



**PREVALENCE OF ANTIBIOTIC RESISTANT *STAPHYLOCOCCUS AUREUS* IN RAW
MEAT SAMPLES INTENDED FOR HUMAN CONSUMPTION**

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ABSTRACT

Food borne diseases are one of the major causes of concern in developing countries resulting in several deaths annually. This study was carried out to evaluate the prevalence of antibiotics resistant *Staphylococcus aureus* in meat samples intended for human consumption in Abakaliki, Ebonyi State, Nigeria. A total of 56 different types of meat were collected from different sales points in Abakaliki metropolis and sent to the laboratory for analysis. Exactly, 1 g of each sample was macerated into slurry and cultured. Isolated organisms were counted, characterized and identified using standard microbiological techniques. Antibiotic susceptibility tests were carried out using the disc diffusion method according to National Committee for Clinical laboratory Standards (NCCLS). The results of microbial count showed that microbial load of samples from Kpirikpiri ranged from 1.81 ± 2.20 to $2.15 \pm 3.00 \times 10^6$ Cfug, while those from Meat Market and Mbukobe ranged from 1.16 ± 4.56 to $2.03 \pm 3.12 \times 10^6$ Cfug and 1.46 ± 5.83 to $2.00 \pm 2.82 \times 10^6$ Cfug respectively. The prevalence of *S. aureus* across the meat sampled from the sales point differed significantly ranging from 21.05 % to 42.11 %. The antibiotics susceptibility test showed that 100 % of the isolates were susceptible to imipenem (IPM), but all isolates from pork from the 3 locations sampled were also 100 % susceptible to meropenem (MEM). However, all the isolates were 100 % resistant to ceftioxin and amoxicillin. The

isolates showed MARI ranging from 0.2 to 0.7 with mean value of 0.6. This study has shown that meat samples sold in Abakaliki contains high prevalence of antibiotics resistant *S. aureus*.

Keyword: Antibiotic resistance, raw meat, *Staphylococcus aureus*, Abakaiki

INTRODUCTION

Staphylococcus is a genus that belongs to the *Micrococcaceae* family. These bacteria are characteristically Gram-positive, non-motile, non-spore forming, spherical cells and aerobic or facultatively anaerobic bacteria which are about 1 µm in diameter and are arranged in irregular clusters [1]. More than 20 species have been recognized in this genus, which are distributed in various habitats. Some colonize the skin surface, cutaneous glands, and human or animal (such as ruminants) mucosal membranes [2].

S. aureus is capable of producing one or more of a family of enterotoxins that known as Staphylococcal enterotoxins (SEs). SEs are classified to classic enterotoxins and new enterotoxins based on their biological and serological characteristics. *S. aureus* is among the leading causes of food-borne diseases (FBDs) worldwide for two reasons. Firstly *S. aureus* is often present in food contaminated by humans. As estimated 30-50% of human population carries *S. aureus*, mainly in the nasopharynx or on the hands where the organisms can persist without causing any damage. Coughing, sneezing or food handling combined with poor hygiene

may cause contamination, especially after heat treatment. When it comes to raw foods, contamination from animal origins is more frequent (i.e. mastitis). The second reason is that *S. aureus* is capable of growing and producing toxins in a wide variety of foods [3].

Food borne diseases are one of the major causes of concern in developing countries resulting in several deaths annually along with billions of dollars of economic burden. In animal production, the emergence and the spread of antimicrobial-resistant pathogens have been associated with the misuse or overuse of antibiotics [4]. Meat is one of the popular food items and consumed worldwide, and commonly contaminated by antibiotic resistant strains of *S. aureus* which pose a great risk in the food web. Those pathogens or the genes associated with antimicrobial resistance (AMR) could enter into the food supply chain through the food-producing animals and food handlers and be transmitted to humans, threatening the effective treatments of infectious diseases [4].

