MICROBIOLOGICAL ASSESMENT OF *staphyloccus arureus* FROM MEAT SOLD IN THE MARKET IN BENIN CITY EDO STATE

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCICENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN- CITY, IN FULFULMENT OF THE REQUIREMENT FOR THE AWARD OF BECHELOR OF SCIENCE DEGREE (B.SC) IN SCINCE LABORATORY TECHNOLOGY.

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CERTIFICATION

This is to certify that this project work was carried out by Elizabeth Kemi DUROTIMI (MISS) with matriculation number of lsc1605930 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin- city

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DEDICATION

This work is dedicated to God Almighty first, my parents, Mr and Mrs DUROTIMI, my Siblings, fellow colleagues and all lovers of science and knowledge in general.

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I am indeed grateful to God Almighty for his guidance, and protection throughout the period of my study and preparation for this work.

I want to sincerely appreciate my lovely parents Mr. and Mrs. Durotimi and my siblings Mrs Mary Adeniyi, Durotimi Daniel and Durotimi Emmanuel, for the love they show, financial and moral support and also for the prayers.

My profound gratitude also goes to my able project supervisor Mrs. D. Ayanru, for her endless support, encouragement morally and academically and prayers. And my sincere appreciation also goes to Mr Akim for his support for the completion of the project work.

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I cannot fail to appreciate my friends and colleagues for their spirit of cooperation, love and encouragement shown. I pray the lord continue to richly bless and keep everyone of you in Jesus name. Amen.

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ABSTRACT

Meat is a nutritive-rich food that contributes vital protein, vitamins and minerals to higher bioavailability than other food sources thereby making it a suitable medium for the growth of microorganisms which serve as a great source of meat contamination and food borne disease. Among the various microorganism that are implicated with meat contamination, *staphylococcus aureus* is one of the most prevalent as it a natural floral in skin and nasal cavity of humans and animal. Due to the unhygienic practices from meat handlers, from the abattoirs to the market places and also vehicles which are in most cases unclean and unfit to convey meat and meat products. This study was conducted to access staphylococcus aureus from raw beef sold in open markets in Benin city, Edo state, Nigeria. A total amounts of 45 samples were collected from the meat, tables and knives. Where the meat samples were collected into a sterile container and the table top and knife sample were collected using a sterile swabs. From 5 different markets in benin city, 26 S. aureus isolates were obtained and subjected to antimicrobial susceptibility test using the Kirby-bauer disc diffusion method . The result of the prevalence staphylococcus aureus from the meat,table ans knife sample were [2/15 (13.3%)], [12/15 (80%)] and [12/15 (80%)] respectively. Having Gentamycin (n=5, 100%) the most effective antimicrobial where all isolates were susceptible to it. Followed by Erythromycin and Nitrofurantoin (n=5, 60%). Although, these isolates also shows resistance to Cefepime (100%) followed by penicillin (80%). Unhygienic practices of meat handlers such as dirty hands, unfit display tables and cutting tools and also improper handling of meat products triggers a very high level of S. aureus in open markets which may eventually affects the health of the consumers. Hygiene sanitary practice is recommended in meat market to prevent the consumers from the risk of acquiring these diseases.

CHAPTER ONE

INTRODUCTION

Meat is a nutritive-rich food that contributes vital proteins, vitamins, and minerals to higher bioavailability than other food sources (McAfee *et al*., 2010; Daniel *et al.,*2011). The high nutritive value of meat, having both essential macro- and micro nutrients, makes it an important part of a balanced diet for most people (Bello *et al*., 2016). In most developed and developing countries, meat constitutes a significant part of the normal diet and contributes more than 15 % energy, 40 % protein, and 20 % fat. The demand for meat in developing countries has continuously grown as the production and consumption pattern of meat increases with the availability of income (Myers and Kent, 2003). However, meat is also a suitable medium for growth of microorganisms.

Meat is not only highly susceptible to microbial spoilage due to its high nutritive value which is suitable for the growth of microorganisms, but also frequently implicated in the spread of food borne illness (Bhandare *et al*., 2007). Contaminated raw meat is one of the main sources of foodborne illness (Bhandare *et al*., 2007; Podpecan *et al*., 2007). During slaughter and processing, all potentially edible tissues are subjected to contamination from a variety of sources within and outside animal. Likewise, the vehicles of transport that convey the meat products from abattoir to market places are in most cases unclean and unfit to prevent microbial contamination (Bello *et al*., 2016). Furthermore, meat is customarily sold and displayed in open markets in Nigeria without proper guarding and hygienic practices from meat handlers. Such include, dirty hands, display tables where meats and meat variety are sold, also cutting tools which are inadequately cleaned and maintained. These underlying factors contribute to the

adherence of microorganisms to meat surfaces which are an eligible cause of meat contamination and hence, pose a potential risk to consumers (Abdulihi *et al*., 2006)**.** The incidence of meat contamination has been investigated worldwide to have been linked to a wide variety of microbial pathogens such as *Pseudomonas species, Acinetobacter species, Brochothrix thermosphacta, Lactobacillus species, Salmonella species, Listeria monocytogens, Yersina enterocolitca, Escherichia coli, Clostridium perfringens, Clostridium botulinum and Staphylococcus aureus* (Carrisoza *et al*., 2017). Among the various microorganisms that are implicated with meat contamination, *Staphylococcus aureus* is the most prevalent as it is a natural flora in skin and nasal cavity of humans and animals (Pal, 2001).

