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ANTIBIOTIC RESISTANT PROFILE AND MOLECULAR DETECTION OF RESISTANCE GENES IN MULTI-DRUG-RESISTANT *Staphylococcus aureus* ISOLATED FROM RETAIL MEAT IN OWERRI

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ABSTRACT

This study examines the antibiogram and molecular detection of Staphylococcus aureus isolated from retailed meat; beef, chicken, and pork in Owerri, Nigeria. Fifty samples were collected from the World Bank, Relief, and Amakaohia markets and assessed for bacterial load, identification, and antibiotic susceptibility. Prevalence rates were highest in beef (41.7%), followed by chicken (33.3%) and pork (25%), with a significant correlation between meat type and contamination ($p < 0.05$). Bacterial counts ranged from 29–300 cfu/g (TVC), 30–350 cfu/g (TCC), and 15–203 cfu/g (TSC), indicating varied hygiene conditions. All isolates showed complete resistance to Ceftriaxone, with high resistance (86.7%) to Azithromycin, Amoxil, and Cefuroxime, and moderate resistance (56.7%) to Rifampicin, Ciprofloxacin, Levofloxacin, Erythromycin, and Gentamycin. Multi-drug resistance was present in 33.3% of isolates, which were resistant to five antibiotic classes. Molecular analysis confirmed MRSA strains through the detection of the mecA gene (533 bp) and phylogenetic similarity to known global strains. These findings underscore retailed meat in Owerri as a reservoir for multidrug-resistant S. aureus, driven by inadequate hygiene and indiscriminate antibiotic use, necessitating stricter regulations, improved handling practices, and enhanced public health surveillance.

Keywords: Meat, Multi drug resistant, antibiogram, prevalence.

Introduction

Staphylococcus aureus is a gram-positive, facultative anaerobic bacterium that is widely recognized as both a commensal and a significant pathogen in human and animal health (Otto, 2020). In addition to its clinical significance, *S. aureus* has also emerged as a critical concern in food safety, particularly in meat and meat products (Hammad and Shimamoto, 2021). The ability of this bacterium to produce a spectrum of virulence factors, including enterotoxins, enhances its potential to cause staphylococcal food poisoning (Turner *et al.* 2019).

Retailed meat is considered a high-risk food product due to its susceptibility to contamination by pathogenic microorganisms during slaughter, processing, transportation, and retail (Zhao *et al.* 2020). Contamination of meat with *S. aureus* can occur through various routes, including cross-contamination from handlers, equipment, and the environment (Ehsan *et al.* 2019). The public health threat posed by *S. aureus* contamination in meat is further compounded by the emergence of antibiotic-resistant strains, including methicillin-resistant *Staphylococcus aureus* (MRSA) and other multidrug-resistant (MDR) variants (Liu *et al.* 2018).

Owerri, the capital city of Imo State in southeastern Nigeria, is a commercial hub with vibrant markets such as Eke Onunwa, where meat is sold under varying hygienic conditions (Iroha *et al.* 2022). The meat sold in these markets is often displayed openly, allowing for potential contamination by airborne microorganisms, vectors, and through human contact (Nworie *et al.* 2021). Moreover, the lack of stringent regulatory frameworks for food safety and the common practice of slaughtering animals in informal settings contribute to the risk of bacterial contamination (Onoh *et al.* 2023). While studies on the microbiological quality of retailed meat in other regions of Nigeria have been conducted, data specific to *S. aureus* contamination and its antibiotic resistance patterns in Owerri are scarce (El-Masry *et al.* 2020).

Given the increasing burden of antibiotic resistance and the critical role of foodborne pathogens in its propagation, this study aims to assess the antibiogram of resistant *Staphylococcus aureus* isolated from retailed meat in Owerri, Nigeria.

Material and Methods

Study Area

The study area is Owerri, Imo State. Geographically, Owerri is located at 5°30′9.42 N and 7˚2′49.02 E. Nigeria's Imo State, located in the center of Igboland, has Owerri as its capital. It is also the biggest city in the state, with Orlu and Okigwe coming in second and third, respectively. Owerri, which is around 100 square kilometers (40 square miles) in size and contains three Local Government Areas, Owerri Municipal, Owerri North, and Owerri West, has an estimated 1,401,873 residents as of 2016 (Federal Republic of Nigeria official gazette, 2007).

Sample collection

Ten (10) samples each of chicken, pork, and beef making total of thirty (30) samples of meat were bought from markets like World Bank, Relief, Amakohia. The selection of the market was based on their strategic location in conjunction with consumer patronage and samples was taken to the Post-graduate Microbiology laboratory Owerri in sterile b

Sample Preparation and Serial Dilution

A standardized microbiological protocol was employed to facilitate the isolation and enumeration of microorganisms from meat samples. Approximately 10 grams of each meat sample were aseptically weighed and transferred into sterile stomacher bags containing 90 mL of buffered peptone water (BPW), a widely used non-selective diluent for microbial recovery from food matrices. The samples were homogenized using a laboratory stomacher for 1–2 minutes to ensure thorough dispersion of microbial cells throughout the suspension (FSIS, 2021).

Following homogenization, serial tenfold dilutions were prepared to reduce microbial load and enable accurate colony enumeration. This was achieved by transferring 1 mL of the homogenized sample into 9 mL of sterile BPW, yielding a 10^{-1} dilution. Subsequent dilutions were prepared in the same manner up to 10^{-6}, depending on the anticipated microbial density. Each dilution was thoroughly mixed using a vortex mixer to ensure homogeneity before plating.

Following incubation, distinct bacterial colonies were streaked onto nutrient agar to obtain pure cultures. The bacterial isolates were then morphologically and biochemically characterized using conventional techniques (Cheesbrough, 2010) and identified using Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 2004).

Microbiological Analysis

For Total Viable Count

To determine the total heterotrophic count, meat samples from chicken, pork, and beef were serially diluted as previously described. The diluted samples were then inoculated onto Nutrient Agar. The inoculated plates were incubated at 37°C for 24 hours, after which the bacterial colonies were counted.

For Coliform Count

Eosin Methylene Blue (EMB) agar was used to detect coliform bacteria to determine the total coliform count. This was accomplished by inoculating diluted water samples into the EMB medium. The inoculation plates were then incubated at 37°C for 24 hours, and the former colonies were counted and recorded (Cheesbrough, 2011).