Infections caused by *Staphylococcus aureus* are becoming more difficult to treat because of increasing resistance to antibiotics [5]. One of the resistance/adaptation strategies of *S. aureus* like other bacteria is the formation of an organized community of aggregated cells, embedded in a hydrated matrix of extracellular polymeric substances (EPS) called biofilms. Biofilm allows bacteria to survive in hostile environment, even in the presence of antibiotics and to colonize new niches by various dispersal mechanisms [6]. β -lactam antibiotics are a broad spectrum group which includes penicillin derivatives such as methicillin and oxacillin and the cephalosporins [7].

Strains unable to resist these antibiotics are classified as methicillin susceptible *Staphylococcus aureus*, or MSSA. Practically all *S. aureus* isolates were methicillin susceptible until 1961, when Jevons found three MRSA strains among 5440 clinical *S. aureus* strains in England [8]. Then the situation changed as humans started to use methicillin. MRSA became prevalent all over the world, and after five decades, more than half of *S. aureus* clinical strains became methicillin resistant. MRSA is born when methicillin-susceptible *S. aureus* has acquired the methicillin resistance gene *mecA* by horizontal gene transfer mediated by a

mobile genetic element staphylococcal cassette chromosome [9].

There has been increase in the incidence of livestock acquired infections all over the world. The ability of *S. aureus* to colonize humans and animals and the detection of MRSA, MDR, enterotoxin-producing, and other emerging *S. aureus* strains in meat-producing animals and retail meat have increased the concern about the spread of those strains into the food supply chain [4]. Treatment of these infections is significantly hampered by the pathogen's propensity to acquire antibiotic resistance. The worst is that in developing countries, the surveillance systems of foodborne diseases hardly exist and it is therefore, difficult to estimate the real magnitude of the problem [10].

Thus, there is need to evaluate the prevalence of antibiotics resistant *Staphylococcus aureus* in meat samples collected from Abakaliki, Ebonyi State so as to make informed recommendation on ways to manage and, if possible, prevent this ugly trends.

MATERIALS AND METHODS

Study Area

Abakaliki is the capital city of Ebonyi State in Southeastern Nigeria, located 64 kilometres (40 mi) Southeast of Enugu. The inhabitants are primarily members of the Igbo nation. The geographical coordinate

of Abakaliki is 6°20'N 8°06'E. It was the headquarters of the Ogoja province before the creation of the Southeastern State in 1967.

Equipment and Instruments

The following equipment and instruments were used in the course of this research; autoclave (Yx-280A, England), incubator (NL-9052-I, England), refrigerator, water bath, hot air oven, petri dishes, sample bottles, test tubes, swab sticks, beakers, mortar and pestle, inoculating loop and Bunsen burner.

Reagents and Chemicals

The following reagents were used; hydrogen peroxide (H₂O₂), safranin, crystal violet, Kovac's reagents, oxidase reagent, Lugol's iodine, acetone, peptone water and distilled water.

Culture media

The culture media used include nutrient agar, Manitol salt agar (MSA), Mueller Hinton Agar (MHA). The culture media was procured from Titan Biotech Ltd., India.

Antibiotics

The antibiotics used in this study include imipenem (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), cefepime (30 µg), meropenem (10 µg), tobramycin (10 µg) ceftazidime (30 µg) and amoxicillin

clavulanic acid (30 µg). All antibiotics were procured from Oxoid limited (Oxoid, UK).

Collection of Raw meat sample

A total of 56 samples of fresh pork, goat meat, chicken and beef samples intended for human consumption were collected from different sales points in Abakaliki including Abakailiki Meat market, Kpirikpiri and Mbukobe and sent to Applied Microbiology Laboratory, Ebonyi State University, Abakaliki for processing and bacteriological analysis.

Sample processing

The meat samples collected were homogenized using mortar and pestle within 2 hours of sample collection. Thereafter, 1 g of each sample was introduced into test tube containing 9 mL of sterile water autoclave at 121°C and 1 atmosphere for 15 minutes. Then 1 mL of the first dilution was dispensed into the next 9 mL of sterile water until the 10th dilution.

