ISOLATION AND CHARACTERIZATION OF *Staphylococcus aureus* FROM SELECTED SEAFOODS IN BENIN CITY

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN,

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JUNE, 2021

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc (HONS) IN MICROBIOLOGY

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CERTIFICATION

This is to certify that this project work was carried out by **Vanessa Oghomwen ARASE** with the matriculation number **LSC1605368** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City..

Dr. E. O Igbinosa
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Date

APPROVAL

This project work was carried out by **Vanessa Oghomwen ARASE** under the supervision of **Dr. E. O Igbinosa** in partial fulfillment of the award of a Bachelor of Science (B.Sc) degree in the Department of Microbiology, University of Benin, Benin City.

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Prof. S.O Omonigho   **Date**

(Head of Department**)**

DEDICATION

This project work is dedicated to God Almighty for His unmerited mercy and grace over my life.

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This research project is a complex entity and emerges from the contributions of many people. First and foremost, praises and thanks to God Almighty, for His showers of blessings throughout the preparation for my undergraduate research project.

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ABSTRACT

Seafood is any form of sea life regarded as food by humans, prominently including fish and shellfish. Seafood is an important source of protein in many diets around the world, especially in coastal areas. Microbiological surveillance of sea food such as crayfish, periwinkle, fish and shrimp's products provides empirical data to enlighten scientific guidance for improving the safety and quality of food.

A total of 52 seafood samples which include 6 shrimps, 15 crayfish, 7 smoked fish, 9 frozen fish, and 15 periwinkles from five local markets in Benin City, Nigeria were assessed. The fish samples were microbiologically assessed using cultural, biochemical and antibiotic susceptibility techniques.

The occurrence of *Staphylococcus aureus* were shrimps [0/6 (0%)], crayfish [4/15 (26.7%)], smoked fish [4/7 (57.1%)], frozen fish [3/9 (33.3%)] and periwinkles [4/15 (26.7%)]. The highest prevalence occurred in smoked fish samples while there was no occurrence observed in shrimp samples.

The distribution of *Staphylococcus* species based on market location were Ekiosa market [2/8 (25%)], Uselu market [1/9 (11.1%)], New Benin market [1/13 (7.7%)], New Market [(4/9) (44.4%)] and Oba market [7/13 (53.9%)]. The highest prevalence was observed at Oba market (53.9%) while the least prevalence was observed at New Benin market (7.7%). The highest antibiotic resistance was demonstrated to penicillin with a resistance rate of 100% while there was no resistance observed towards nitrofurantoin and gentamicin. The multiple antibiotics resistance (MAR) index of *Staphylococcus aureus* in this study ranged from 0.7 – 0.3. All the isolates were resistant to at least two antibiotics and demonstrated an MAR index ≥ 0.3.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

Seafood is any form of sea life regarded as food by humans, prominently including fish and shellfish. Shellfish include various species of molluscs (e.g. bivalve molluscs such as clams, oysters, and mussels and cephalopods such as octopus and squid), crustaceans (e.g. shrimp, crabs, and lobster), and echinoderms (e.g. sea cucumbers and sea urchins). Historically, marine mammals such as cetaceans (whales and dolphins) as well as seals have been eaten as food, though that happens to a lesser extent in modern times. Harvesting of wild seafood is usually known as fishing or hunting, while the cultivation and farming of seafood is known as aquaculture or fish farming (in the case of fish). Seafood is often colloquially distinguished from meat; vegetarians who consume seafood as the only source of meat are said to adhere to pescetarianism. Seafood is an important source of (animal) protein in many diets around the world, especially in coastal areas.

The Nigerian domestic seafood market is a mix of the modern and the traditional. In the urban centers the wealthy buy western style products (at western prices plus), but the majority of the population, (rural communities and those in poorer urban areas) depend upon traditional markets, mostly selling traditional products. Shrimp, as a very perishable product, is mostly sold smoked, unless sold close to point of capture where fresh/live product can be on sale. Distribution relies upon small traders who buy and deliver small quantities of dried/smoked seafood to rural markets using local transport (Angelo *et al.,* 2016). This is a trade dominated by women, the fish mammies, who tend to control all artisanal post-harvest activities throughout West Africa, often within family businesses where the men folk fish while the women manage and sell. Rural markets are neither hygienic nor clean, with food safety a matter for buyers. There has been some impact of western food products upon the traditional sector. Recently, improved distribution and cold storage networks have allowed a huge increase in marketing of low-cost frozen and Nigeria has become a leading importer of this category. The wealthy urban market is, in contrast, western in structure and approach to hygiene products are frozen and retailed in consumer packs (Iwamoto *et al.,* 2010).

Microbiological surveillance of sea food such as crayfish, periwinkle, fish and shrimps products provides empirical data to enlighten scientific guidance for improving the safety and quality of food. Surveillance data may be useful in explaining research priorities based on risk assessments and enlightening the development of seafood safety standards (Angelo *et al.,* 2016). In recent years, the demand for seafood products has consistently increased in open markets in southern Nigeria. From the farm to the consumer, processing, transportation, and storage of seafood products possibly enables nutrient content and growth conditions to support unwanted microbial proliferation. The patronage of seafood centers within Benin City have also increased over time, as parents and guardians have become busy and as such prefer to buy convenient food instead of preparing it. Four out of every five Nigerian visit a fast food center at least once a day. Due to the increased consumption of seafood in most parts of the world, there has been an increased need to determine its safety, especially when prepared and sold in fast food centers and cafeteria In Nigeria, there is little or no knowledge of foodborne diseases and their transmission among food handlers working in food centers, and no rules are provided from the establishment of food centers. Most proprietors of seafood centers are not duly licensed, and their staff properly selected. All these necessitated the need to examine the microbiological quality of food from food centers to reduce the risk of food poisoning.

Due to the fact that these seafood products are not always given additional bactericidal treatment prior to consumption, the contamination of seafood products by foodborne pathogens continues to draw attention. *Staphylococcus aureus, Listeria monocytogenes, Vibrio* species*, Escherichia coli, Campylobacter jejuni and Salmonella enterica* contamination accounts for the greatest number of seafood products (Yang *et al.,* 2016). *Staphylococcus aureus* is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as "grape-like." On media, these organisms can grow in up to 10% salt, and colonies are often golden or yellow (*aureus* means golden or yellow). These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18℃ and 40℃ (Rasigade and Vandenesch, 2014). *Staphylococcus* food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus*. About 25% of people and animals have *Staphylococcus* on their skin and in their nose. It usually does not cause illness in healthy people, but *Staphylococcus* has the ability to make toxins that can cause food poisoning (Tong *et al.,* 2015).