For Total Staphylococcal Count

To selectively isolate *Staphylococcus* species, particularly *Staphylococcus aureus*, Mannitol Salt Agar (MSA) was employed due to its high salt concentration (7.5% NaCl), which inhibits the growth of most non-halotolerant organisms while allowing *Staphylococcus* spp. to thrive. Also, MSA contains mannitol and phenol red, enabling differentiation based on mannitol fermentation, characteristic of *S. aureus* (Baird-Parker, 1990). Aliquots of 0.1 mL from appropriate dilutions were plated onto MSA using the spread plate technique and incubated at 35–37°C for 24–48 hours under aerobic conditions (Cheesbrough, 2011).

Morphological Identification of isolates

The bacterial isolates were identified using colonial, cellular characteristics, Gram Staining, Motility test, and biochemical properties. Colonial and cellular characteristics were used in the identification of microbial isolates, and they include: Colony's shape, colour, consistency, surface appearances, and sizes of the colony (diameter in mm) (Ohazuruike *et al.,* 2017)

Gram's staining

This differential staining technique differentiates microorganisms into Gram-positive and Gram-negative (Ohazuruike *et al.,* 2017). The Gram staining method described (Ohazuruike *et al.,* 2017) was adopted with the aid of a sterile inoculating wire loop; smears of the isolates were made on clean, grease-free glass slides, air-dried, and heat-fixed by passing the slides 2 – 3 times over a Bunsen burner flame. Afterwards, each smear was covered with a Crystal violet (primary stain) for 30 seconds and quickly washed off with clean water. The smear was flooded with iodine (mordant) for 60 seconds. After which, they were decolorized with 75% alcohol for 30 seconds, which was washed off quickly with clean water and counterstained with safranine for 30 seconds. The safranine stain was washed off quickly with clean water. The back of the slides was wiped and placed in a draining rack to air-dry. The smear was then examined microscopically using the oil immersion objective (X100). Gram-positive cells showed purple while gram-negative cells showed red colour (Ohazuruike *et al.,* 2017).

Biochemical tests

Some of the biochemical tests used in the identification of bacteria isolates are:

Catalase test

The method, as described by Prescott *et al.* (2015), was adopted. This test differentiates catalase-producing bacteria like *Staphylococci* from non-catalase-producing bacteria such as *Streptococci*. The catalase produced acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. A drop of 3% hydrogen peroxide was placed on each end of a microscope slide with a sterile wire loop. Colonies of the test organisms were transferred to one end of the microscope slide, and

the other end was not inoculated but served as a control. Gas bubbles indicate a positive catalase test, while the absence indicates a negative catalase test.

Citrate utilization test

This test is used to identify members of the family *Enterobacteriaceae.* The test is carried out to-demonstrate the use of citrate as a sole source of carbon by alkalinization of the medium and ammonia as the only source of nitrogen by the bacteria. The method as described by (Ohazuruike *et al.,* 2017) was adopted. The test was carried out by inoculating sterilized Simmon's citrate agar with the test organisms using a sterile wire loop, incubating at 370 for 48 hours, and observing for colour changes. Positive result shows a change of the medium colour from green colour of two royal blue, indicating the presence of citrate-utilizing bacteria.

Coagulase test

The test demonstrates the ability of bacteria to produce coagulase as a defence mechanism, by clotting the area of plasma around it by converting fibrinogen to fibrin, thereby enabling them to resist phagocytosis. It is used for the identification of *Staphylococcus aureus*. The method, as described by Ohazuruike *et al.* (2017), was adopted. Distilled water was placed on each end of the microscope slide. A colony of test organisms was emulsified in each drop of distilled water placed on the ends of the microscopic slide to make thick suspensions. A 100cfu/l of plasma was added to one of the suspensions and mixed gently. No plasma was added to the same suspension serving as a control. Clumping the mixture within 10 seconds will indicate a positive coagulase test, while the absence of clumps within 10seconds indicates a negative result.

Indole test

The method of Ohazuruike *et al.* (2017) was adopted. Testing for indole production is important in the identification of enterobacteria. The test organism is cultured in a medium that contains tryptophan. Indole production is detected by Kovac's reagent, which contains 4-p-dimethylaminobenzaldehyde; it reacts with the indole to produce a red coloured compound. The test organisms were inoculated in a bijou bottle containing 3ml of sterile tryptone water, which was incubated at 37^0C °C for 48 hours. After incubation, 0.5ml of Kovac's reagent was added, the tubes were gently shaken, and the appearance of a red surface layer within 10 minutes indicates a positive indole test.

Oxidase test

The method, as described by Ohazuruike *et al.* (2017), was adopted. A piece of filter paper was soaked with a few drops of oxidase reagent (tetra-ethyl-p-phenylenediamine dihydrochloride). A colony of the test organism was picked with a sterile glass rod and smeared on the filter paper. A blue-purple colour develops within a few seconds if the organism is an oxidase producer due to

the oxidation of the phenylendiamine, while the absence of a blue-purple colour indicates a negative result.

Methyl Red/ Voges-Proskauer (MR/VP)

This test determines which fermentation pathway utilizes glucose (Ohazuruike *et al.,* 2017). It is used to differentiate bacteria capable of fermenting glucose with enough acid to lower the pH of the medium to 4 - 4.5, and those that ferment glucose without much acid production. Methyl red contains glucose and peptone. The method, as described by Ohazuruike *et al.* (2017), was adopted. The bacteria isolates were inoculated into 2mls of glucose phosphate (peptone water) and were incubated at 37^0C °C for 48hours. After incubation, four drops of methyl red indicator were added to the tube. The solution was homogenized and observed immediately for colour change. The appearance of a red colour indicates a positive result, while the appearance of a yellow colour indicates a negative result. For Voges-proskauer test, the method described by Ohazuruike *et al.* (2017) was adopted, the bacteria isolates were added to 2ml of glucose phosphate (peptone water) and it was incubated at 37^0C for 48hrs, after incubation, 40% KOH and 3ml of 5% alcoholic alpha-naphthol were added, the appearance of a pink colour after 2-5 minutes indicates a positive result.