Isolation of *Staphylococcus aureus* from samples

Exactly 1 mL of the 5th diluent was dispensed onto freshly prepared nutrient agar plate using the pour plate method. This was incubated at 37°C for 24 hour for growth to appear. Individual colonies were sub-cultured on Mannitol salt agar plate for purification using the streak culture method. Colonies

Mbukobe) showed that beef samples collected from Kpirikpiri recorded significantly ($P < 0.05$) higher number of colonies ($2.15 \pm 3.00 \times 10^6$ Cfug) followed by goat meat sample collected from Meat market ($2.03 \pm 3.12 \times 10^6$ Cfug), pork sample from Kpirikpiri ($2.01 \pm 5.34 \times 10^6$) and beef samples from Mbukobe (2.00 ± 2.82 Cfug), while pork samples from meat market recorded the lowest microbial burden ($1.16 \pm 4.56 \times 10^6$ Cfug) as shown in **Table 1**.

Also, the result showed that among chicken samples from the different collection points, Kpirikpiri sample recorded significantly ($P < 0.05$) high microbial load ($1.81 \pm 2.20 \times 10^6$ Cfug) followed by Meat market sample, while mbukobe sample had the least microbial load ($1.46 \pm 5.83 \times 10^6$ Cfug). Meanwhile, goat meat samples from meat market recorded the highest colony counts ($2.03 \pm 3.12 \times 10^6$), while goat meat from Mbukobe recorded the lowest microbial load ($1.62 \pm 3.71 \times 10^6$ Cfug), beef samples collected from Kpirikpiri had the highest microbial content ($2.15 \pm 3.00 \times 10^6$ Cfug) while meat market had lowest microbial load ($1.85 \pm 5.74 \times 10^6$). In the same vein, pork sample from Kpirikpiri also recorded highest number of colony counts ($2.01 \pm 5.34 \times 10^6$ Cfug), while Meat market sample had lowest counts ($1.16 \pm 4.56 \times 10^6$ Cfug) as

shown in **Table 1**. There was significant difference in the microbial load of the meat samples collected from these locations.

Distribution of *S. aureus* among meat samples collected from three different meat outlets

The result of the prevalence of *S. aureus* in the meat samples across the locations showed that among chicken samples collected from three different locations (viz; Meat market, Kpirikpiri and Mbukobe), samples collected from Kpirikpiri and Mbukobe recorded the highest prevalence (34.78 %) while meat market sample recorded the lowest (30.44 %) prevalence. Meanwhile, among goat samples all the isolates recorded the same percentage prevalence (33.33 %) across the locations, but in beef samples, Mbukobe samples showed highest (40 %) prevalence of *S. aureus*, while samples from Meat market and Kpirikpiri recorded the same prevalence (30 %) each. However, the prevalence of *S. aureus* among pork samples was significantly ($p < 0.05$) higher in Mbukobe sample (42.11 %) followed by Kpirikpiri sample (36.84 %), while Meat market sample recorded the lowest prevalence of 21.05 % as shown in **Figure 1**.

Antibiotics susceptibility pattern of the *S. aureus* isolates

The result of the antibiotics sensitivity of the isolates against including CTX, CAZ, TOB,

IPM, FOX, FEP, MEM AMC showed that the all the isolates from the different location and across different meat sample were 100 % susceptible to IPM. Also, all the isolates from pork samples collected from the 3 location were 100 % susceptible to MEM as well as isolates from Kpirikpiri and Mbukobe chicken samples, while only 75 % of the isolates from meat market chicken were susceptible to MEM, However, none of the isolates was susceptible to FOX and AMC as shown in **Table 2**.

The result showed that majority of the *S. aureus* isolates from Kpirikpiri chicken samples were less susceptible to CTX and CAZ (25 %) each, TOB and FEP were (12.5 %), Goat meat samples collected from Mbukobe recorded the lowest susceptibility against CTX and TOB (25 %), but shows no susceptibility to CAZ, FOX, FEP and amoxicillin clavulanic acid.