Staphylococcus aureus is a unique and adaptive bacterium in the antibiotic era that has been documented to develop resistance to various classes of A13 units in human and animal therapy (Bhargiva *et al*., 2011). Of various food products surveyed, meat and meat products are widely known to be an important reservoir of *Staphylococcus aureus* and involved in several outbreaks (Aydin *et al*., 2011; Hennekinne *et al.,* 2012; Wang *et al*., 2013; Sallam *et al.,* 2015). The microbiological investigation of *Staphylococcus aureus* from meat can implement a definitive system for monitoring of meat contamination. Although, prevalence values of *Staphylococcus aureus* in meat and meat variety may vary according to conditions such as: sample sizes, sample types and geographic location of investigation, (Wu *et al.,* 2018). Many studies have documented high levels of hazard in meat sold in wet market in Benin-city which is a contributive public health concern in Nigeria at large because diseases arising from the contamination of food such as diarrhea, is now an important distributor to the huge burden of sickness and death. However, there is less information on the knowledge, attitude and practices (KAP) around meat safety (Lin

et al., 2009). Hence, the aim of this study is to assess *Staphylococcus aureus* from raw beef sold in open markets in Benin city, Edo state, Nigeria.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Sources of Meat Contamination

Owing to its composition, meat is recognized as one of the best media for microbial growth leading to meat spoilage and foodborne illness. The ability of microorganisms to adhere to surfaces where meat is deposited during selling in open and unhygienic areas such as markets is a non-negligible cause of meat contamination (Mouafo *et al*., 2020). Spoilage and contamination can be caused by a wide variety of factors, such as improper handling, exposure to air and high temperature, or conditions that trigger chemical reactions or microbial contamination, although the most common cause is the presence of microorganisms together with metabolite production (Ukut *et al*., 2010). Live stock contact and meat handling are two ways by which *staphylococcus aureus* may colonize humans, *S. aureus* is also found on many other surface environment which individuals comes in contact with on a daily basis. Another reports indicates the presence of staphylococcus aureus on the hands of food handlers (Lies and Tondener, 2007). Also, Campos *et al*., (2009) indicated that food handlers are a major cause of food contamination. Food borne disease outbreaks reported in the United States, for instance, were often associated with mishandling with 79 % from commercial or institutional establishment and 20 % from homes (Haapala and Probart, 2004).

Meats products sold to the public in open markets or by streets vendors are grossly contaminated with coliform bacteria as well as other bacterial forms. Research in microbial science has discovered that meats products are contaminated with pathogenic Gram- positive and Gram-negative bacteria. This also implies that these meats are viable source of various diseases

(Okonko *et al*., 2008). Some of these diseases could spread and acquire epidemic status which poses serious health hazards since improper handling and improper hygiene might lead to the contamination of meats products; and this might eventually affect the health of the consumers (Okonko *et al.,* 2008;Okonko *et al.,* 2009).

2.2 Microorganisms associated with Meat

Bacterial which commonly contaminate meat while it is being processed, cut, packaged, transported, sold and handled include: *Salmonella spp*., *Shigella spp*., *E. coli*, *Proteus*, *Staphylococcus albus* and *Staphylococcus aureus*, C. *welchii*, *B. cereus* and faecal streptococci. These bacteria are all commonly carried by humans. Infectious bacteria from the soil include *C. botulinum*. As these microorganisms colonize a piece of meat, they begin to break it down, leaving behind toxins that can cause enteritis or food poisoning, potentially lethal in the rare cases of botulism (Lawrie and Ledward, 2006).*Staphylococcus aureus* is a significant cause of food-borne diseases just as much as it is a prevalent microbial pathogen responsible for mostly the contamination of meat and meat products. The microbe is estimated to cause 241,000 illnesses in the US (Byrd-bredbenner *et al,* 2013). The medical institute has recognized food borne diseases "The potential for foods to be involved in the emergence or reemergence of microbial threats to health is high, in large part because there are many points at which food safety can be compromised" (Rosooly, 2000).

