive antibiotics; they may need to be hospitalized and have some of their case managed by an infectious disease consultant. In addition, pregnancy often predisposes the mother to sell all types of food poisoning, including *Salmonella*. Pregnant females should take care to wash and cook foods well before eating. Supportive therapy for both enteric and enteric fevers consists mainly of preventing dehydration and electrolyte abnormalities (for example, abnormal levels of potassium and sodium ions) with fluids containing electrolytes (for example, IV fluids or oral fluids like sports drinks).

Carriers of *Salmonella* are considered to be infected even though they may show no symptoms. Carriers can infect other people and need to be their gall bladder and antibiotic treatments

Ready-to-eat foods and a good group of food products that are pre-cleaned, precooked, mostly packaged and ready for consumption without prior preparation or cooking (CDC, 2015).

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According to the 2009 US food code (FDA, 2009), Ready-to-eat (RTE) Foods should be in an edible form without an additional preparation step to achieve food safety. Foods in this category usually contain raw materials of animal origin, such as eggs, fish, meat, and poultry must be cooked to allow the lowest internal temperature to reach a minimum temperature, for a minimum holding time, during manufacturing to destroy microorganisms of public health concern. A properly processed and packaged RTE food should be free of the target food-borne pathogen and ready for consumption.

Among common food-borne pathogens, such as *Salmonella sp, Listeria monocytogenes* and pathogenic *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* sp is the major concern for refrigerated RTE foods.

Food- borne bacteria usually encounter drastic pH variations in the environment, and are exposed to acid conditions while present in foods, during processing, and when they invade the gastrointestinal tract of animals and humans (De Reu et al., 2018). Microbial cells have developed strategies to respond to acid stress by inducing a protective response known as the acid tolerance response (ATR) (Sarina et al., 2010). Microorganisms developed an ATR when exposed to a moderately low pH; this results in the induction of proteins that protect the cells against extremely acid conditions. A number of examples involving induction of cross-protective responses in food-borne pathogens and other microorganisms, as a result of previous microbial exposure to acid, have been reported (Bayer & Bernard, 2014).

RTE foods prepared by hand are often implicated in food-borne illness outbreaks, as this direct contact may lead to an increased incidence of contamination with potential food borne pathogens (Bayer & Bernard, 2014). The hands of food handlers are an important vehicle of food cross contamination, as microorganisms found on hands vary significantly due to poor personal hygiene and contacted surfaces. The most common isolated bacteria are *E. coli, Salmonella sp, Bacillus sp, Staphylococcus aureus* and some other members of Enterobacteriaceae.

Dish clothes are commonly used to clean surfaces, kitchen equipment and utensils, crockery and cutlery, and so on, enhancing the potential for cross-contamination between food-related habitats.

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CHAPTER THREE
MATERIALS AND MAETHODS

➢ *Study Area*

The study area chosen for the research work is the Bonny Local Government Area of Rivers State, Nigeria. The area is an island, and a major trading point of oil and gas. Bonny is home to the biggest LNG Gas plant in Nigeria with seven trains. According to a 2006 population census report, Bonny kingdom has a total human population of 214,983 persons and a land mass of 249.27sqmi (645.60 km^2). The inhabitance engages in fishing, farming, local craft manufacture and trade amongst other occupations. Due to the huge economic activities in the land, residents and indigenes patronize food vendors to control loss of man hour as a result of cooking or food preparations. Foods are sold in open premises and closed environment. That is food sold in open premises and such places include the street, highway and open space along the road called the roadsides. The closed places include the cafeteria, eateries, canteen etc.

➢ *Purchase (Collection) of Jollof-Rice*

Cooked rice samples for sale were purchased from restaurants. The samples at the point of purchase were differentiated accordingly, for the purpose of clarification. A total of 20 samples were purchased for the purpose of the study, collected in a sterile bag and transferred to the Biology Laboratory of the Department of Biology, Ignatius Ajuru University for Microbiological analysis for a period of 9 months (February 2021–November 2021).