However, among the beef samples, Mbukobe isolates were not susceptible to CTX, CAZ,

TOB, FOX and amoxicillin clavulanic acid **Table 2** while among pork samples from the different locations Meat market sample showed 25 % susceptibility to CTX and TOB, 12.5 % (CAZ) 0 % susceptibility against FOX, FEP and AMC but 100 % susceptible to IPM and MEM as shown in **Table 2**.

Multiple Antibiotics resistance (MAR) index of the *S. aureus* isolates

The result of the mean multiple antibiotic resistance index of the isolates showed that the MAR index ranged from 0.2 to 0.7 with mean MARI of 0.6. The isolates from goat meat and beef samples from the different location as well as pork samples from Meat market and Mbukobe and chicken samples from Kpirikpiri showed high MAR index of 0.7. While, isolates from chicken samples collected from Mbukobe recorded the lowest mean MAR index of 0.2 as shown in **Table 3**.

Table 1: Microbial load of the different meat samples collected from the different locations

| Samples | Chicken (x10 ⁶)CFU/g | Goat meat(x10 ⁶) CFU/g | Beef (x10 ⁶) CFU/g | Pork (x10 ⁶) CFU/g |
|------------|----------------------------------|------------------------------------|--------------------------------|--------------------------------|
| Meat MKT | 1.76±4.96 | 2.03±3.12 | 1.85±5.74 | 1.16±4.56 |
| Kpirikpiri | 1.81±2.20 | 1.91±2.53 | 2.15±3.00 | 2.01±5.34 |
| Mbukobe | 1.46±5.83 | 1.62±3.71 | 2.00±2.82 | 1.50±4.24 |

Key: MKT= Market

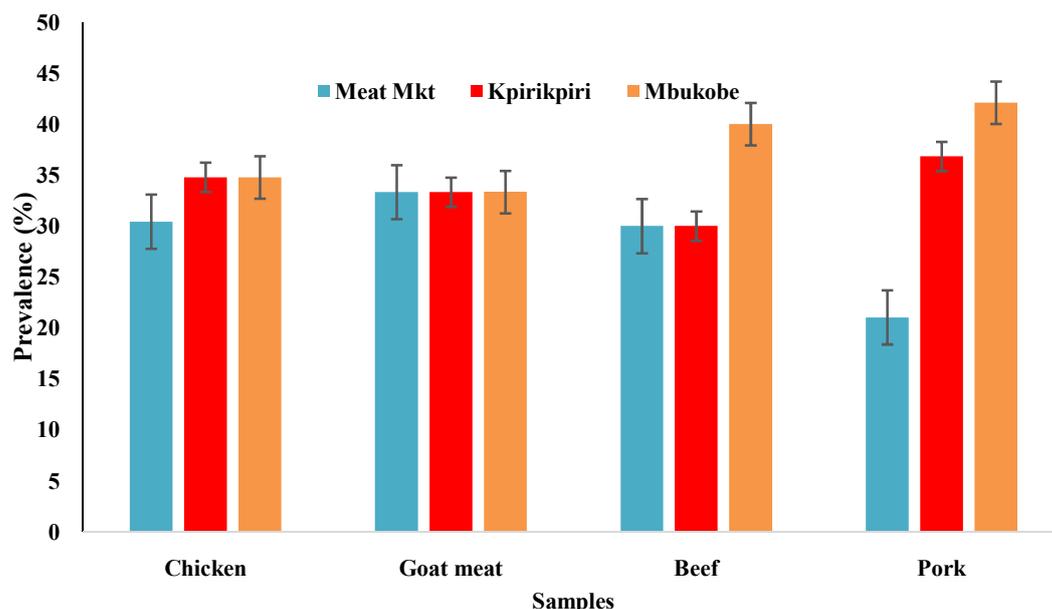


Figure 1: Prevalence of *Staphylococcus aureus* among different meat samples

Table 2: Antibiotics Susceptibility pattern of isolates from different meat samples