2.3 Staphylococcus aureus

S. aureus is a commensal and opportunistic pathogen that can cause wide spectrum of infections, from superficial skin infections to severe, and potentially fatal, invasive disease (Lowy, 1998). This ubiquitous bacterium is an important pathogen due to combination of "toxin-mediated

virulence, invasiveness, and antibiotic resistance." This organism has emerged as a major pathogen for both nosocomial and community acquired infections. *Staphylococcus aureus* does not form spores but can cause contamination of food products during food preparation and processing and can grow in a wide range of temperatures (7 ^{0}C to 48.5 ^{0}C; optimum 30 ^{0}C to 37 ^{0}C), pH (4.2 to 9.3; optimum 7 to 7.5), and sodium chloride concentration up to 15 % NaCl (Kadariya *et al.,* 2014). *Staphylococcus aureus* is a desiccation tolerant organism with the ability to survive in potentially dry and stressful environments, such as the human nose and on skin and inanimate surfaces such as clothing and surfaces (Chaibenjawong and Foster, 2011). These characteristics favor growth of the organism in many food products (le-Loir *et al.,* 2003). *S. aureus* is able to grow and express virulence in a wide variety of food such as mixed food, meat and meat products (Guven *et al*., 2010). Also, it is widely known to be prevalent causing several disease outbreaks (Ayadin *et al*., 2011; Hennekinne *et al*., 2012). In 2013, there was 12.5 % of food borne bacteria outbreak that was caused by *Staphylococcus aureus* in China which ranked as the third most virulent pathogen after *vibro parahaemolysis* (27.8 %) and *Salmonella* (23.1 %) (Weiroei *et al*., 2018).

2.4 *Staphylococcal* Food-Borne Disease (SFD)

SFD is one of the most common FBD and is of major concern in public health programs worldwide (Balban and Rosooly, 2000; Le- Loir *et al*., 2003; Hennekinne *et al*., 2012). It is one of the most common causes of reported FBD in the United States (Balban and Rosooly, 2000; Bean *etal*., 1990; Murray, 2005). The first documented event of SFD due to the consumption of contaminated cheese was investigated by Vaughan and Sternberg in Michigan, USA, in 1884 (Hennekinne *et al*., 2012) A typical FBD caused by *S. aureus* has a rapid onset following ingestion of contaminated food (usually 3– 5 hours). This is due to the production of one or more

toxins by the bacteria during growth at permissive temperatures (le-Loir *et al*., 2003). However, the incubation period of SFD depends on amount of toxin ingested (Murray, 2005). Very small dose of SEs (Staphylococcal enterotoxins) can cause SFD.

Several studies have documented prevalence of S. aureus in many food products including raw retailed meat indicating that consumers are at potential risk of *Staphylococcus aureus* colonization and subsequent infection. Presence of pathogen in food products imposes potential hazards for consumers and causes grave economic loss and loss in human productivity via food borne disease. Symptoms of SFD include nausea, vomiting and abdominal cramp with or without diarrhea (Kadariya *et al*., 2014).

2.5 *Staphylococcusaureus* Enterotoxins (SEs)

S. aureus produces wide arrays of toxins. *Staphylococcal* enterotoxins (SEs) are a family of nine major serological types of heat stable enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ) that belong to the large family of pyrogenic toxin superantigens (Balban and Rosooly, 2000; Argudin *et al*., 2010) Pyrogenic toxins cause superantigenic activity such as immunosuppression and nonspecific T-cell proliferation (Le-Loir *et al.,* 2003) It is hypothesized that superantigenic activity of SEs helps facilitate transcitosis that allows the toxin to enter the bloodstream, thus enabling it to interact with antigen-presenting cells and T cells leading to superantigen activity (Argudin *et al*., 2010). The majority of effects of SEs in SFD is believed to be triggered by initiating a focal intestinal inflammatory response due to their superantigenic activity or by affecting intestinal mast cells causing their degranulation (Dings *et al*., 2000). SEs are highly stable and highly heat-resistant and resistant to environmental conditions such as freezing and drying (le-Loir *et al.,* 2003). They are also resistant to proteolytic enzymes such as

pepsin or trypsin and low pH, enabling them to be fully functional in the gastrointestinal tract after ingestion. The heat stability characteristic of *S. aureus* imposes a significant threat in food industries (Balban and Rosooly, 2000). Mechanisms of SEs causing food poisoning are not clearly known. However, it is believed that SEs directly affect intestinal epithelium and vagus nerve causing stimulation of the emetic center (Murray, 2005). All *staphylococcal* enterotoxins cause emesis. An estimated 0.1 μg of SEs can cause staphylococcal food poisoning in humans (Dings *et al*., 2000). Many research studies have detected the prevalence of different types of toxin genes that are produced by *staphylococcus aureus* in different types of meat. For example, in one of the studies by Pu *et al* (2011), He concluded that the high percentage of staphylococcal enterotoxins from Louisiana retail meat was 66 % for Seg and Sei, 20 % for Seh, 15 % for Sed, 13 % for Sej and 1 % for Sea (Wang *et al*., 2010). Another research in Korea reported types of toxin gene in raw meats which included toxin shock syndrome -1 (TSS-1) and enterotoxin (Hwang *et al*., 2007)

Recently, several Multidrug resistant (MDR) of *Staphylococcus aureus* were reported in food poisoning outbreaks and isolated from food products in previous research (Suver *et al*., 2008).