1.2 Aim and Objectives of the study

The aim of this study was to determine the prevalence and antimicrobial resistance profile of *Staphylococcus aureus* from sea food sold in local markets in Benin City, Edo State.

The specific objectives of this study were to:

1. isolate and characterize *Staphylococcus aureus* from seafood;
2. evaluate the prevalence of *Staphylococcus aureus* from the seafood;
3. determine the antimicrobial susceptibility profile of the isolates,
4. determine the multiple antibiotic profile of the isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sea food

Seafood is often colloquially distinguished from meat as vegetarians who consume seafood as the only source of meat are said to adhere to pescetarianism. Seafood is an important source of (animal) protein in many diets around the world, especially in coastal areas (Mizan *et al*., 2015).

2.2 Categories of seafood

2.2.1 Fish

Fish are aquatic vertebrates which lack limbs with digits, use gills to breathe, and have heads protected by hard bone or cartilage skulls (Bakre *et al.,* 2018). The main seafood groups can be divided into larger predator fish (sharks, tuna, billfish, mahi-mahi, mackerel, and salmon) and smaller forage fish (herring, sardines, sprats, anchovies, and menhaden). The smaller forage fish feed on plankton, and can accumulate toxins to a degree. The larger predator fish feed on the forage fish, and accumulate toxins to a much higher degree than the forage fish. Some seafood groups are cod, flatfish, grouper and stingrays. Demersal fish feed mainly on crustaceans they find on the sea floor, and are more sedentary than the pelagic fish. Pelagic fish usually have the red flesh characteristic of the powerful swimming muscles they need, while demersal fish usually have white flesh (Mizan *et al*., 2015).

Freshwater fish live in rivers, lakes, reservoirs, and ponds. Fresh fish is a highly perishable food product, so it must be eaten promptly or discarded; it can be kept for only a short time. In many countries, fresh fish are filleted and displayed for sale on a bed of crushed ice or refrigerated. Fresh fish is most commonly found near bodies of water, but the advent of refrigerated train and truck transportation has made fresh fish more widely available inland (Bakre *et al.,* 2018).

2.2.2 Shellfish

Shellfish resources found in Nigerian inshore and offshore waters can be classified into various groups which include moluscans and crustaceans

2.2.2.1 Moluscans

Apart from their subsistence harvest by coastal populations they are not abundant in such number to warrant commercial exploitation. Members of the families' cassidae, cepridulidae, cymatidae, fissurellidae, haliotidae, melongenidae, muricidae, strombidae and volutidae are suitable ingredients used in seafood dishes of coastal communities. Mollusc shells are chiefly made up of layers of calcium carbonate. On account of this, it is used (in powdery form) by nutritionists in poultry feeds. Some gastropods shells are collected and sold as ornaments.

The bivalves consist mainly of oysters e.g *O. tulipa* and the less saline species *Crassostrea gasar*. The mangrove oyster *Crassostrea gasar* is a regular harvest of coastal swamp areas in Nigeria. Oyster meat fetches high prices in Europe, North America and Japan. Apart from the coastal areas, oysters are virtually unknown as food in Nigeria. The bivalves of food value in Nigerian coastal states include the ark clams (*Senila senilis* and *Anadara senegalensis*), cockles (*Cardium costatum*), donacid clams (*Iphigenia delesserti* and *Donax rugosus*). Most of these organisms are intertidal where their exploitation is at subsistence level (Bakre *et al.,* 2018).

The univalves lie in or on the sediment of the mangrove swamp; they are usually submerged and exposed at low tides. Dominant members of the in fauna in Nigerian estuaries are *Pachymelina quadriserata* and *Tympanotamus fuscatus* (periwinkle). They are mostly eaten as food. Most of its empty shells are inhabited by hermit crabs.

2.2.2.2 Crustaceans

Commercially important species of marine and freshwater crustaceans belongs to the order decapoda. They include the families penaeidae, palaemonidae, portunidae (swimming crabs) and the palinuridae (lobsters). Shrimps in Nigeria include *Peneaus notialis* (white shrimp), *Peneaus monodon* (black tiger shrimp), *Peneaus kerathurus* (tiger/stripe shrimp), *Parapeneopsis atlantica* (Guinea/ brown shrimp), *Peneaus duorarum* (pinkshrimp), *Peneaus longirostris* (red/ royal shrimp), *Plesiopenaeus edwardsianus* and *Solonocera africana*. The artisanal fishermen shrimp between 0-5 nm while the industrial fleet shrimp from 5nm and beyond. Shrimp grounds cover 2500 km^2 off Nigeria. Shrimp stocks are found in abundance off Badagry to Lagos – Lekki lagoon systems and mouth of rivers on the mouth of the delta from Benin to Pennington and from River Bonny to the Cross River estuary (Tobor, 1992). Shrimp resources are abundant around river mouths and lagoon entrances. The species mostly exploited are the pink shrimp, *Penaeus notialis*, which is most abundant and most valued economically; Guinea shrimp, *Parapenaeopsis atlantica*; tiger shrimp, *Penaeus kerathurus* and the royal shrimp, *Parapenaeus longirostris*. *Penaeus notialis* prefers the supra-thermocline muddy sand with fine particles and abundant organic matters at 25°C. Concentrations are particularly high in the Niger Delta at 20-30m.

Parapenaeopsis atlantica is prevalent at 10-40m depth while *Parapenaeus longirostris* is found in deep waters from 60-400 m. Exclusively exploited by small scale operators with passive cane or netting gear in the estuary and with small trawls in the surf zones, the estuarine prawn *Nematopaleamon hastatus*, a major shrimp fishery is heavily fished in the creeks and limited to depths up to about 50m. It constitutes about 50 percent in terms of estuarine catches. According to Zabbey, (2006), an important and interesting feature of late has been the arrival of wild *Penaeus monodon* specimens in trawler catches. *Penaeus monodon* (black tiger shrimp) appeared 4 years ago, and apparently occurs mainly in the Calabar/eastern delta zone where it comprises as much as 10% of trawler catches. This is an Asiatic exotic that could have only arrived by man's agency (African current patterns preclude natural introduction), and presumably escaped from a West African (Gambian, Senegalese or Cameroonian) shrimp farm. Other species that can be ranked as important in the small-scale shrimp sector in the sense that they are harvested for sales include *Desmocaris trispinosa*, (Guinea swamp shrimp), *Palaemon maculatus* (Zaire prawn) and *Palaemonetes africanus* (creek shrimp). Some shrimp species such as *Leander tenuicornis* (biocellate prawn) and *Palaemon elagans* (rockpool prawn) do occur incidentally in local catches, which according to Powell (1982), are never intentionally fished for. The shrimp season in the estuaries is during the dry season between November and May. At sea, it is all year round with peaks during the rains from May to September.