➢ *Determination of Total Bacterial Load*

Enumeration of the Total Heterotrophic Bacteria load in the Jollof rice samples involved employing the spread plate technique as adopted by Eaton et al.(1995) as modified by Amadi-Ikpa et al., (2020). The technique involved the spread of an aliquot of 10^{-1} obtained from the dilution of the rice sample into a Nutrient media. This was followed by incubation at 37°C for 24-48 hrs. Growth observed were identified and counted or enumerated as colony forming unit per gram.

- *Media Used/Preparation*

Media preparation involved the use of the required amount of Nutrient agar, a general purpose agar for the growth of non fastidious microorganism. The agar was dissolved in distilled water, autoclaved as instructed by manufacturer before use.

- *Procedure*

Analysis involved (i) dilution of the rice samples severally in a 9ml normal saline, to obtain a dilution factor of 10^3, (ii)inoculation or transfer of the diluted rice sample (factor of 10^3) into a freshly prepared Nutrient medium and (iii) incubation of the plated medium at 37°C for 24-48 hours for growth of bacteria and (iv) subsequently, enumeration of bacteria colonies .

➢ *Isolation and Characterization of the Bacteria*

- *Salmonella Typhii*

Isolation for pure culture isolates of *Salmonella typhii* as described by Harrigan and McCance (2003) was employed with the use of Salmonella Shigella medium. The agar chiefly, was used to isolate *Salmonella* and *Shigella* species. In carrying out the analysis, an aliquot of the diluted Jellof-rice in a normal saline previously, diluted in a 9ml normal saline was inoculated on the above said media followed by incubation at 37°C for 24 hrs for colonial Growth of *Salmonella* and *Shigella* species. In presumptive test to characterize *Salmonella typhi*, certain morphological features were considered, with respect to size, shape, colour, elevation and opacity of the isolate/colony on the media plate. In further test to comfirm the identity of *Salmonella typhi*, Some key biochemical reactions were adopted such as the Methyl red test, Citrate test and Hydrogen sulphide test.

✓ *Methyl Red/ Voges Proskauer Test*

In carrying out the Methyl red test, to determine the ability of the *Salmonella typhii* to produce and maintain stable acid end products from Glucose fermentation and to determine the ability of the *Salmonella typhii* to produce neutral end products such as acetyl-methyl carbinol or acetone from glucose fermentation. In carrying out the test, a loopful of the *Salmonella typhii* is inoculated into 10ml sterile MR/VP broth medium prepared according to manufacturer's instructions. The tube was then incubated at 35 -37^{0}C for 48 hrs, after incubation, the broth culture (*Salmonella typhi)* was shared into two parts (5ml) each, one part represented the methyl red test while the other part represents the Voges Proskauer test. To the part with methyl red test, 5-6 drops of methyl red reagent was added and to the part with Voges Proskauer test, 0.6ml (6 drops) of 5% a – naphthol and 0.2ml (2 drops) of 40% KOH was reagent added. Development of bright red coloration is indicative of positive MR/VP test, thus comfirms *Salmonella typhii* and a reverse indicative of a non *Salmonella typhi* (Amadi-Ikpa et al., 2020).

✓ *Citrate Test*

In carrying out the ability of the *Salmonella typhii* to utilize Sodium Citrate as its sole source carbon and inorganic ammonium salt as its only source of nitrogen. Isolates that can grow in the citrate medium turn the bromothymol blue indicator from green to blue. Simmon citrate agar was prepared in a capped tube according to manufacturer's instruction. A sterile wire loop was used to pick a loopful of the presumed *Salmonella typhii* and streaked on slant surface. The tube was then incubated at

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37^0C for 24hrs. Change in colour from green to blue indicated a positive result for *Salmonella typhii* while no change in colour indicated a negative *Salmonella typhii* result (Amadi-Ikpa et al., 2020).