2.6 Antibiotic Resistance of *staphylococcus aureus*

A study from Louisiana (Wang *et al.,* 2010) found that most *staphylococcus aureus* in different types of meat were commonly resistance to penicillin (71 %), ampicillin (68 %), tetracyclin (67 %), erythromycin (30), clindamycin (18 %), oxacillin with up to 2 % of sodium chloride (14 %), levofloxacin (13 %), ciprofloxacin (1 %). A study on the impact of food related illness in the USA was done due to a number of serious incident in recent years. Consequently, the US foods and drug administration (FDA) has increased efforts to survey and improve the quality of

contaminated products (Scallan *et al.,* 2011) Both *staphylococcus* and MRSA (methicillin-resistant *staphylococcus aures)*have been isolated from chicken pork, beef and others. For instance, one study in Japan found out two isolate of MRSA out of 444 samples of raw chicken and beef meat at 145 different supermarkets (Kitai *et al*., 2005). Recently, few studies reported variable prevalence of *staphylococcus aureus* and MRSA in retail meats at different US location including Louisiana (Pu *et al*., 2009), Maryland, USA (Kelman *etal*., 2011), Detroit, Michigan (Bhargava *et al.,* 2011), Lowa (Hanson *etal*., 2011), Minnoesota and New jersey (O'Brien *et al*., 2012), Georgia (Jackson *et al*., 2013) and North Dakota (Buyukcangaz *et al*., 2013; Velasco *et al*., 2014

CHAPTER THREE

3.0 MATERIALS AND METHODS

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 2000 mm.

3.2 Sample collection

A total of forty five (45) samples were collected from five different markets in Benin City. The sampling points and its distribution were 15 meats samples, 15 tables' swab and 15 knives swab samples respectively. The markets include New Benin market located between New Lagos Road and Upper Mission Road; Uselu market located at Uselu; Oba market located at Ring Road; Aduwawa market located at Benin-Auchi Road and Oregbeni market located at Ikpoba Okha along Benin-Agbor Road. The samples were collected from the table tops, knives and meat. The tables and knives samples were collected aseptically using a sterile swab stick by rubbing it against the tables and knives surfaces while the meat samples were collected into a sterile container and immediately transported to the laboratory in an ice pack for analysis within 4 hours.

3.3 Preparation of culture media used

The culture media used in this study include tryptone soy broth (TSB) (Merck,Darmstadt, Germany), mannitol salt agar (MSA) (Lab M, Lancashire, United Kingdom), nutrient agar (NA) (Lab M, Lancashire, United Kingdom) and mueller-hinton agar (MHA) (Lab M, Lancashire, United Kingdom). The media were used for isolation, preservation and antibiotic susceptibility

testing. The culture media were prepared and sterilized according to the manufacturer's instructions.

3.4 Isolation of *Staphylococcus aureus*

The samples were inoculated into 50 test tubes containing tryptone soy broth (TSB). TSB was prepared by dissolving 30 g of the media powder in 1000 mL distilled water which was subsequently sterilized by autoclaving at 121 ^{o}C for 15 minutes. The tables and knives swab samples were inoculated into 5 mL TSB and incubated at 37 ^{o}C for 18–24 h. Ten grams of the meat samples was weighed and homogenized in 90 mL sterile distilled water for about 30 minutes, then 500 µL from the homogenized samples were inoculated into 4.5 mL TSB and incubated at 37 ^{o}C for 18–24 h. After incubation, a loopful of the culture broth was streaked onto sterile MSA (Manitol salt agar) plates and incubated at 37 ^{o}C for 24 h in an inverted position. MSA was prepared by dissolving 108 g of the medium powder in 1000 mL distilled water and sterilized by autoclaving at 121 ^{o}C for 15 minutes. The MSA culture plates were incubated at 37 °C for 18-24 h. The plates were observed for growth and fermentation of mannitol to produce yellow colonies on bright yellow zones, a presumptive evidence of *Staphylococcus aureus* as described by Fratamico *et al.* (2005). Discrete presumptive colonies of *Staphylococcus aureus* were purified on freshly prepared nutrient agar plates using streak plate method and incubated at 37 ^{o}C for 24 h and discrete colonies which developed were stored on nutrient agar slants then refrigerated at 4 ^{o}C for further use.