Sugar Fermentation Test

This test was employed to check for the ability of an organism to ferment sugar. Triple Sugar Iron (TSI) is the agar used in this test. This test measures the ability of the organism to produce gas, Hydrogen sulphide, to ferment Glucose, lactose, and sucrose, and to ascertain if its Slant and Base are acidic or basic. The agar was sterilized at 121^0C at 15mins. The test organism was inoculated into a slanted test tube. A colour change from pink to yellow indicates the utilization of several sugars. A black duct in the slanted area indicates the presence of H_2S. Moreover, a gaseous bubble at the bottom or slant of the test tube indicates the presence of gases, while displacement in Durham's tube indicates gas production (Ohazuruike *et al.,* 2017).

Antibiotics Susceptibility Test

After Isolation and Identification, an antimicrobial susceptibility test was carried out using the disk diffusion method described by Ohazuruike *et al.* (2017) on Muller-Hinton agar medium. The following antibiotics were employed for sensitivity analysis: Rifampicin, Gentamycin, Amoxil, Ciprofloxacin, Streptomycin, Erythromycin, Azithromycin, Cefuroxine, and Levofloxacin.

The growth was standardized by diluting the culture with normal Saline to match the turbidity of 1.0×10^6 cfu/ml (0.5McFarland standards). The 0.1ml was collected and spread on the surface of Muller Hinton agar (Oxoid LTD, Basingstoke, UK) using a sterile glass rod. The antibiotic disc was placed carefully to make good contact with the agar surface using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition.

The agar plates were left on the bench for 30 minutes to allow for diffusion of the antibiotics and were incubated at 37 °C for 24 hours. The results were interpreted as sensitive and resistant. Results were recorded by measuring and comparing the incubation zone with the CLSI susceptibility.

Molecular Identification

Bacterial DNA Extraction

DNA extraction was carried out using the ZR Bacteria DNA MiniPrep Kit (Inqaba, South Africa). Pure bacterial colonies were lysed in a ZR Bashing Bead tube with lysis solution and homogenized at maximum speed for 5 minutes. After centrifugation, the supernatant was filtered and mixed with DNA binding buffer before being passed through a Zymo-Spin IIC column for DNA purification. The column was sequentially washed with pre-wash and wash buffers, and DNA was finally eluted with 100 µl of elution buffer. The purified DNA was stored at –20°C for subsequent molecular analyses.

16S rRNA Amplification

The 16S rRNA gene of the bacterial isolates was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') in a 50 µL reaction volume for 35 cycles on an ABI 9700 thermal cycler. The PCR mixture contained X2 DreamTaq Master Mix (Inqaba, South Africa), 0.4 µM of each primer, and the extracted DNA template. Cycling conditions included an initial denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds; annealing at 52°C for 30 seconds; extension at 72°C for 30 seconds; and a final extension at 72°C for 5 minutes. Amplified products were separated on a 1% agarose gel at 120V for 15 minutes and visualized under UV illumination.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, and similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) represents the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor, 1969).

Results

The result of bacterial load of beef samples (BEF1–BEF10) from three markets is presented in Figure 1. BEF3 from the World Bank Market showed the highest Total Viable Count (230 cfu/g) and Total Coliform Count (180 cfu/g), indicating significant contamination. Meanwhile, BEF10 from Amakaohia Market recorded the highest Staphylococcal Count (203 cfu/g). These results

reflect varying hygiene practices, with World Bank and Amakaohia markets showing potential handling concerns. Result of the bacterial loads in chicken samples (CHK1–CHK10) from three markets revealed that CHK5 (Relief Market) had the highest Total Viable Count (300 cfu/g). At the same time, CHK3 (World Bank Market) showed the highest Total Coliform Count (350 cfu/g) and Staphylococcal Count (175 cfu/g), indicating potential hygiene issues. In contrast, chicken samples from Amakaohia Market exhibited lower microbial counts, suggesting better sanitary handling. (Figure 2). For bacterial counts in pork samples (POK1–POK10) from three markets in Owerri, the results showed the highest Total Viable Count (180 cfu/g) and Total Coliform Count (190 cfu/g) in samples from the Relief Market, suggesting possible hygiene lapses. The highest Staphylococcal Count (135 cfu/g) was found in a sample from the World Bank Market. Lower counts in other samples may suggest comparatively better microbial quality in some locations (Figure 3).

The morphological and biochemical profiles of bacterial isolates from beef, chicken, and pork, presented in Table 4a, identified five genera, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, *Escherichia coli*, *Salmonella spp.*, and *Klebsiella spp.*. *In contrast,* Table 4.2b showed the different types of retailed meat where *Staphylococcus aureus* was isolated.

Results showed that *Staphylococcus aureus* predominated for 22.2%, while *Pseudomonas spp.* and *Klebsiella spp.* recorded the lowest prevalence, each with 6 isolates (11.1%) (Figure 5). Similarly, beef samples had the highest contamination, followed by chicken and pork (Figure 6)

For antibiotics susceptibility, the results show that all 30 isolates were resistant to Ceftriaxone, indicating a complete lack of efficacy of this antibiotic against the tested isolates. High resistance rates were also noted for Azithromycin, Cefuroxime, and Amoxil, with 26 out of 30 isolates (86.7%) showing resistance. The remaining antibiotics, including Rifampicin, Ciprofloxacin, Levofloxacin, Erythromycin, and Gentamycin, each demonstrated moderate resistance rates of 56.7%, with 17 resistant and 13 susceptible isolates.

The correlation between the prevalence of *Staphylococcus aureus* and different meat types in retailed meat samples from Owerri is presented in Table 5. The table includes the prevalence percentages for each meat type, along with the corresponding Chi-Square (χ^2) values and p-values, which assess the statistical significance of the relationship between meat type and bacterial prevalence. The results indicate that beef had the highest prevalence of *S. aureus* at 41.7%, with a Chi-Square value of 7.45 and a p-value of 0.024, suggesting a statistically significant association between beef and *S. aureus* contamination. Chicken, with a prevalence of 33.3%, showed a Chi-Square value of 4.35 and a p-value of 0.038, also indicating a significant relationship, albeit slightly weaker than that for beef. On the other hand, pork exhibited the lowest prevalence at 25%, with a Chi-Square value of 2.10 and a p-value of 0.150, which is not statistically significant. These findings suggest that the prevalence of *S. aureus* is significantly higher in beef and chicken

compared to pork, underlining the potential risks associated with different types of meat in terms of contamination.