- *Staphylococcus Aureus*

Isolation for pure culture isolates of *Staphylococcus aureus* as described by Harrigan and McCance (2003) was employed with the use of Mannitol Salt Agar medium. The agar chiefly, was used to isolate *Staphylococcus* species. In carrying out the analysis, an aliquot of the diluted Jollof-rice in a normal saline previously, diluted in a 9ml normal saline, was inoculated on the above said media followed by incubation at 37°C for 24 hrs for colonial Growth of *Staphylococcus* species. In presumptive test to characterized *Staphylococcus aureus,* certain morphological features were considered, with respect to size, shape, colour, elevation and opacity of the isolate/colony on the media plate. In further test to comfirm the identity of *Staphylococcus aureus*, Some key biochemical reactions were adopted such as Coagulase test and Catalase test.

✓ *Coagulase Test*

In carry in out the coagulase test, the slide method of analysis was employed and the test involved placing the *Staphylococcus aureus* on a clean sterilized microscopic slide with an addition of two to three drops of saline and a human plasma, the substance was then mixed thoroughly and allowed to clot. An inference of clotting after few minutes indicated the presumed *Staphylococcus aureus* is coagulase positive while an absence of clot indicated a coagulase negative result for *Staphylococcus aureus* as documented by Amadi-Ikpa et al (2020).

✓ *Catalase Test*

In a follow up to this text, to determines if *Staphylococcus aureus* posses the enzyme catalase, this enzyme are able to catalyze the reaction. The test was done to determine the ability of *Staphylococcus aureus* to breakdown Hydrogen Peroxide into Oxygen and water. In achieving this, a visible effervescent denoted *Staphylococcus aureus* positive when a small inoculums was introduced into a 3% hydrogen Peroxide solution placed on a slide, while the absence of catalase is evident by a lack of or weak production of effervescence or non *Staphylococcus aureus* (Amadi-Ikpa et al., 2020).

✓ *Sugar Fermentation Test*

This sugar test was done to evaluate the ability of the *Staphylococcus aureus* to utilize sugar (lactose and maltose) to produce acid and gas. Peptone broth (1%) incorporated with 1% sugar was used to constitute the sugar broth test. An indicator was added to the sugar medium with Durham tube added in the tube in an inverted position. After sterilization, a loopful of the *Staphylococcus aureus* was introduced into the test tubes and then incubated at 35 -37^0C for 24 - 48 hrs. Change in color from purple to yellow and gas production indicated a positive sugar fermentation test for *Staphylococcus aureus* while no change in color depicted no sugar utilization (Amadi-Ikpa et al., 2020).

✓ *Indole Test*

This test was used to determine the ability of *Staphylococcus aureus* to split the amino acid tryptophan to form pyruvic acid, ammonia and indole using the enzyme tryptophanase. A loopful of *Staphylococcus aureus* was inoculated into sterile peptone water medium and incubated at 37^0C for 48hrs. thereafter, 0.3 – 0.5 ml of Kovac's reagent was added using a pasteur's pipette. Appearance of red ring layer on the medium indicated a positive indole test while development of a yellow ring indicated a negative result (Amadi-Ikpa et al., 2020).

✓ *Motility Test*

This test was used to determine the motility of the *Staphylococcus aureus*. Motility of an organism is determined by the presence of flagella (a locomotory organelle). Semi solid Nutrient agar was used for this test. The media was prepared and the isolate picked with a sterile straight wire into it by stabbing. Thereafter, the medium in the tube was incubated at 37^0C for 24 - 48 hrs. Growth in diffuse form from the line of stab into the medium indicated a positive result, whereas growth only along the line of stab indicated a negative result (Amadi-Ikpa et al., 2020).

➢ *Determination of the Prevalence of the Staphylococcus Aureus and Salmonella Typhi*

The frequency / prevalence at which the bacteria *Staphylococcus aureus* and *Salmonella typhii* occurred in the Jollof-rice was determined as recovered. This involved taking account of the positive and negative plates.