Prawns are entirely fished from the wild and harvested as far inland as 250 km in the various river systems and lakes mainly in the southern part of Nigeria. Production areas are restricted to Asejire (Oyo state), Port-Harcourt (Rivers state), Calabar (Cross River state) and Lokoja (Kogi state). A few species such as *Atya gabonensis* occur inland around the Middle-Belt areas especially Makurdi. The locally occurring freshwater prawn of economic importance in Nigeria is *Macrobrachium vollenhovenii* (African river prawn). A second one of less importance is *M. macrobrachion*, which occurs in brackish waters in Badagry, Port-Harcourt, and Calabar areas. Other species of less economic importance are *M. felicinum* (Niger River prawn), which is found around the Niger-Delta areas, and *Atya garbonensis* found around the Middle-Belt areas (Zabbey, 2007). They are fished using mainly fishing traps, which are usually baited with fish, coconut, cassava, onions and palm-kernel nuts. To a lesser extent, they are caught in cast and gill nets as incidentals while targeting some other fin fishes. During the breeding season (at peak of rainfall) some of the species, particularly the African river prawn, *Macrobrachium* *vollenhovenii* migrates to the brackish waters perhaps to breed. At this time it is caught with *M. macrobrachion* though relatively in fewer quantity (USAID, 2002).

The marine species of true crabs (brachyurians) are divisible into three (3) groups. These are the terrestrial (land-based) crabs found on the fringes of estuaries, and are mainly members of the family gecarcinidae, grapsidae and ocypopidae e.g. Ocypode africana; the swimming species in inshore waters and even in estuaries, all belonging to the family portunidae, and typical examples of these species in Nigerian waters include *Callinectes pallidus* (blue crab), *Callinectes amnicola* (big-fisted swim crab) and *Cardiosoma armatum*. The third group is the deep sea crabs (geryonidae) and is part of the benthic ecosystem on the continental slope.

Spiny lobsters (palinuridae) and locust lobsters (syllaridae) inhabit the coastal waters of Nigeria. They are entirely of marine origin. Only one species, *Pallinurus regius*, the royal spiny lobster, is common and prefers rocky substrates in depths of 5-15m. As a stock, it has no commercial importance in view of its low magnitude, although lobster meat is of high table quality (Li *et al.,* 2020).

2.3 Microbiology of seafood

Shrimps, fish sea food is one of the most essential nutritional needs of every community. Often, consumption of contaminated shrimp cause gastrointestinal diseases in human. Staphylococcal food poisoning causes vomiting, diarrhoea and abdominal cramps within two to six hours after the ingestion of food contaminated with SEs (Angelo *et al*., 2016). This bacterium does not require any particular nutritional or environmental factors for growth. The bacteria can also grow in substrates with a low water activity of 0.86, over a wide temperature range of 7 to 48^{o}C, and at pH values ranging from 4.2 to 9.3.

Several studies in other countries have investigated the potential paths of transmission of this dangerous strain by human carriers or environment, such as transport and packaging, contaminated hands of workers, and the contact of infected respiratory secretions with seafood products. In some parts of the world, more than 50% of food poisoning is caused by SEA. In Great Britain and America, SEA and SEB are the cause of more than 69% of all food poisonings. The symptoms are more severe in children, pregnant women, elderly, and patients who are undergoing tumor therapy or are taking immune suppressing drugs; due to quick digestion and proper absorption of protein and minerals, these population groups consume more shrimp, therefore the safety of these products becomes more critical. On the other hand, because of its tissue, shrimp meat has a high potential for corruption. Improper conditions during fishing and storage and non-standard transportation provide a good ground for pathogens growth unfortunately improper cooking method used for these products is the most important reason for causing disease.

All these factors increase the risk of gastroenteritis and food poisoning caused by contaminated food. Food borne diseases are a large group of the global diseases and are one of the most important problems in every community. *Staphylococcus aureus* is considered as the third most important cause of food borne illnesses reported worldwide. This bacterium is one of the most common agents in food poisoning outbreak. In Iran, food poisoning outbreaks by *Staphylococcus aureus* have increased during recent years. This might be due to changes in the environment, the development of the food service industry, and communal feeding. PCR-based techniques are commonly used for typing, as they are easy, fast, and cost-effective.

Seafood is responsible for a significant amount of foodborne diseases and represents a great concern from a public health perspective. Bacterial load of raw fish depends on the environmental condition and microbial quality of the water where fish is hunted, temperature of the water, salt content of the water, distance of hunting area from areas contaminated with human and animal feces, fishing method and cooling conditions (Saito *et al*., 2011). Vibrio cholerae, *Vibrio parahemolyticus*, *Vibrio vulnificus*, *Listeria monocytogenes*, *Clostridium botulinum* and *Aeromonas hydrophila* are pathogenic bacteria that found naturally in the sea and rivers and they may infect to humans carried by fisheries (Da Silva *et al*., 2010).

2.4 *Staphylococcus aureus*

Staphylococcus aureus is considered to be the most important pathogen among staphylococci responsible for a variety of severe infections including foodborne poisoning, skin and soft tissue infections, nosocomial riers of *Staphylococcus aureus* play a crucial role as a source of infection in dissemination of this pathogen among humans and to food through direct and indirect contact (Becker and von Eiff, 2011). Therefore, *Staphylococcus aureus* can be found commonly in various foods including meat (Jackson *et al*., 2013) and fish. It is clear that contamination of food with this pathogen is often related to improper handling and storage conditions, as well as inadequate hygienic conditions and post-production microbial contamination (Ray, 2004).

The present study also provides information on the incidence, enterotoxigenic potentials and antimicrobial resistance of *Staphylococcus aureus* in fish as a vital source of nutrition to humans due to their proteinaceous nature. Although many researchers have reported the presence of *S. aureus* in various foods such as meat and dairy products, there are a limited number of reports on contamination of fish with *Staphylococcus aureus* (Saito *et al.,* 2011).