3.4 Identification of *Staphylococcus aureus*

Presumptive *Staphylococcus aureus* were further screened using Gram reaction with potassium hydroxide (3 % KOH) test and catalase test. Potassium hydroxide test was carried out by making

a drop of 3 % potassium hydroxide on a clean microscopic slide and a loopful of the organism was emulsified to the potassium hydroxide to make a dense suspension using a sterile inoculating loop. The suspension become slimy if the test organism is positive to KOH test. Presumptive *Staphylococcus aureus* isolates were KOH negative. Catalase test was carried out by making a suspension of 18 h old culture of the test organisms using sterile distilled water on a clean glass microscope slide and few drops of hydrogen peroxide were added using a dropping pipette. Formation of bubbles indicates positive result. Presumptive *Staphylococcus aureus* isolates were catalase positive. Coagulase test was carried out by placing two separate drops of saline on a clean glass slide. Using a sterile inoculating loop, discrete colony of presumptive *Staphylococcus aureus* was emulsified in one of the saline drops to make bacteria suspension. Using a Pasteur pipette, a drop of plasma was added to the bacteria suspension and saline drop then mixed gently. Formation of clumps in the bacteria suspension within 10-15 seconds indicates coagulase positive result.

3.5 Antimicrobial Susceptibility Test

Presumptive *Staphylococcus aureus* were subjected to antimicrobial susceptibility screening using the Kirby-Bauer disc diffusion method. An approximately 0.5 McFarland's standard turbidity suspension of 18 h colonies of the test isolates was obtained and aseptically spread on Mueller-Hinton agar plates. Mueller-Hinton agar was prepared by dissolving 38 g of the medium powder in 1000 mL of distilled water and subsequently sterilized by autoclaving at 121^{o}C for 15 minutes. The antibiotics discs were impregnated on the Mueller-Hinton agar plates aseptically. The antibiotics tested include Penicillin G (10 ug), Gentamicin (10 ug), Erythromycin (15 ug), Tetracycline (30 ug), Cefepime (30 ug), Clindamycin (2 ug) and Nitrofurantoin (300 ug) (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 as resistant, intermediate and susceptible according to Clinical Laboratory Standards Institute (CLSI, 2018).

CHAPTER FOUR

4.0 RESULTS

A total of forty five (45) samples were collected from five different markets in Benin City. The occurrence of *Staphylococcus aureus* from samples obtained at Aduwawa market as shown in Table 1. The occurrence distribution include: meat [0/3 (0 %)], table [2/3 (66.7 %)] and knife [2/3 (66.7 %)]. The highest prevalence occurred in table and knife samples while there was no occurrence observed in meat samples.

The occurrence of *Staphylococcus aureus* from samples obtained at Oba market is shown in Table 2. The occurrence distribution include meat [1/3 (33.3 %)], table [3/3 (100 %)] and knife [2/3 (66.7 %)]. The highest prevalence occurred in table samples while the least occurrence was observed in meat samples.

The occurrence of *Staphylococcus aureus* from samples obtained at New Benin market was shown in Table 3. The occurrence distribution include meat [0/3 (0 %)], table [2/3 (66.7 %)] and knife [2/3 (66.7 %)]. The highest prevalence occurred in table and knife samples while there was no occurrence observed in meat samples.

The occurrence of *Staphylococcus aureus* from samples obtained at Uselu market is shown in Table 4. The occurrence distribution include meat [1/3 (33.3 %)], table [2/3 (66.7 %)] and knife [3/3 (100 %)] .The highest prevalence occurred in knife samples while the least occurrence was observed in meat samples.

The occurrence of *Staphylococcus aureu*s from samples obtained at Oregbeni market is shown in Table 5. The occurrence distribution include meat [0/3 (0 %)], table [3/3 (66.7 %)] and knife [3/3 (100 %)]. The highest prevalence occurred in table and knife samples while there was no occurrence observed in meat samples.

Table 1. Prevalence of *Staphylococcus aureus* in Aduwawa market.

Sampling points	Number of samples tested	Number of positive samples	Prevalence (%)
Meat	3.00	0	0
Table	3.00	2.00	66.7
Knife	3.00	2.00	66.7
Total	**9.00**	**4.00**	**44.4**

Table 2. Prevalence of *Staphylococcus aureus* in Oba market.

Sampling points	Number of samples tested	Number of positive samples	Prevalence (%)
Meat	3.00	1.00	33.3
Table	3.00	3.00	100
Knife	3.00	2.00	66.7
Total	**9.00**	**6.00**	**66.7**

Table 3. Prevalence of *Staphylococcus aureus* in New Benin market.

Sampling points	Number of samples tested	Number of positive samples	Prevalence (%)
Meat	3.00	0	0
Table	3.00	2.00	66.7
Knife	3.00	2.00	66.7
Total	**9.00**	**4.00**	**44.4**

Table 4. Prevalence of *Staphylococcus aureus* in Uselu market.