| Samples | Location | n=8 (%) | | | | | | | |
|-----------|------------|---------|---------|---------|--------|------|---------|---------|------|
| | | CTX | CAZ | TOB | IPM | FOX | FEP | MEM | AMC |
| Chicken | Meat MKT | 7(87.5) | 6(75) | 4(50) | 8(100) | 0(0) | 2(25) | 6(75) | 0(0) |
| | Kpirikpiri | 2(25) | 2(25) | 1(12.5) | 8(100) | 0(0) | 1(12.5) | 8(100) | 0(0) |
| | Mbukobe | 6(75) | 6(75) | 6(75) | 8(100) | 0(0) | 4(50) | 8(100) | 0(0) |
| Goat meat | Meat MKT | 3(37.5) | 1(12.5) | 3(37.5) | 8(100) | 0(0) | 1(12.5) | 7(87.5) | 0(0) |
| | Kpirikpiri | 4(50) | 1(12.5) | 2(25) | 8(100) | 0(0) | 0(0) | 8(100) | 0(0) |
| | Mbukobe | 2(25) | 0(0) | 2(25) | 8(100) | 0(0) | 0(0) | 8(100) | 0(0) |
| Beef | Meat MKT | 4(50) | 1(12.5) | 4(50) | 8(100) | 0(0) | 3(37.5) | 8(100) | 0(0) |
| | Kpirikpiri | 5(62.5) | 5(62.5) | 3(37.5) | 8(100) | 0(0) | 1(12.5) | 5(62.5) | 0(0) |
| | Mbukobe | 0(0) | 0(0) | 4(50) | 8(100) | 0(0) | 0(0) | 8(100) | 0(0) |
| Pork | Meat MKT | 2(25) | 1(12.5) | 2(25) | 8(100) | 0(0) | 0(0) | 8(100) | 0(0) |
| | Kpirikpiri | 2(25) | 0(0) | 3(37.5) | 8(100) | 0(0) | 0(0) | 8(100) | 0(0) |
| | Mbukobe | 2(25) | 2(25) | 2(25) | 8(100) | 0(0) | 2(25) | 8(100) | 0(0) |

Key: IPM=Imipenem, CTX = cefotaxime, FOX = ceftazidime, FEP = cefepime, MEM = Meropenem, TOB = tobramycin, CAZ = ceftazidime, AMC= amoxicillin clavulanic acid. MKT = Market, n = number of meat sample per location; % = percentage susceptibility

Table 3: Multiple antibiotics resistance index of the *S. aureus* isolates

| Samples | Location | MARI | Antibiotics |
|-----------|------------|------|---------------------------------|
| Chicken | Meat MKT | 0.3 | TOB, FOX and AMC |
| | Kpirikpiri | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| | Mbukobe | 0.2 | FOX and AMC |
| Goat meat | Meat MKT | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| | Kpirikpiri | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| | Mbukobe | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| Beef | Meat MKT | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| | Kpirikpiri | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| | Mbukobe | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| Pork | Meat MKT | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |
| | Kpirikpiri | 0.5 | TOB, FOX, FEP and AMC |
| | Mbukobe | 0.7 | CTX, CAZ, TOB, FOX, FEP and AMC |

Key: MKT = Market

DISCUSSION AND CONCLUSION

Staphylococcal food borne intoxication is one of the most common forms of bacterial food-borne disease in many countries [13], and in livestock, *Staphylococcus aureus* has been described as a significant cause of skin and soft tissue infections, mastitis and systemic infections and is considered a key foodborne pathogen [14]. The demand for animal proteins is increasing globally at a relatively high rate for human consumption. Concern about the threat of antibiotic-resistant strains of *Staphylococcus* species has increased in recent years [15]. The present study was carried out to evaluate the prevalence of antibiotics resistant *Staphylococcus aureus* in raw meat samples intended for human consumption in Abakaliki, Ebonyi State.