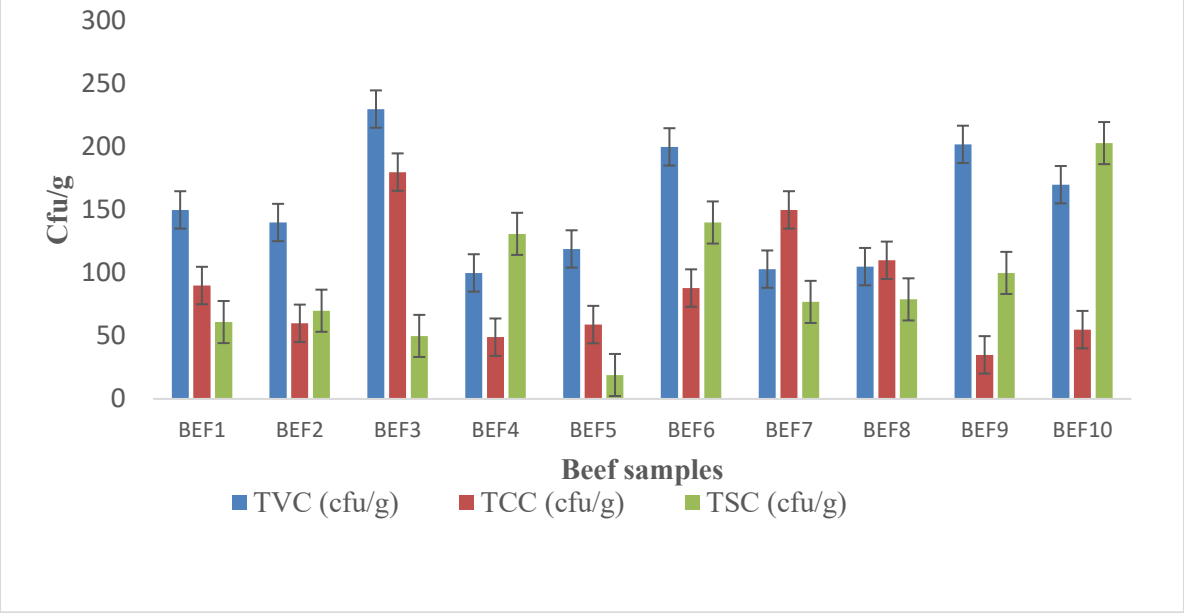


Figure 1: Enumeration of bacteria load of beef samples from different markets

Keys: TVC = Total Viable Count

TCC = Total Coliform Count

TSC = Total Staphylococcal Count

NG = No growth

BEF 1 -03 = World Bank Market

BEF 04 - 06 = Relief Market

BEF 07-10 = Amakaohia Market

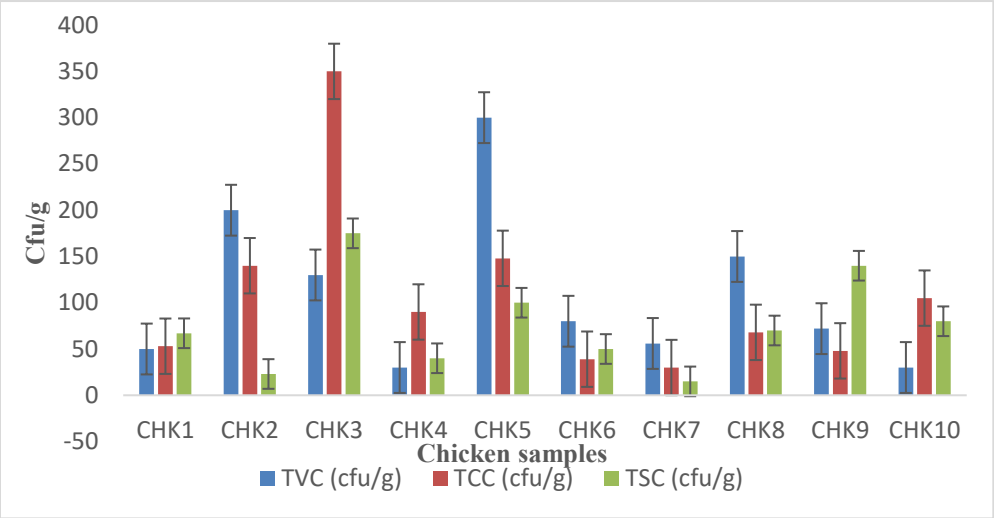


Figure 2: Enumeration of Bacteria load of chicken samples from different markets

Keys: TVC = Total Viable Count

TCC = Total Coliform Count

TSC = Total Staphylococcal Count

CHK 1 - 03 = World Bank Market

CHK 04 -06 = Relief Market

CHK 07-10 = Amakaohia Market

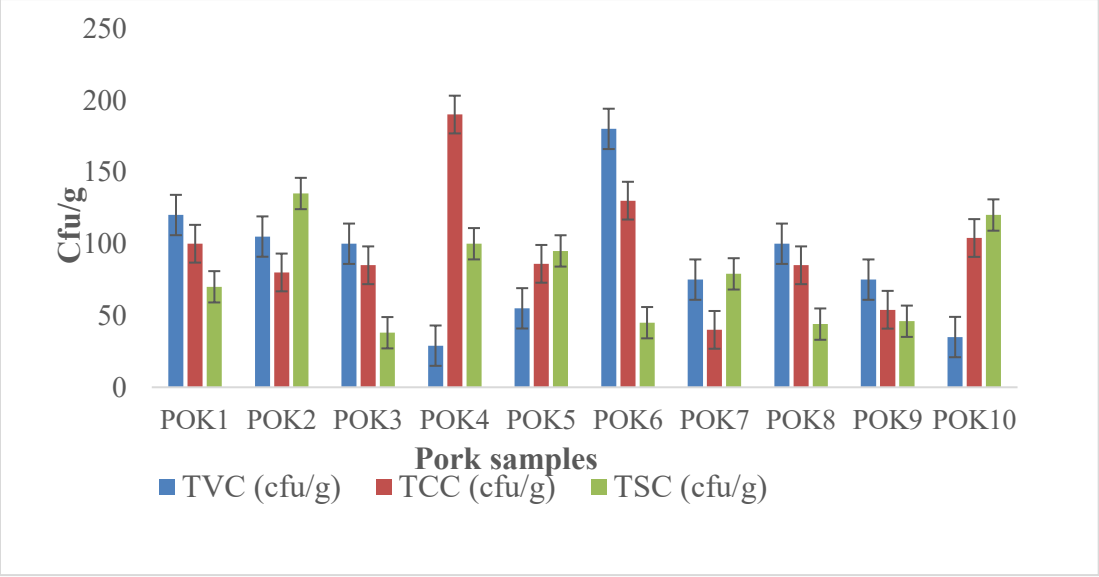