2.4.1 Characteristics of *Staphylococcus aureus* in the food environment

Staphylococcus is a spherical, nonsporulating, nonmotile bacterium (coccus) that, when observed under the microscope, occurs in pairs, short chains or grape-like clusters. These facultative aero-anaerobic bacteria are Gram- and catalase positive. Staphylococci are ubiquitous in the environment and can be found in the air, dust, sewage, water, environmental surfaces, humans and animals. Common biochemical tests used in the identification tests include catalase positive (all pathogenic *Staphylococcus* species), coagulase positive to distinguish *Staphylococcus aureus* from other *Staphylococcus* species, novobiocin sensitive to distinguish *Staphylococcus saprophyticus* and mannitol fermentation positive to distinguish *Staphylococcus aureus* from *Staphylococcus aureus epidermidis.*

To date, more than 50 species and subspecies of staphylococci have been described according to their potential to produce coagulase. Their classification thus distinguishes between coagulase-producing strains, designated as coagulase-positive staphylococci (CPS) and noncoagulase-producing strains, called coagulase-negative staphylococci (CNS). Among CNS, some species are known to play an important role in the fermentation of meat and milk-based products and are therefore considered as food grade. The enterotoxigenic potential of CNS has always been a subject of controversy. Several investigations failed to detect enterotoxin production or enterotoxin-like gene in CNS (Becker *et al*., 2001). However, some studies found that certain CNS strains were able to produce enterotoxins which could lead to food poisoning. More recently, another study demonstrated that, among 129 CNS strains isolated from fermented foodstuffs, only one carried SE genes (Even *et al*., 2010).

2.4.2 Reservoirs

Staphylococcus aureus belongs to the normal flora found on the skin and mucous membranes of mammals and birds. This bacterium can be disseminated in the environment of its hosts and survives for long periods in these areas. Several biotypes isolated from different hosts (human, poultry, cattle and sheep/goat) have been described within *Staphylococcus aureus* species demonstrating the close adaptation of the bacterium to its host. They were identified according to four biochemical tests (staphylokinase, ß-haemolysin production, coagulation of bovine plasma and growth type on crystal violet agar) following the simplified biotyping scheme described by Devriese (1984). However, many strains cannot be assigned to these host-specific biotypes and belong to nonhost-specific (NHS) biotypes, i.e., those associated with several hosts. Later, a poultry-like biotype associated with meat products and meat workers was tentatively designated as a 'slaughterhouse' biotype by (Isigidi *et al*., 1990). Indeed, introduction of an additional biochemical test, protein A production, and phage typing allowed researchers to differentiate the poultry biotype from this new biotype. However, as the protein A test is no longer commercially available, and as phage typing cannot be routinely used, these two biotypes cannot be easily distinguished.

2.5. Analytical methods for SE detection

Diagnosis of SFP is generally confirmed either by the recovery of at least 105 *Staphylococcus aureus* g−1 from food remnants or by the detection of SEs in food remnants. In some cases, confirmation of SFP is difficult because *S. aureus* is heat sensitive, whereas SEs are not. Thus, in heat-treated food matrices, *S. aureus* may be eliminated without inactivating SEs. In such cases, it is not possible to characterize a food poisoning outbreak by enumerating CPS in food remnants or detecting se genes in isolated strains. *S. aureus* is usually enumerated using microbiological techniques with dedicated media such as Baird Parker or rabbit plasma fibrinogen agar. However, methods are used to detect bacterial toxins in food include bioassays, molecular biology and/or immunological techniques.

2.5.1 Bioassays

Bioassays are based on the capacity of an extract of the suspected food to induce symptoms such as vomiting, gastrointestinal symptoms in animals and/or superantigenic action in cell cultures. Historically, SEs have been detected based on their emetic activity in monkey-feeding and kitten-intraperitoneal tests and, more recently, using animal models such as house musk shrews Suncus murinus. Symptoms of SFP appear if the dose of SEA ingested by the animals is above 2.3 μg, a considerably higher amount than those involved in human food poisoning (Ostyn *et al.,* 2010). Thus, this technique is not appropriate for characterizing SFPOs.

2.5.2 Molecular methods

Molecular biology methods often involve the polymerase chain reaction (PCR). These methods usually detect genes encoding enterotoxins in strains of *Staphylococcus aureus* isolated from contaminated foods. However, these methods have two major limitations: first, staphylococcal strains must be isolated from food, and second, the results inform as to the presence or absence of genes encoding SEs, but do not provide any information on the expression of these genes in food. This method therefore cannot be the sole method for confirming *S. aureus* as causative agent in an outbreak. However, the PCR approach is a specific, highly sensitive and rapid method that can characterize the S. aureus strains involved in SFPOs, thereby providing highly valuable information. In outbreaks described by (Ostyn *et al*., 2010), SEE has been found in the common source vehicle and the see gene was present in the tested S. aureus isolates. In such a case, se gene determination helps to confirm the role of an SE rarely encountered. Very recent efforts have been directed to determining directly which se genes are found in suspected foods. Following the huge SFP event which occurred in Japan in July 2000 (more than 13 000 people were intoxicated by powdered or liquid milk), (Ikeda *et al*., 2005) developed a PCR-based methodology whereby sea, seg, seh and sei genes could be detected in the incriminated powdered skim milk, although cultivable *S. aureus* were not recovered from the sample. Moreover, to evaluate the toxic potential of strains isolated from SFPOs, various authors (Derzelle *et al*., 2009) have recently designed primers to perform PCR and reverse transcription PCR (RT-PCR) for SE genes.

Finally, (Duquenne *et al.,* 2010) developed an efficient method for extracting bacterial RNA accessible for RT-quantitative PCR (RT-qPCR) from cheese and adapted a simple, sensitive and reproducible, method for quantifying relative transcript levels to evaluate S. aureus enterotoxin gene expression during cheese manufacture. These approaches demonstrate possible transcription of mRNA from those genes, but do not indicate whether those strains were able to produce detectable or poisonous levels of toxins in food.