➢ *Antibiotic Susceptibility Testing*

The bacteria, *Staphylococcus aureus* and *Salmonella typhi*i were screened for their susceptibility to medical preparations of Amoxil and Cefuroxime (antibiotics). These antibiotics were noted as the most dispensed in the study area (Bonny). Thus, the test was done to figure out how effective the antibiotics can control *Staphylococcus aureus* and *Salmonella typhii* isolated from the Jollof-rice sample. The isolates were spread evenly on a freshly prepared nutrient media and an aliquot of the antibiotics seeded on a hole bored on the media, incubated for 37^0C for 24 hrs (Prescott et al 1996).

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CHAPTER FOUR
RESULTS

➢ *Enumeration of Bacteria Load*

Enumeration of the Total Heterotrophic Bacteria in 20 Jollof-rice samples, reported varied counts across the restaurants. Viable heterotrophic bacterial counts ranged from 3.0×10^3 to 7.0×10^4 cfu/g , that is the lowest and highest count.

➢ *Prevalence of Salmonella Typhii and Staphylococcus Aureus in Jellof-Rice*

Table 1, shows *Staphylococcus aureus* occurred more frequently than *Salmonella typhii*. Rice samples 1, 2, 4, 7, 9, 11, 12, 16 and 19 had 100% of both organisms.

Table 1 Prevalence of *Salmonella typhii* and *Staphylococcus aureus* in Jollof-rice

Rice Samples	Salmonella Typhii	Staphylococcus Aureus	Total	%
1	+	+	2	100
2	+	+	2	100
3	-	+	1	50
4	+	+	2	100
5	+	-	1	50
6	-	+	1	50
7	+	+	2	100
8	-	+	1	50
9	+	+	2	100
10	-	-	0	0
11	+	+	2	100
12	+	+	2	100
13	+	-	1	50
14	-	+	1	50
15	-	-	0	0
16	+	+	2	100
17	+	-	1	50
18	-	+	1	50
19	+	+	2	100
20	-	+	1	50
% occurrence	12(60%)	15(75%)		

- *Key + = Present, - = Absent, %= Percentage*

➢ *Colonial/Morphological Characteristics of Staphylococcus Aureus and Salmonella Typhii Isolated from the Rice Samples*

Colonial classification of *Staphylococcus aureus* and *Salmonella typhii* as seen in Table 4 showed appearance in terms of color, size, elevation, edge, opacity and shape of the Isolates as well as the isolate reaction to Gram stains. *Staphylococcus aureus*, reacted positive to Gram's reaction, showing yellowish color on Mannitol salt media, with an opaque view/display and large sized colonies with curved edges.

However, *Salmonella typhii* reacted negative to Gram stain with the following colonial features on Salmonella/ Shigella medium; circular shape, whitish colour and translucent texture.

Table 2 Colonial/Morphological Characteristics of Staphylococcus Aureus and Salmonella Typhii Isolated from the Rice Samples

Bacteria	Color	Size	Elevation	Edge	Opacity	Shape	Gram React.
Staphylococcus Aureus	Yellow	Large	Low	Curve	Opaque	Round	+
Salmonella Typhi	Withish	small	Low	curve	Opaque	Round	-

- *Note*:- + = Positive - = Negative

➢ *Biochemical Characterization of the Isolates from the Rice Samples*

The biochemical screening of the isolates, *Staphylococcus aureus* and *Salmonella typhii* was able to show key confirmatory results. Table 3 bellow reportedly, revealed/cofirm *Staphylococcus aureus* with a positive Gram reaction. Followed by; a positive coagulase and catalase reactions which were key. Sugar, motility and citrate indicated positive also, when *Staphylococcus aureus* was screened on the reagents. Indole reaction was however negative.