Figure 3: Enumeration of Bacteria load of pork samples from different markets

Keys: TVC = Total Viable Count

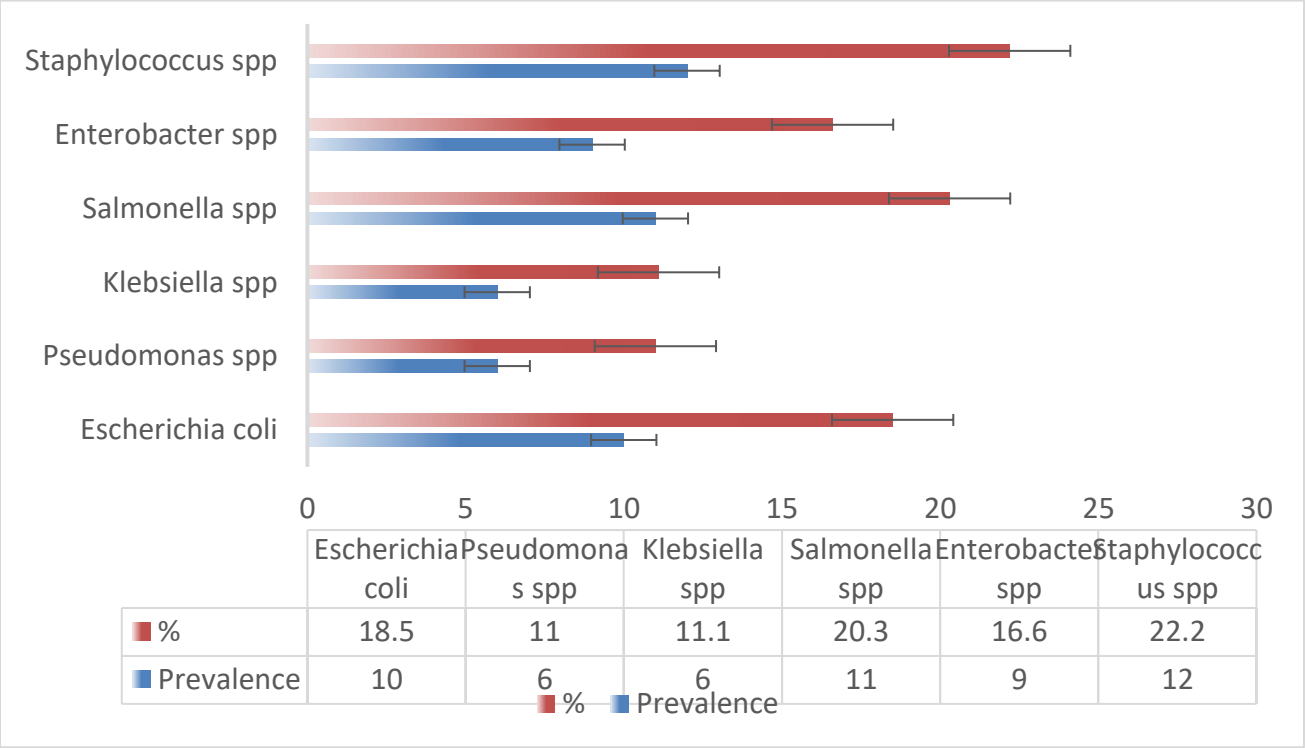
TCC = Total Coliform Count

TSC = Total Staphylococcal Count

CHK 1 -03 = World Bank Market

CHK 04 -06 = Relief Market

CHK 07-10 = Amakaohia Market



	Escherichia coli	Pseudomonas spp	Klebsiella spp	Salmonella spp	Enterobacter spp	Staphylococcus spp
%	18.5	11	11.1	20.3	16.6	22.2
Prevalence	10	6	6	11	9	12

Figure 4: **Prevalence of bacteria isolates from different retailed meat samples.**

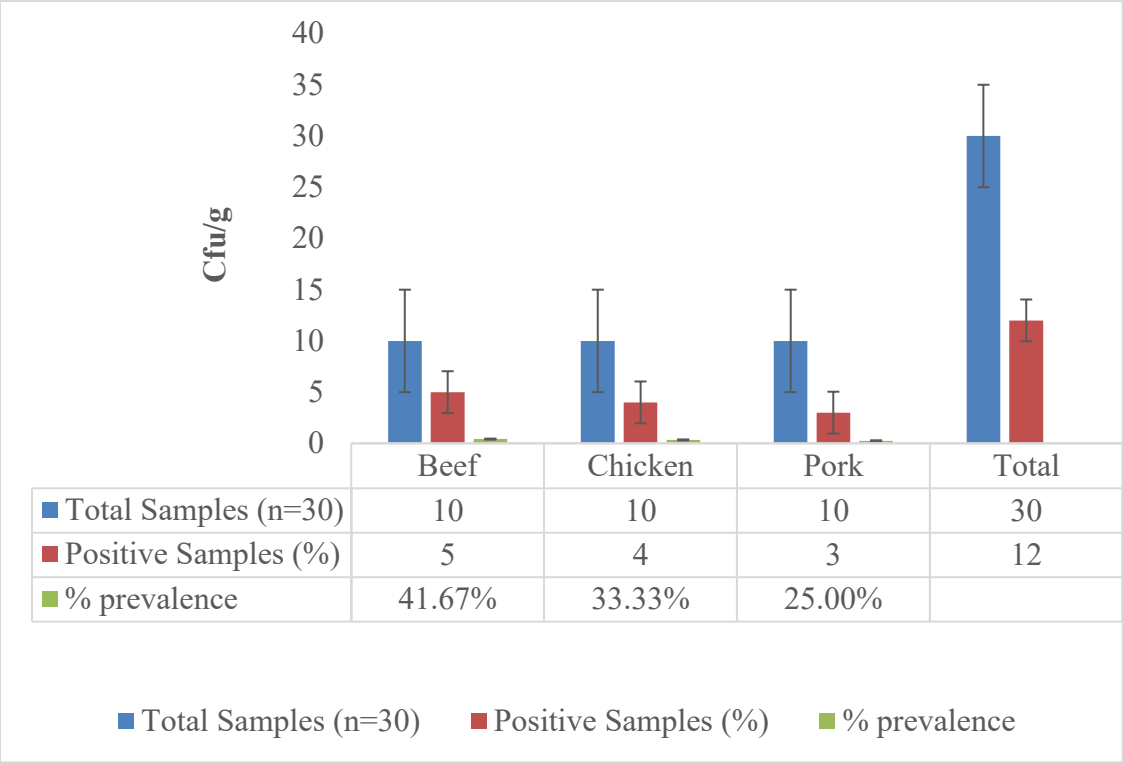


Figure 6: **Prevalence of Staphylococcus isolates from different retailed meat samples**