Sampling points	Number of samples tested	Number of positive samples	Prevalence (%)
Meat	3.00	1.00	33.3
Table	3.00	2.00	66.7
Knife	3.00	3.00	100
Total	**9.00**	**6.00**	**66.7**

Table 5. Prevalence of *Staphylococcus aureus* in Oregbeni market.

Sampling points	Number of samples tested	Number of positive samples	Prevalence (%)
Meat	3.00	0	0
Table	3.00	3.00	100
Knife	3.00	3.00	100
Total	**9.00**	**6.00**	**60**

The occurrence distribution of *Staphylococcus aureus* from the samples based on market location is shown in Figure 1. The distribution include Aduwawa [4/9 (44.4 %)], Oba [6/9 (66.7 %)], New Benin [4/9 (44.4 %)], Uselu [6/9 (66.7 %)] and Oregbeni [6/9 (66.7 %)]. The highest prevalence occurred in Oba, Uselu and Oregbeni market samples while the least was observed in Aduwawa and New Benin market samples.

The occurrence distribution of *Staphylococcus aureus* based on sampling points is shown in Figure 2. The distribution include meat [2/15 (13.3 %)], knife [12/15 (80 %)] and table [12/15 (80 %)]. The highest prevalence occurred in table and knife samples while the least was observed in meat samples.

Table 6 represents resistant profile of *Staphylococcus aureus.* The resistance demonstrated by the isolates were penicillin G [4/5 (80 %)], gentamicin [0/5 (0 %)], erythromycin [1/5 (20 %)], tetracycline [1/5 (20 %)], cefepime [5/5 (100 %)], clindamycin [2/5 (40 %)] and nitrofurantoin [1/5 (20 %)]. The highest resistance was demonstrated to cefepime and penicillin G with a resistance rate of 100 % and 80 % respectively. There was no resistance demonstrated towards gentamicin.

The multiple antibiotics resistance (MAR) profile of *Staphylococcus aureus* was shown in Table 7. It was observed that two isolates [2/5 (40 %)] was resistant to at least four antibiotics. However, one isolate [1/5 (20 %)] was resistant to one antibiotics. The multiple antibiotics resistance profile of *Staphylococcus aureus* in this study ranged from 0.6 – 0.1. Four isolates were resistant to at least two antibiotics and demonstrated an MAR index ≥ 0.3.