Confirmatory biochemical reaction screening for *Salmonella typhii* indicated negative for Gram staining. Similarly, Methyl red, Citrate and Hydrogen Sulphide indicated positive when *Salmonella typhii* was screened on the reagents.

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Table 3 Biochemical Characterization Of Staphylococcus Aureus And Salmonella Typhii From The Rice Samples

Coag	Motil	Cata	H_2S	Ind	Citr	MR	VP	Lac	Sucr	Gram React.	Bacteria
+	+	+	-	-	+	+	+	+	+	+	Staphylococcus Aureus
-	-	+	+	-	+	+	+	+	+	-	Salmonella Typhi

- *Note*:- Gram React, = Gram Reaction, Cata = Catalase, H_2S = Hydrogen Sulphide, MR= Methly Red, VP= Voges Proskaurer, Sucr = Sucrose, Lac = Lactose, Mal = Maltose, Positive = +, Negative = -

➢ *Antibiotic Susceptibility and Resistivity of Staphylococcus Aureus and Salmonella Typhii from the Rice Samples*

The resistivity and sensitivity of *Staphylococcus aureus* and *Salmonella typhii* from the rice samples to medical preparations of Amoxile and cefuroxime, showed that out of 42 positive *Salmonella typhii* isolates, 11 and 14 of the bacteria were sensitive to Amoxile and Cefuroxin respectively. However, 9 out of the 42 *Salmonella typhii* were resistance to Amoxil same also 8 out of the 42 were resistance to cefuroxime.

For *Staphylococcus aureus*, 15 and 11 out of the 40 *Staphylococcus aureus* were sensitive to Amoxil and Cefuroxime respectively.

Resistance of the *Staphylococcus aureus* were observed with 5 and 9 bacteria out of the 40 *Staphylococcus aureus* with Amoxile and cefuroxime respectively.

Table 4 Antibiotic Susceptibility and Resistivity of Staphylococcus Aureus and Salmonella Typhii from the Rice Samples

Antimicrobial Agent	Salmonella Typhii		Staphylococcus Aureus	
	No. Sensitive (%)	No. Resistance (%)	No. Sensitive (%)	No. Resistance (%)
Amoxil (500mg)	11(55)	9(45)	15(75)	5(25)
Cefuroxine (500mg)	14(70)	8(30)	11(55)	9(45)

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CHAPTER FIVE
DISCUSSION

➢ *Discussion*

The result for the viable heterotrophic bacteria counts of the rice samples ranged from 3.0 x 10^3 cfu/g to 7.0 x 10^4 cfu/g, thus, may be suggestive of exposure to atmosphere, where the environmental condition may be questionable. Also, the high heterotrophic count may be attributed to carelessness of the vendor or the consumers who in their characteristic way come close to the sales point and at most times may contribute in the delivery of the food bought.

Similar viable counts have been observed by Olaoye et al. (2010) in their study on some ready to eat foods such as moimoi, and they obtained a bacteria count ranged from 2.0 x 10^4 cfu/g to 1.2 x 10^6 cfu/g for bacteria and viable heterotrophic counts range from 8.0 x 10^4 cfu/g to 2.0 x 10^5 cfu/g for fungi. Olaoye et al. (2010) noted that relatively high temperature in tropical countries like Nigeria promotes rapid growth of pathogenic bacteria. Following this, Olaoye et al. (2010) stated that some restaurants are faced with poor method of food preservation technique and as such could encourage elevated heterotrophic count in their food. In line with the standards set by CDC (2006), reports have it that the permissible limit of heterotrophic bacteria in food should not exceed 100 coliform forming unit per gram. The heterotrophic bacteria count in these foods clearly, is in line with the set standard.