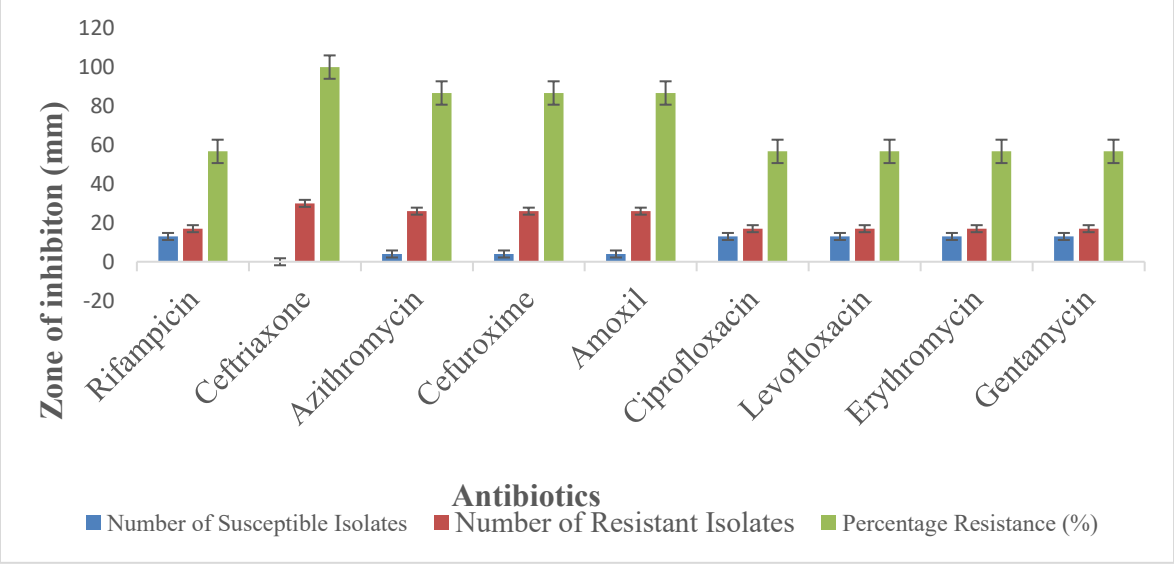


Figure 6: Antibiogram of *Staphylococcus* isolates against selected antibiotics

TABLE 1: Morphological and Biochemical Characteristics of Isolates from Different Sources of Meat Samples.

ISOLATE CODE	Biochemical Test							ST	Motility Test	Gram Staining	Colour	Morphology	Probable Organism
	CA	CO	OX	IN	CI	MR	VP	GLU					
BEF2,3,6,8,9, CHK3,5,7,9, POK2,5,9	+	–	+	+	+	+	+	+	–	+	Creamy yellow	Cocci in cluster	*Staphylococcus aureus*
BEF 1,4, CHK 2,4 POK5,10	+	–	–	+	–	–	+	+	+	–	Green – yellowish	Rod in chain	*Pseudomonas aeruginosa*
BEF 2,3,10 CHK 1,3,7 POK 1,3,8	+	–	–	–	+	–	+	A/G	+	–	Pink	Large rods in chain	*Enterobacter spp*
BEF 8,5,4, CHK 3,5,8,10 POK 5,6,9	+	–	+	+	–	+	–	A/G	+	-	Green black	Tiny rods in chain	*Escherichia coli*
BEF 1,4,2	+	_	_	_	+	+	_	+	+	-	Pale yellow	Straight Rods	*Salmonella spp*

6,9, CHK 5,6,9 POK 5,7,9													
BEF CHK POK	1,4 2,3,5 6	+	-	-	-	+	-	+	A/G	-	-	pink	Rod	*Klebsiella* spp.

KEY: CA = Catalase test, CO = Coagulase test, OX = Oxidase test, IND = Indole test, CI = Citrate test, MR = Methyl red test, VP = Voges-Proskauer test, ST = Sugar fermentation test, GLU = glucose, + = Positive, - = negative, A/G = produce Acid and gas

TABLE 2: Morphological and biochemical characteristics of *Staphylococcus aureus* from different sources of meat samples.

ISOLATE CODE	Biochemical Test								Motility Test	Gram Staining	Colour	Morphology	Probable Organism
	CA	CO	OX	IN	CI	MR	VP	GLU					
BEF 2,3,6,8,9, CHK 3,5,7,9, POK 2,5,9	+	–	+	+	+	+	+	+	–	+	Creamy yellow	Cocci in cluster	*Staphylococcus aureus*

Table 3: Molecular Sequence 16S rRNA identity of bacteria

S/N	Isolate Number	Percentage (%)	NCBI match
1.	BEF01	100	*Staphylococcus aureus* OQ891488
2.	CHK07	100	*Staphylococcus aureus* CPQ113244
3.	POK07	87	*Staphylococcus aureus* PQ123258

KEY: CA = Catalase test, CO = Coagulase test, OX = Oxidase test, IND = Indole test, CI = Citrate test, MR = Methyl red test, VP = Voges-Proskauer test, ST = Sugar fermentation test, GLU = glucose, + = Positive, - = negative, A/G = produce Acid and gas