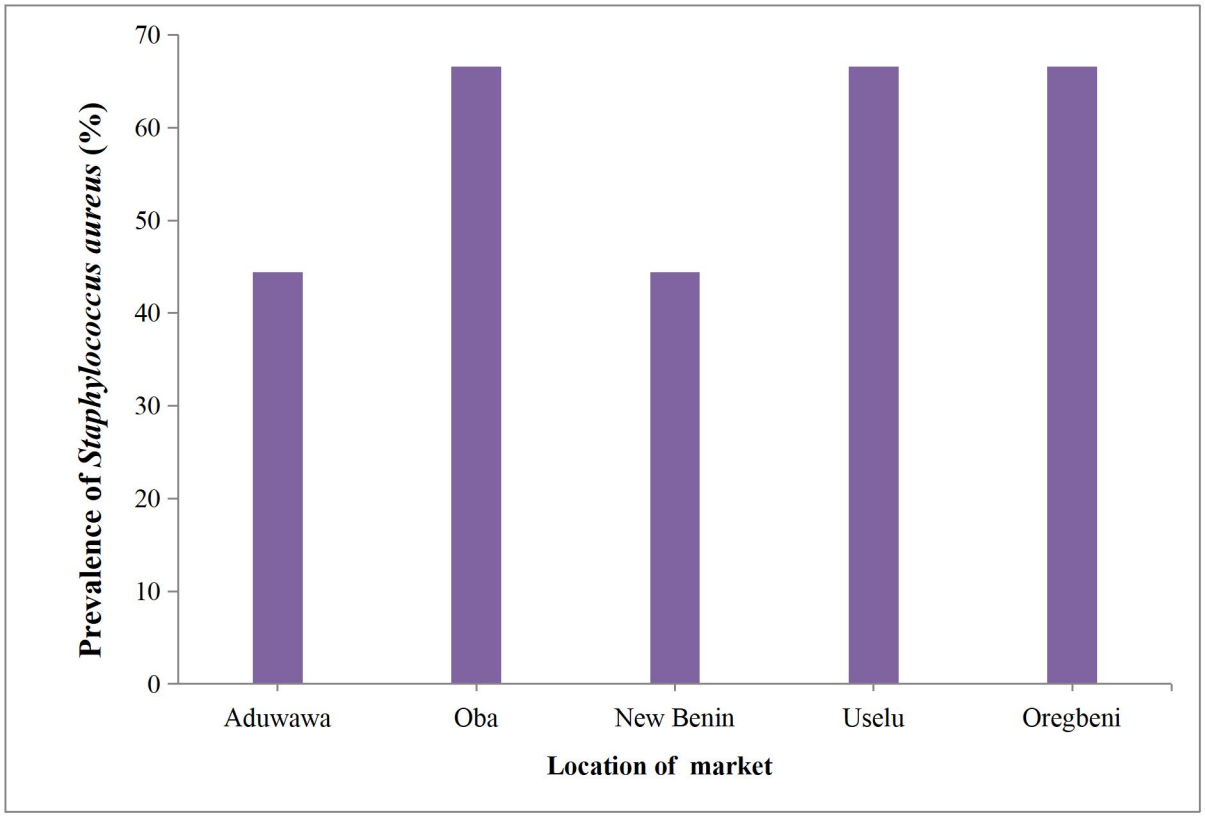

Figure 1. Prevalence of *Staphylococcus aureus* based on market location.

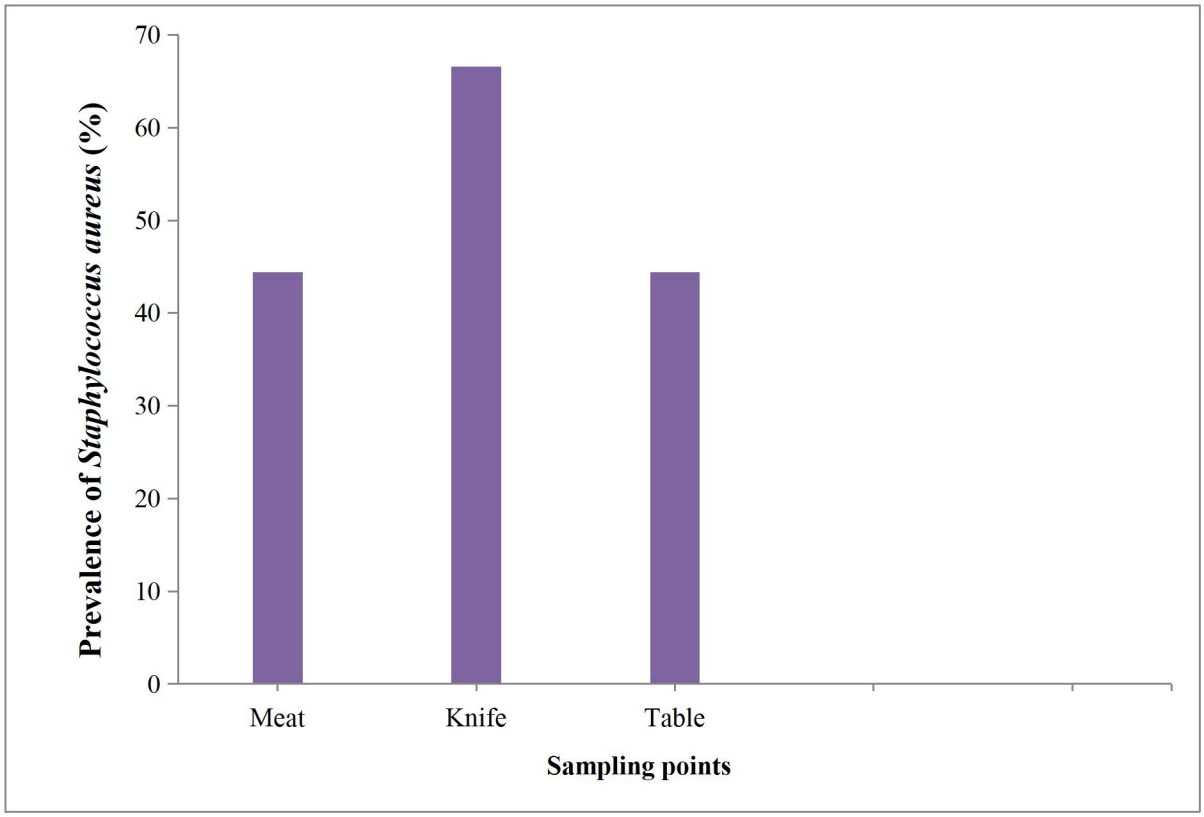

Figure 2. Prevalence of *Staphylococcus aureus* based on sampling points

Table 6. Antimicrobial susceptibility profile of *Staphylococcus aureus*

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

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

Table 7. Multiple antimicrobial resistance of *Staphylococcus aureus*

Isolate code	Number of antibiotics	Resistance phenotype	MAR Index
NET_2	4	PEN/ERY/FEP/CLI	0.6
OBT_2	4	PEN/TET/FEP/CLI	0.6
ORK_2	3	PEN/FEP/NIT	0.4
USK_2	2	PEN/FEP	0.3
ADT_3	1	FEP	0.1