The result showed that the microbial counts ranged from $2.15 \pm 3.00 \times 10^6$ Cfu/g in beef

samples collected from Kpirikpiri to $1.16 \pm 4.56 \times 10^6$ Cfu/g in pork collected from meat market **Table 1** while. the prevalence of *S. aureus* ranged from 30.44 % to 34.78 % **Table 2**. This suggest that the meat samples sold in this region are highly contaminated with microorganisms depicting a possibility of poor hygiene animal handling. The prevalence of *S. aureus* in the different meat samples was lower than the report by [16] who reported a 79 %, 63 % and 44 % prevalence of *S. aureus* in chicken, pork and beef samples respectively and [17] also reported prevalence of *S. aureus* ranging from 50 to 90 % in different processed meat intended for human consumption. The result also indicated poor meat handling across the locations which have resulted in cross contamination as evident in the result. Meat contamination by microorganisms is common since these organisms are

ubiquitous. In line with this assertion, [18] stated that most foods contain viable bacteria unless thoroughly heated or pasteurized and further stated that, otherwise, raw meat will serve as an important vehicle for transmission of pathogenic organisms to consumers. Although the microbial load and the prevalence of *S. aureus* seems a little higher in some locations than the other **Tables 1 and 2**, there was no significant difference in their microbial burden and *S. aureus* prevalence, suggesting a situation of possible generalized careless handling of meat which has led to cross contamination and heavy microbial burden.

The result of the antibiotics susceptibility of the isolates showed that all the isolates were resistant to some conventionally used antibiotics including majority of amoxicillin clavulanic acid (AMC) and FOX but were highly susceptible to the carbapenems including imipenem (IPM) and meropenem (MEM) where all the isolates were susceptible to IPM. Meanwhile, among the meat samples, chicken and pork isolates from Kpirikipiri were more resistant to the antibiotics compared to those isolated from Meat market and Mbukobe, while goat meat and beef isolates from Mbukobe showed highest rate of antibiotics resistance, 50 to 100 % resistant to the antibiotics used.

Similar to this observation, [17] reported that 100% of their isolates were resistant to kanamycin, 92% for penicillin and neomycin, 84% for oxacillin and erythromycin and 68% for ampicillin and nalidixic acid, 64% for oxytetracycline and sulphamethoxazol. This indicates that the occurrence of antibiotics resistance *S. aureus* may be a global phenomenon and this can be attributed to global use of antibiotics in animal husbandry. In line with this assertion, the occurrence of antimicrobial-resistant bacteria is associated with the use of antimicrobial agents in food producing animals [19]. Human infections involving antimicrobial resistant bacteria have increased and are now common. The emergence of antibiotic resistance has been recognized to be the result of extensive prophylactic and therapeutic use of antimicrobials as growth promoters in food-producing animals [20, 21].

The result of MAR index ranged from 0.2 to 0.7 with mean MAR index of 0.6. The isolates from goat meat and beef recorded higher MAR index followed by pork, while chicken had the lowest MAR index **Table 3**. Similar to this observation, [17] reported a multiple antibiotic resistance MAR index of isolated *S. aureus* ranged from 0.071 to 1 with an average of 0.59. This indicates that greater percentage of the *S. aureus* are

multiple antibiotics resistant strains. Since antibiotic resistance from animals can be disseminated to humans through food products, environment and by direct contact to agricultural workers [22]. Hence, there is need to device effective means of putting this under control in order to curtail this ugly trend.

CONCLUSION

This study has shown that there is high prevalence of pathogenic *S. aureus* in meat samples sold in Abakaliki intended for human consumption. All the meat samples had high microbial load; were able to resist the effect of many antibiotics and also showed high MAR index. This is particularly a threat to the general public as the health of the masses is at stake. There is need to take drastic measure to curtail this situation.

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