2.5.3 Immunological methods

The third and most commonly used method for detecting SEs in food is based on the use of anti-enterotoxin polyclonal or monoclonal antibodies. Commercially available kits have been developed according to two different principles: (1) enzyme immunoassay (EIA) comprising ELISA and enzyme-linked fluorescent assay (ELFA); and (2) RPLA. It is widely recognized that the use of immunological methods to detect contaminants in food matrices is a difficult task, mainly because of the lack of specificity and sensitivity of the assay. Many drawbacks impair the development and use of these techniques for detecting SEs. First, highly purified toxins are needed to raise specific antibodies to develop an EIA; purified toxins are difficult and expensive to obtain. Moreover, and until very recently, only antibodies against SEA to SEE, SEG, SEH and SElQ were available (Schlievert and Case, 2007).

The ELISA test will not detect the other SEs, which could partly explain why some outbreaks remained uncharacterized without a known aetiological agent. Another drawback is the low specificity of some commercial kits, where false positives may occur depending on food components as it is well known that some proteins, such as protein A, can interfere with binding to the Fc fragment (and, to a lesser extent, Fab fragments) in immunoglobulin G from several animal species, such as mouse or rabbit, but not rat or goat. Other interferences are associated with endogenous enzymes, such as alkaline phosphatase or lactoperoxidase. Whatever the detection method used and owing to the low amount of SEs present in food, it is crucial to concentrate the extract before performing detection assays. For this purpose, various methodologies have been tested. Among them, only extraction followed by dialysis concentration has been approved by the European Union for extracting SEs from food. However, up to now, after enumerating CPS strains, conclusive diagnosis of SFPs has mainly been based on demonstrating the presence of SEs in food using commercial EIA kits designed to detect SEA to SEE (Bennett, 2005) or using a confirmatory in-house ELISA method to differentiate and quantify these types of SEs.

2.5.4 Mass spectrometry-based methods

Owing to the drawbacks with currently available detection methods and the lack of available antibodies against the newly described SEs, other strategies based on physicochemical techniques have been developed very recently. Among these, mass spectrometry (MS) has newly emerged as a very promising and suitable technique for analysing protein and peptide mixtures (Mamone *et al*., 2009). It is among the most sensitive techniques currently available because it provides specific, rapid and reliable analytical quantification of the amount of enterotoxins (Brun *et a*l., 2007). The development of two soft ionization methods, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), and the use of appropriate mass analyzers such as time-of flight (TOF) have revolutionized the analysis of biomolecules. Given the wide range of methodologies available, a single MS technique cannot be used for all proteins. The MS method thus requires the development of a series of techniques, individually suited for each particular case. In the case of food analysis, the situation is complex because the matrix can contain many proteins, lipids and many other molecular species that interfere with the detection of the targeted toxin and may distort quantification. Sample preparation remains the critical step of the analysis. Several authors have tried to improve this step, by, for example, optimizing digestion parameters (Norrgran *et al*., 2009) or by adding a purification step (Oeljeklaus *et al*., 2009). The strategy of incorporating an isotopically labeled internal standard into the samples has also been developed. In the case of SE detection, some authors have developed MS tools to detect these toxins in culture supernatants and in spiked samples, such as water or apple juice. For example, (Bernardo *et al*., 2002) developed a MALDI-TOF method for detecting S. aureus virulence factors such as enterotoxins and demonstrated that this technique was suitable for detecting SEs other than SEA to SEE in culture supernatants. (Callahan *et al*., 2006) detected and quantified SEB using liquid chromatography coupled to ESI/MS detection in apple juice used as a model food matrix. In this study, enterotoxin types SEA and SEB were detected in spiked cheese. More recently, (Brun *et al*., 2007) developed an MS approach able to perform absolute quantification of SEA and TSST1 in spiked water or urine samples. To improve characterization and quantification of SEs, this latter methodology was successfully used to carry out absolute quantification of SEA in a naturally contaminated cheese sample (Dupuis *et al*., 2008) and applied to a recent case of food poisoning outbreak (Hennekinne *et al*., 2009). In this outbreak, MS tools in combination with tools presented earlier were used by the European Union Reference Laboratory for CPS. This MS-based method overcame specific technical limitations of existing ELISA for SE characterization but its throughput and cost per analysis compared unfavourably with ELISA. This last method was no doubt the gold standard of low-cost and high-throughput techniques for the detection and quantification of protein compounds down to subnanomolar concentrations in large sample cohorts. However, the timescale for ELISA assay development was of the order of 1 year and high developmental costs precluded systematic ELISA optimization. This cost also made ELISA less suitable for the characterization of small panels such as SFP elucidation. In this regard, the versatility and low development cost of the absolute quantification methodology positioned it as a good alternative to ELISA for these specific applications, keeping in mind that purified SEs standards were also needed to establish the accuracy and specificity of MS-based methods. Thus, combining classical microbiology for enumerating CPS strains with immunological techniques, molecular biology and mass spectrometry-based methods, the diagnosis was reinforced and these outbreaks could be attributed to the presence of SEs.

2.6 *Staphylococcus* contamination

Staphylococcus aureus is not part of the natural microbiota of live fish, its presence in fish and fishery products is due to unhygienic handling, cross-contamination during storage, or contamination by workers who are asymptomatic carriers of coagulase-positive *S. aureus* strains. *S. aureus* has been found in cultured fish, fresh seafood, ready-to-cook and ready-to-eat seafood products, seafood processing environments, and the hands of seafood workers. *Staphylococcus aureus* has been found in cultured fish, fresh seafood and ready-to-eat seafood products, seafood processing environments, and the hands of seafood workers. *Staphylococcus aureus* contamination of fish occurs after harvesting and the rate of contamination increases as the fish pass through various stages of processing and packing and is highest in processed fish. The prevalence in imported seafood of *S. aureus* strains resistant to multiple antibiotics has raised concerns that seafood can be responsible for the global spread of such resistant strains.

2.7 Methicillin Resistance *Staphylococcus aureus*

Staphylococcus aureus is responsible for life-threatening human infections acquired in hospitals and within the community (Moran *et al*., 2006). The first methicillin-resistant *Staphylococcus aureus* (MRSA) infection was described in 1961 (Jevons, 1961) and since then, human infections caused by multi-drug-resistant MRSA have become common (Waness, 2010). The MRSA phenotype results from the acquisition of the staphylococcal cassette chromosome mec (SCCmec), which harbors the mecA gene that encodes the low-affinity penicillin-binding protein 2a (PBP2a) (Katayama *et al*., 2000). The structure of numerous SCCmec elements reveals that this element is following multiple evolutionary trajectories.