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

CHAPTER FIVE

DISCUSSION

This study was done to evaluate the microbial susceptibility of *S. aureus* on retailed meat sold in the markets in Benin City. The existence of a high prevalence of *S. aureus* in the current study (57.78 %) can be attributed to the condition of the market tables, equipment and machines which had been used during meat processing, preparing, and the unhygienic practices employed by sellers and handlers. Animal health, dressing skills, personnel hygiene, abattoir cleanliness, and adequate storage and holding temperature during distribution and retail influence the constitution and number of microorganisms present (Hudson *et al*., 1996). This is similar to the result of Collins (1995) who isolated Grampositive and Gram-negative bacteria, including S. aureus from retailed meat and meat products. Nkanga and Uraih (1981) also reported high prevalence rate of *S. aureus* in meat samples from traditional market in Benin City, Nigeria. In Eastern Cape, South Africa, Pekana and Green (2018) found that 20.4 % of beef samples obtained from the abattoir were contaminated with Staphylococcus aureus. The occurrence distribution S. aureus based on sampling points is shown in Figure 2. with the knives and tables having the highest prevalence (80 %) respectively, with the meat having the least occurrence (13.33 %). Compared with studies from other countries, the presence of S. aureus in retail meat was lower than the study of Ge *et al*. (2017) who reported that 27.9 % of retailed meat samples in the USA were positive for S. aureus, and also lower than Tang *et al.* (2017)'s research who reported that 68 % of samples in Denmark were positive. Attributed to the sample sizes, sample types, and geographic locations of investigation, this maybe the reason for these differences.

Staphylococcocal food-borne dieases is an important health care and community acquired infection in every region of the world (Hassan, *et al*., 2013; Center for Disease Control, 2015). Studies have shown that toxins produce by *S. aureus* in meat possess a potential health hazard to consumers (Lee, 2003) and isolation of such strains is useful as a parts of risk analysis of meat or beef as it should be (Zouharova and

Rysanek, 2008). In this study, some retailers were not alarmed by the presence of increased bacterial loads in meat, since they believed that cooking would eradicate the organisms present. However, Prescott and Klein (2002) have reported that most strains of *Staphylococcus aureus* implicated in staphylococcal enteritis are responsible for the synthesis of extracellular toxins, which are actually heat-resistant proteins that remain pathogenic when consumed even in cooked foods (Prescott and Klein 2002).

As to the antimicrobial susceptibility profile of the isolates as shown in table 6, out of 5 isolates, 4 (80 %) had resistance to at least one of the 7 antimicrobials tested. Mekuria *et al.* (2013) reported that about 90% of *S. aureus* isolated from patients were resistant in 1980s. Eighty percent (80 %) of the isolates tested, showed resistance to Penicillin G. This is in agreement with a separate study where 70-73 % of *S. aureus* stains isolated from various foods were resistant to Beta lactam such as Penicillin and ampicillin (Pereira *et al.*, 2009). All isolates were resistant to Cefepime. Gentamycin was the most effective antimicrobial of the 7 antibiotics tested as all isolates (100 %) were susceptible in this study. This is in agreement with a previous study by Beyene *et al.* 2017 were all 43 *Staphylococci* isolates were susceptible to gentamycin. Only 20% of the isolates showed resistance to erythromycin, tetracycline and nitrofurantoin. This agrees with reports by Adzitey *et al.* (2019), were 33.33 % of the total isolates were susceptible to tetracycline. Adugna *et al*. (2018) also observed intermediate susceptibility for erythromycin and tetracycline in *Staphylococcus* aureus isolated from beef in Ethiopia. This concurred with the results in this study, with tetracycline, erythromycin, clindamycin and nitrofurantoin having an intermediate susceptibility of 60 %, 20 %, 20 % and 20 % respectively. The intermediate resistant isolates were neither resistant or susceptible. Such isolates have the tendency to become resistant and pose a challenge when it comes to treatment when they are involved in infection (Adzitey *et al.,* 2015). According to a research carried out by Datta *et al*. (2012), thirty-five of the tested isolates of *staphylococci* (47 %) were resistant to only two antibiotics, 25 (33 %) to three antibiotics, and multidrug resistance (MDR) was confirmed in 15 isolates (20 %). Regecová *et al*. (2009) also found 47 % to two antibiotics, and multidrug resistance was 25 % from fish meat Staphylococci isolates. In Korea, Heo *et al*. (2008) found 7.8 % MDR *S. aureus* isolates from domestic and imported meats. Waters *et al*. (2011) found 52 % MDR (resistant against 3 or more drug) *S.*

aureus from poultry and meats. This correlates with the present study were 2 of the 5 isolates showed multi-drug resistance (MDR) to 4 out 7 antibiotics tested. The other 3 isolates showed multi-drug resistance to at least 2 of the 7 antimicrobials used.

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