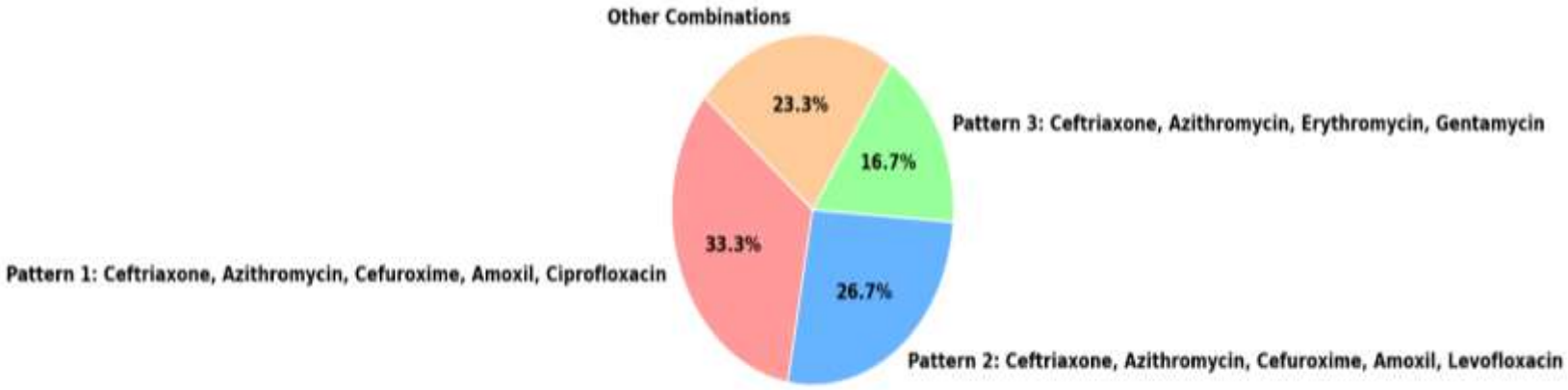


Fig 7: Multi drugs resistance patterns in *Staphylococcus aureus* isolates

Table 4: Correlation between prevalence of *Staphylococcus aureus* and meat type

Meat Type	*Prevalence (%)*	*Chi-Square (χ^2)*	*p-value*
Beef	*41.7%*	*7.45*	*0.024*
Chicken	*33.3%*	*4.35*	*0.038*
Pork	*25%*	*2.10*	*0.150*

Table 5: Antibiotic Susceptibility Profile of *S. aureus* Isolates (n =30)

Antibiotic	*Number of Susceptible Isolates*	*Number of Resistant Isolates*	*Percentage Resistance (%)*
Rifampicin	*13*	*17*	*56.7*
Ceftriaxone	*0*	*30*	*100*
Azithromycin	*4*	*26*	*86.7*
Cefuroxime	*4*	*26*	*86.7*
Amoxil	*4*	*26*	*86.7*
Ciprofloxacin	*13*	*17*	*56.7*
Levofloxacin	*13*	*17*	*56.7*
Erythromycin	*13*	*17*	*56.7*
Gentamycin	*13*	*17*	*56.7*

Discussion

The study of the antibiogram of *Staphylococcus aureus* isolated from retailed meat in Owerri, Nigeria, reveals concerning patterns of antibiotic resistance. *S. aureus* is a significant pathogen in foodborne illnesses and can lead to a variety of infections, including skin and soft tissue infections, pneumonia, and sepsis. In this study, isolates from different types of retailed meat: beef, chicken, and pork were tested for their susceptibility to several commonly used antibiotics.

The parameters assessed include Total Viable Count (TVC), Total Coliform Count (TCC), and Total Staphylococcal Count (TSC). TVC values ranged from 100 to 230 cfu/g, with the highest level observed in sample BEF3 (230 cfu/g), obtained from World bank market. TCC ranged between 35 and 180 cfu/g, with BEF3 again recording the highest count (180 cfu/g). TSC values were found to range from 19 to 203 cfu/g, with the peak count recorded in BEF10 (203 cfu/g) from Amakaohia market. These findings suggest varying degrees of bacterial contamination among the sampled locations. Notably, samples from World Bank Market exhibited elevated TVC and TCC levels, particularly in BEF3, while Amakaohia Market demonstrated the highest staphylococcal load in BEF10, indicating potential hygiene and handling concerns specific to each market as seen Figure 1.

As for chicken samples, total Viable Count (TVC) values ranged from 30 to 300 cfu/g, with CHK5 from Relief Market recording the highest count (300 cfu/g). Total Coliform Count (TCC) ranged between 30 and 350 cfu/g, with CHK3 from World Bank Market exhibiting the highest coliform level (350 cfu/g). Similarly, Total Staphylococcal Count (TSC) ranged from 15 to 175 cfu/g, with CHK3 again showing the highest value (175 cfu/g). The elevated microbial counts observed in CHK3 suggest significant bacterial contamination, indicating possible lapses in hygiene and handling practices at the World Bank Market. Conversely, samples from Amakaohia Market generally recorded lower microbial loads across all parameters, pointing to comparatively better sanitary conditions as revealed in Figure 2.

While the Pork meat, the Total Viable Count (TVC) ranged from 29 to 180 cfu/g, with the highest count observed in sample POK6 from Relief Market (180 cfu/g). Total Coliform Count (TCC) varied between 40 and 190 cfu/g, with POK4, also from Relief Market, recording the highest value (190 cfu/g). Total Staphylococcal Count (TSC) ranged from 38 to 135 cfu/g, with the peak value found in POK2 from World Bank Market (135 cfu/g). The elevated microbial loads, particularly the high TCC in POK4 and TVC in POK6, suggest possible hygiene or sanitation lapses in pork handling and processing within Relief Market. In contrast, the relatively lower counts in other samples indicate better microbial quality in some of the other market locations as presented in Figure 3

The bacterial counts in Figures 1-3 align with previous studies on meat contamination in open markets. A study by Clarence et al. (2009) on beef in Nigerian markets reported TVC ranging from

10^2 to 10^5 cfu/g, with coliforms and *Staphylococcus* spp. frequently detected, consistent with the current findings of TVC (29–300 cfu/g), TCC (30–350 cfu/g), and TSC (15–203 cfu/g) across beef, chicken, and pork. The elevated TCC in chicken (CHK3, 350 cfu/g) and pork (POK4, 190 cfu/g) suggests fecal contamination, corroborating findings by Adesokan et al. (2014), who linked high coliform counts in poultry to poor slaughterhouse hygiene. The presence of *S. aureus* in multiple samples (Table 2) is consistent with Hassan et al. (2017), who identified *S. aureus* as a common contaminant in meat due to human handling, particularly in markets with inadequate sanitation.