Staphylococcus aureus is also a major cause of bovine mastitis worldwide, which results in substantial economic losses for the dairy industry. Methicillin-resistant *Staphylococcus aureus*-causing bovine mastitis was first reported in 1972 (Devriese *et al*., 1972) and MRSA transmission appears to occur between animals, and from animals to humans (Lee, 2003; Voss *et al*., 2005; Lozano *et al.,* 2011a). Carriage of MRSA by livestock has been shown to correlate with the MRSA colonization of farmers and farm families, veterinarians, and health-care workers. Sequencing of 7 housekeeping gene amplicons has been used to group MRSA isolates into clonal complexes (CC) using “based upon related sequence types,” or BURST, analysis (Enright *et al*., 2000). These analyses have revealed that certain strains, referred to as livestock-associated MRSA, belong to a small number of CC, with the most prevalent being CC398, and it is known that CC398 strains also cause infections in humans. A recent report suggests that

CC398 strains emerged from a human evolved methicillin-susceptible Staph. aureus (MSSA) strain and that livestock-associated MRSA sublineages arose during antimicrobial selection in livestock (Price *et al*., 2012).

2.8 Virulence factor

Staphylococcus aureus possesses many virulence factors and the most notable are the five major classical types of staphylococcal enterotoxins (SEs: SEA to SEE), the non-classical SE-like toxins (SEl: SEG to SEU), and other virulence genes such as toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins and cytolytic toxins (leukocidin and hemolysins). Staphylococcal enterotoxins (SEs) are heat stable proteins that are mainly associated with food poisoning outbreaks, while TSST-1 is a superantigenic exotoxin that causes toxic shock syndrome. The exfoliative toxins are responsible for staphylococcal scalded skin syndrome that typically affects infants and young children, lukPV cytotoxin causes leukocytosis with necrotic lesions in the skin or mucosa while hemolysins involve epithelial barrier disruption.

Staphylococcus aureus is one of the leading etiologic agents of hospital infections (Le Loir *et al*., 2003). It is known to cause a number of pathological conditions in humans and animals like bacteremia, urinary system infections, systemic diseases, osteomyelitis (Hageman *et al*., 2006). Toxins produced by *S. aureus* are one of the most frequent causes of bacterial food poisonings (Hennekinne *et al*., 2012). Foodstuff contamination may result from poor hygiene during production processes or the retail and storage of food. The pathogenic nature of S. aureus is related to the high genotypic and phenotypic heterogeneity of its strains. This results mainly from the abilities of staphylococci to exchange genetic material through mobile genetic elements. Growing antibiotic resistance in *S. aureus* strains is a worldwide problem. The most worrying are strains resistant to methicillin (Methicillin-resistant *Staphylococcus aureus* -

MRSA). The presence of antibiotic resistant strains among food derived microorganisms suggests that it may play a much more important role in transferring the antibiotic resistance encoding genes than previously thought (Chajęcka-Wierzchowska *et al.,* 2016). Antibiotics are used for prophylactic purposes in swine, cattle, rabbit and poultry production as well as in vegetable and fruit cultivation and beekeeping (Ding and He, 2010) and, thus, the meat of farm animals, fruit, vegetables and water may be a source of resistant strains. Food and feed safety is essential, and the presence of MRSA in the food chain may contribute to the increasing dissemination of MRSA worldwide (Oniciuc *et al*., 2017). The multi-drug resistant S. aureus strains may have an increased ability to spread. This does not only provide therapeutic challenges for clinicians but may be very detrimental to human health. The study was designed to determine the occurrence of *S. aureus* and MRSA strains in ready-to-eat food products as sushi, salads, and hamburgers in Poland. Moreover, the presence of genes which encode resistance to methicillin and tetracycline's was investigated.

Staphylococcus aureus is a well-known opportunistic foodborne pathogen, and is involved in numerous nosocomial and community-associated (CA) outbreaks worldwide (Paterson *et al*., 2014). The widespread use of antibiotics, and particularly inappropriate use or overuse, has facilitated the emergence of pathogens resistant to antibiotics, such as methicillin-resistant *S. aureus* (MRSA).

2.8.1 **Risk factors**

Risk factors in the production of ready to eat (RTE) cooked and processed meat products include inadequate cooking, ineffective cooling after cooking, lack of temperature control during storage and distribution and poor standard of hygiene during post-processing handling and packing.

For meat products that are cured and cooked, incorrect levels of added curing substances (salt and nitrite) also contribute (MLA, 2015) Temperature abuse may allow growth of *S. aureus* and potential SE production.

2.8.2 Risk mitigation

Cooking applied in the production of RTE cooked and processed meat products is lethal to *Staphylococcus aureus*, but not to SE. Time and temperature abuse of food products should be avoided by applying good practices of temperature control in food manufacturing and handling. Good manufacturing practices and good hygienic practices will also play a role in preventing SFP.

2.9 *Staphylococcus aureus* and its food poisoning toxins characterization

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases in the world following the ingestion of staphylococcal enterotoxins (SEs) that are produced by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mainly *Staphylococcus aureus* (Jablonski and Bohach, 1997) and very occasionally by other staphylococci species such as Staphylococcus intermedius (Khambaty *et al*., 1994). When outbreaks occurred during large social events, chaotic situations resulted requiring the rapid implementation of medical care for a high number of cases (Bonnetain *et al*., 2003; Do Carmo *et al*., 2004). The first description of food-borne disease involving staphylococci was investigated in Michigan (USA) in 1884 by Vaughan and Sternberg. This food poisoning event was because of consumption of a cheese contaminated by staphylococci.

Proof of the involvement of staphylococci in food poisoning was first brought by Barber in 1914. He demonstrated with certainty that staphylococci were able to cause poisoning by his consumption of unrefrigerated milk from a cow suffering from mastitis, an inflammation owing to staphylococci. However, the correlation between staphylococci-containing food and symptomatology was not recognized until other examples of food poisoning occurred later in the twentieth century.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study area

The study was carried out in Benin City, Edo State. It lies between latitude 6°20'00 North and longitude 5°37'20 East of the Greenwich Meridian. It has a temperature of about 27°C and an annual rainfall of over 2000mm.

3.2 Sample collection

A total of 52 seafood samples which include 6 shrimps, 15 crayfish, 7 smoked fish, 9 frozen fish, and 15 periwinkles from five local markets in Benin City, Nigeria were assessed. The markets include New Benin market located between New Lagos Road and Upper Mission Road; Ekiosa market located between Second East Circular Road, Third East Circular Road and Sakponba Road; New market located along Second East Circular Road; Oba market located at Ring Road; and Uselu market located at Uselu, Ugbowo, Benin City. The samples were collected in sterile containers and conveyed immediately in ice box to Applied Microbial Processes and Environmental Health Research Laboratory at the Department of Microbiology, Faculty of Life Sciences, University of Benin, Nigeria for analysis within 4 h after collection.