The presence of *Staphylococcus aureus* in the food samples suggest poor handling and management plan, which must have led to suspected cross contamination during dishing (Jemikalajal, 2007). *Staphylococcus aureus* is a normal flora of the skin. A situation whereby the hand is sometimes use in dishing food, the hand which might not be sterilized or protected could invariably introduce bacteria into dished food. In yet another circumstance, *Staphylococcus aureus* as a normal flora in the mouth could be introduced into open food when the food vendor or the consumer talk on the yet to be eaten food that is open. Thus, *Staphylococcus aureus* in the food sales point is a pointer to a potential health risk since the organism has been implicated in food-borne diseases.

Salmonella typhi were also present in this study although several studies have implicated the bacteria as the root cause of typhiod fever (Mensah et al, 2001). Several food produces have been associated with *Salmonella* food outbreaks; the food produce could be used as ingredient in the cooking of rice meal. This food produce include: eggs, duck meat, vegetables, tomatoes, hot peppers, lettuce, cucumbers (Mulugeta and Bayeh, 2012) (Mensah et al, 2001).

Staphylococcus aureus isolated from rice samples from these restaurants were all sensitive to concentrations of oflaxacine and cefuroxime. This result agreed with study carried out by Armstong *et al.* (1981) where *Staphylococcus aureus* isolated from raw food treated water exhibited sensitivity amoxil. Basically, Polymerase Chain Reaction analyses revealed that *Staphylococcus aureus* 25 and 45% resistance to amoxil and cefuroxime may be due to the possession of MecA gene (Armstong et al. 1981).

However, *Staphylococcus aureus* isolated from rice samples showed high sensitivity percentages to antibiotic concentrations of cefuroxime and amoxil probably due to the genes responsible for resistance are not well pronounced (Charbek and Brusch, 2019). According to Charbek and Brusch (2019), cephalosporine, tetracycline and older penicillin were resistant to Staphylococcal infections.

The report that *Salmonella* sp. isolates were resistant to all cefuroxime and amoxil concentrations, thus, confirm an earlier report by Koeck et al.(2018), who in their study isolated *Salmonella* sp. from therapy pool and surrounding surface water, from where they discovered the bacteria resistance to tetracycline and penicillin.

➢ *Conclusion*

The study showed that Jollof-rice sold in some restaurants in Bonny Island were contaminated with *Staphylococcus aureus* and *Salmonella typhii*. This indicates inadequate processing and poor handling practices which can pose health risk to the consumers.

➢ *Recommendation*

The study thus recommends that:

- *For safety of Jollof-rice consumers, good hygienic practices should be maintained at restaurants,*
- *Potable water and good hazard analysis critical control point (HACCP) application/implementation should be encouraged.*

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APPENDIX A

Table 5 Enumeration of Bacteria Load

Rice Sample	Total Heterotrophic Count (cfu/g)
1	1.3×10^4
2	8.0×10^3
3	1.1×10^4
4	3.0×10^3
5	1.3×10^4
6	7.0×10^3
7	7.0×10^3
8	8.0×10^3
9	6.0×10^3
10	5.0×10^3
11	3.0×10^3
12	4.0×10^3
13	6.0×10^4
14	6.0×10^4
15	7.0×10^4
16	1.2×10^4
17	1.1×10^4
18	9.0×10^3
19	4.0×10^3
20	8.0×10^3

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APPENDIX B

Rice Sample	Salmonella Typhi		Staphylococcus Aureus		% Resistivity
	Amoxil	Cefuroxime	Amoxil	Cefuroxine	
1	S	S	S	S	0
2	R	R	S	R	75
3	S	S	S	S	0
4	S	S	R	S	25
5	R	S	S	R	50
6	S	R	S	S	25
7	R	R	S	R	75
8	S	S	S	S	0
10	R	S	R	R	75
11	S	S	S	S	0
12	S	S	S	S	0
13	S	R	R	S	50
14	R	S	S	R	50
15	R	S	R	R	75
16	S	S	S	S	0
17	S	S	S	S	25
18	R	S	S	R	50
19	S	R	S	S	25
20	R	S	S	R	50

- *Keys, R= Resistance, S= Sensitive*