3.3 Isolation of *Staphylococcus aureus*

The fish samples were microbiologically assessed by using a sterile swab stick to rub the body surface and the interior part of the fishes via the mouth cavity. The swab samples were inoculated in 5 mL tryptone soy broth (TSB) (Merck, Darmstadt, Germany) and incubated 37°C for 18–24 h. Ten grams of other samples (shrimps, periwinkles and crayfish) was weighed and homogenized in 90 mL sterile distilled water for about 30 minutes. An aliquot of 100 µL from each of the homogenized samples were aseptically pipetted into 5 mL TSB then incubated at

37°C for 18–24 h. TSB was prepared by dissolving 30 g of the media powder in 1000 mL distilled water which was subsequently sterilized by autoclaving at 121°C for 15 minutes. After incubation, a loopful of the bacterial growth from TSB was streaked on mannitol salt agar (MSA) (Lab M, Lancashire, United Kingdom) plates using sterile inoculating loop. MSA was prepared by dissolving 108 g of the media powder in 1000 mL distilled water and sterilized by autoclaving at 121°C for 15 minutes. The MSA culture plates were incubated at 37°C for 18-24 h. After incubation, distinct golden yellow colonies were considered to be presumptive *Staphylococcus aureus* isolates. Presumptive *Staphylococcus aureus* were purified on nutrient agar (Lab M, Lancashire, United Kingdom). Nutrient agar was prepared by dissolving 28 g of the media powder in 1000 mL of distilled water and subsequently sterilized by autoclaving at 121°C for 15 minutes. The purified isolates were stored on nutrient agar slants until ready for further use.

3.4 Identification of *Staphylococcus aureus*

The presumptive *Staphylococcus aureus* isolates were screened using Gram reaction with potassium hydroxide (3% KOH) test and catalase test and coagulase test.

3.5 Antimicrobial susceptibility tests

Staphylococcus aureus were subjected to antimicrobial susceptibility screening using the Kirby-Bauer disc diffusion method. Suspension of the test isolates with an approximated turbidity 0.5 McFarland's standard was obtained and aseptically spread on mueller-hinton agar plates (Lab M, Lancashire, United Kingdom). Mueller-hinton agar was prepared by dissolving 38 g of the media powder in 1000 mL of distilled water and subsequently sterilized by autoclaving at 121°C for 15 minutes. The antibiotics discs were impregnated on the mueller-hinton agar plates aseptically. The antibiotics tested include clindamycin (2 µg), tetracycline (30 µg), nitrofurantoin (300 µg), cefepime (30 µg), erythromycin (15 µg), penicillin G (10 units) and gentamicin (10 µg) (Oxoid, Hampshire, United Kingdom). The plates were incubated for 18-24 hours at $37^{0}C$. Diameter of zones of inhibition were measured and interpreted according to Clinical Laboratory Standards Institute (CLSI, 2018).

CHAPTER FOUR

4.0 RESULTS

4.1 The occurrence and distribution of *Staphylococcus aureus*

The occurrence of *Staphylococcus aureus* based on cultural and biochemical characterization was shown in Table 1. The distribution of occurrence include shrimps [0/6 (0%)], crayfish [4/15 (26.7%)], smoked fish [4/7 (57.1%)], frozen fish [3/9 (33.3%)] and periwinkles [4/15 (26.7%)]. The highest prevalence occurred in smoked fish samples while there was no occurrence observed in shrimp samples.

The distribution of *Staphylococcus aureus* based on market location was shown in Table 2. These include Ekiosa market [2/8 (25%)], Uselu market [1/9 (11.1%)], New Benin market [1/13 (7.7%)], New Market [4/9 (44.4%)] and Oba market [7/13 (53.9%)]. The highest prevalence was observed at Oba market (53.9%) while the least prevalence was observed at New Benin market (7.7%). In overall, the total *Staphylococcus aureus* positive samples were 13/52 (25%).

Table 1. Occurrence of *Staphylococcus aureus* from seafood

Samples	**Samples assessed**	***Staphylococcus aureus* positive samples**
Shrimp	6	0
Crayfish	15	4
Smoked Fish	7	4
Frozen Fish	9	3
Periwinkle	15	4
Total	52	15(28.9%)

Table 2. Distribution of *Staphylococcus aureus* from seafood based on market location

Market	**Samples assessed**	***Staphylococcus aureus*** **positive samples**
Ekiosa Market	8	2
Uselu Market	9	1
New Benin Market	13	1
New Market	9	4
Oba Market	13	7
Total	52	15(28.9%)

4.2 Antimicrobial resistance profile of *Staphylococcus aureus*

The resistant profile of *Staphylococcus aureus* was shown in Table 4. The resistance demonstrated by the isolates were clindamycin [2/5 (40%)], tetracycline [3/5 (60%)], nitrofurantoin [0/5 (0%)], cefepime [4/5 (80%)], erythromycin [3/5 (60%)], penicillin G [5/5 (100%)] and gentamicin [0/5 (0%)]. The highest resistance was demonstrated to penicillin with a resistance rate of 100% while there was no resistance observed towards nitrofurantoin and gentamicin. However, one isolate demonstrated an intermediate resistance towards nitrofurantoin.

The multiple antibiotics resistance (MAR) index of *Staphylococcus aureus* was shown in Table 4. It was observed that one isolate [1/5 (20%)] was resistant to at least five antibiotics while two isolates [2/5 (40%)] demonstrated resistance to four antibiotics. However, two isolates [2/5 (40%)] were resistant to two antibiotics. The multiple antibiotics resistance profile of *Staphylococcus aureus* in this study ranged from 0.7 – 0.3. All the isolates were resistant to at least two antibiotics and demonstrated an MAR index ≥ 0.3.

Table 3. Antimicrobial susceptibility profile of *Staphylococcus aureus*

Antimicrobial class	Antibiotics	Susceptibility profile of *Staphylococcus aureus* (*n*=5)		
		Sensitive (%)	Intermediate (%)	Resistance (%)
Lincosamides	CLI	2(40)	1(20)	2(40)
Tetracyclines	TET	0(0)	2(40)	3(60)
Nitrofurans	NIT	4(80)	1(20)	0(0)
Cephalosporins	FEP	1(20)	-	4(80)
Macrolides	ERY	2(40)	0(0)	3(60)
Penicillins	PEN	0(0)	-	5(100)
Aminoglycosides	GEN	5(100)	0(0)	0(0)

Key: CLI: clindamycin (2 µg), **TET:** tetracycline (30 µg), **NIT**: nitrofurantoin (300 µg), **FEP**: cefepime (30ug), **ERY**: erythromycin (15 µg), **PEN**: penicillin G (10 units) and **GEN**: gentamicin (10 µg).

Table 4. Multiple antimicrobial resistance of *Staphylococcus aureus*

Isolate code	Number of antibiotics	Resistance phenotype	MAR Index
VF_1	5	CLI/TET/FEP/ERY/PEN	0.7
MP_3	4	CLI/TET/ERY/PEN	0.6
CF_1	4	TET/FEP/ERY/PEN	0.6
MFF_1, $B10_2$	2	FEP/PEN	0.3

Key: CLI: clindamycin (2 µg), **TET:** tetracycline (30 µg), **NIT**: nitrofurantoin (300 µg), **FEP**: cefepime (30ug), **ERY**: erythromycin (15 µg), **PEN**: penicillin G (10 units) and **GEN**: gentamicin (10 µg).

CHAPTER FIVE

5.0 DISCUSSION

The proper assessment of food safety is of vast importance in community health as several illnesses have been attributed to consumption of contaminated food products. In this study, the occurrence of *Staphylococcus aureus* were shrimps (0%), crayfish (26.7%), smoked fish (57.1%), frozen fish (33.3%) and periwinkles (26.7%). In this study, the overall prevalence of *Staphylococcus aureus* was 25%. This is similar to studies of Arfatahery *et al*. (2015) and Bujjamma and Padmavathi, (2015) which reported *Staphylococcus aureus* prevalence of 24.6% and 24.47% respectively in shrimps and fishes. However, *Staphylococcus aureus* had already been detected in a lower proportion (6.1%) in the study of Gutiérrez *et al*. (2012) on food contact surfaces in dairy, meat, and seafood industries. The highest prevalence occurred in smoked fish samples while the least was observed in shrimp samples. This high prevalence in smoked fish samples could be attributed to exposure unhygienic conditions by the handlers during the course of processing. This agrees with the report of Albuquerque *et al*. (2007) that seafood requires adequate sanitary conditions from the moment of catch, through preparation, sale and also consumption in order avoid contamination. The consumption of contaminated seafood has resulted in significant number of disease outbreaks (Solano *et al*., 2011; Rhee and Woo, 2010). Similarly, Simon and Sanjeev (2007) stated that the quality and safety of seafood can be directly influenced by the lack of hygienic habits of fish handlers, improper storage and cross contamination via work surfaces, including benches, tables and unwashed knives.

The distribution of *Staphylococcus* species based on market location was Ekiosa market (25%), Uselu market (11.1%), New Benin market (7.7%), New Market (44.4%) and Oba market (53.9%). The highest prevalence was observed at Oba market (53.9%) while the least prevalence was observed at New Benin market (7.7%). The varying level of prevalence in diverse market location could be attributed to the hygienic practices, storage methods and extent of exposure to contaminating conditions in those locations. Bujjamma and Padmavathi, (2015) have earlier emphasized on the need to properly handle, store and market seafood with extreme care in order to avoid contamination because they are highly perishable and easily undergo biological degradation. This agrees with the report of Albuquerque *et al.* (2007) that *Staphylococcus* species are one of the most important food borne opportunistic potential pathogens in seafood and the detection of the bacteria in food products could be an indication of spoilage.

The antibiotic resistance demonstrated by the *Staphylococcus aureus* isolates was clindamycin (40%), tetracycline (60%), nitrofurantoin (0%), cefepime (80%), erythromycin (60%), penicillin G (100%) and gentamicin (0%). The highest resistance was demonstrated to penicillin with a resistance rate of 100% while there was no resistance observed towards nitrofurantoin and gentamicin. Previous report by Mišic´ *et al*. (2017) noted that penicillin-resistant *S. aureus* strains which were earlier prevalent in health-care sector have spread into the community. This study agrees with the previous study by Sharma *et al*. (2015) that demonstrated a significant resistance of *S. aureus* to penicillin. *Staphylococcus* isolates from the study of Beyene *et al*. (2017) have earlier evidently demonstrated resistance to penicillin (95.3%). Similarly, Tassew *et al*. (2016) reported resistance of *S. aureus* to penicillin. *S. aureus* resistance to penicillin could be due to the production of beta lactamase enzyme carried on transmissible plasmids, which inactivates penicillin and other beta-lactam antimicrobials (Igbinosa and Beshiru, 2019).

The relatively high resistance exhibited by clindamycin in this study is in agreement with the findings of Mišic´ *et al*. (2017) which stated that antibiotics that could serve as alternatives to penicillin such as methicillin and clindamycin have been reported for emerging resistance by *S. aureus* and some other staphylococci species. This antimicrobial resistance in this study could have been as a result of indiscriminate and prolonged use of antimicrobials.

The multiple antibiotics resistance (MAR) profile of *Staphylococcus* species shows that one isolate (20%) was resistant to at least five antibiotics while two isolates (40%) demonstrated resistance to four antibiotics. The multiple antibiotics resistance profile of *Staphylococcus* species in this study ranged from 0.7-0.3. All the isolates were resistant to at least two antibiotics and demonstrated an MAR index ≥ 0.3. The high MAR index could be attributed to extensive exposure of the microbial source to antibiotics. Previous report by Gufe *et al.* (2019) affirmed that MAR index greater than 0.2 indicates that the bacterial strain tested originated from the high-risk sources where antibiotics are used frequently.

5.1 CONCLUSION

The detection of *Staphylococcus aureus* in this study highlights the potential risk of foodborne pathogen that could arise from the consumption of seafood. Seafood being a product with short shelf life makes the strategy of quality control for the presence of pathogens more difficult. Periodic training on food safety and hygiene for seafood handlers is essential because it will enhance the awareness on the health risk associated with the consumption of contaminated food products. The resistance of *Staphylococcus aureus* to multidrug could be attributed to indiscriminate usage of drugs in human and veterinary medicine therefore strict restrictions should be implemented in order to control the abuse of antibiotics.

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