However, the bacterial counts in this study are generally lower than those reported in some studies, such as Egbule et al. (2020), who found TVC up to 10^6 cfu/g in beef from open markets, possibly due to differences in sampling conditions or market hygiene practices. The variation in bacterial loads across markets (e.g., higher counts in World Bank Market for chicken) suggests localized differences in handling, storage, or environmental conditions, a phenomenon also observed by Okonko et al. (2010).

The morphological and biochemical characteristics of bacterial isolates from beef, chicken, and pork, confirming the presence of potentially pathogenic species including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, *Escherichia coli*, *Salmonella spp.*, and *Klebsiella spp* as revealed in figure 2 presents. Each was identified based on characteristic colony appearance and reactions to standard biochemical tests. while table 4.2b showed that different type of retailed meat was *staphylococcus aureus* was isolated only.

The identification of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella spp.*, and *Klebsiella spp.* in Figure 1 aligns with studies like that of Odumosu et al. (2016), who reported these pathogens in meat from Nigerian markets, attributing their presence to cross-contamination during processing or storage. *Pseudomonas aeruginosa* and *E. coli* are indicative of environmental and fecal contamination, respectively, while *Salmonella spp.* poses a significant public health risk, as noted by the World Health Organization (2015) in studies on foodborne pathogens. The consistent detection of *S. aureus* across all meat types (Figure 2) mirrors findings by Normanno et al. (2007), who reported its prevalence in meat due to its resilience in various environmental conditions.

These results indicate significant bacterial contamination in beef, chicken, and pork from the studied markets, with *S. aureus*, *E. coli*, *Salmonella spp.*, and other pathogens posing potential health risks. These findings emphasize the need for improved hygiene practices in meat handling and storage, consistent with recommendations from previous studies to mitigate foodborne illness risks in market settings.

The bacterial isolates analyzed in this study demonstrated a genetic similarity among them. Phylogenetic analysis, based on 16S rRNA gene sequencing, showed 100% coverage relatedness for *Staphylococcus aureus* OQ891488, CP113244 and PQ123258 sequence coverage, respectively, when compared to publicly available sequences on NCBI (Table 3). However, using 16srRNA

sequence in phylogenetic analysis lacks good resolution that helps to identify isolates on the basis of their strains level. The molecular diagnostic approach is mostly specific and sensitive; its technique provides rapid diagnostic results. Although it is expensive, it offers a comprehensive assessment related to the conventional method (Ujoh *et al.,* 2022).

All tested *S. aureus* isolates retained their resistance phenotypes over multiple subcultures in antibiotic-containing media. Growth was absent on antibiotic-free plates only in plasmid-cured control experiments (not shown), confirming that: The resistance genes were stably inherited, there was no spontaneous curing during routine lab culture and Plasmid-mediated resistance is likely persistent in the studied meat-derived isolates. The study confirms no plasmid curing occurred in antibiotic-resistant *Staphylococcus aureus* strains isolated from retailed meat in Owerri. This stability under selective pressure highlights the public health concern of persistent, transmissible resistance traits in foodborne pathogens.

The results showed high resistance rates, particularly to Ceftriaxone, Azithromycin, Cefuroxime, Amoxil, and Ciprofloxacin. These antibiotics are frequently used in clinical settings to treat infections caused by *S. aureus*, and their reduced efficacy in the meat isolates suggests the presence of multi-drug resistant (MDR) strains in the food chain. One notable finding was the complete resistance of *S. aureus* isolates to Ceftriaxone, a third-generation cephalosporin. Ceftriaxone is an important antibiotic used in the treatment of infections caused by *S. aureus*, but the resistance observed in this study suggests the possibility of extended-spectrum beta-lactamase (ESBL) production by the bacteria. ESBLs are enzymes that hydrolyze the beta-lactam ring, rendering these antibiotics ineffective. This finding is consistent with other studies, such as Olayinka *et al.* (2020), which reported high levels of resistance to Ceftriaxone in *S. aureus* isolates from retail meat in Lagos. However, this study contrasts with the findings of Adeyanju *et al.* (2020), who reported lower resistance levels to Ceftriaxone in their isolates from meat in southwestern Nigeria. These regional differences may be attributed to variations in local antibiotic use practices, differences in meat handling, and other environmental factors.

The study also found a high resistance rate of 86.7% to Azithromycin, Cefuroxime, and Amoxil, antibiotics commonly used to treat respiratory and soft tissue infections. Such resistance patterns are worrisome because these antibiotics are often prescribed for outpatient management of mild-to-moderate infections. The high levels of resistance to these drugs may be reflective of the overuse or misuse of antibiotics in both veterinary and human medicine. In line with this, a study by Owoseni *et al.* (2021) also reported significant resistance of *S. aureus* from retail meats in southwestern Nigeria to common antibiotics like Amoxil and Cefuroxime. Such findings highlight the broader issue of antibiotic resistance across different regions in Nigeria, suggesting that *S. aureus* is a major contributor to the growing global problem of antimicrobial resistance (AMR). Specialty

Additionally, moderate resistance rates were observed for antibiotics such as Rifampicin, Ciprofloxacin, Levofloxacin, Erythromycin, and Gentamycin. Resistance to fluoroquinolones like Ciprofloxacin is particularly troubling, as fluoroquinolones are often used as second-line agents in the treatment of severe infections caused by resistant organisms. A similar resistance to fluoroquinolones was reported by Akinmoladun *et al.* (2020), who found moderate levels of Ciprofloxacin resistance in *S. aureus* isolates from retail meat samples in Nigeria. This trend underscores the increasing prevalence of quinolone resistance, which has become a significant issue globally. Fluoroquinolones work by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes essential for bacterial replication. The resistance to these antibiotics may be due to mutations in the genes encoding these enzymes, reducing the drug's ability to bind and inhibit bacterial growth.

The presence of multi-drug resistance (MDR) strains of *S. aureus* in retailed meats is a particularly concerning finding, as it complicates treatment options and raises the risk of more severe infections in humans. In this study, MDR was defined as resistance to at least one antibiotic from three or more classes, and several of the isolates from retailed meats showed this pattern. The development of MDR in *S. aureus* is a direct result of the overuse and misuse of antibiotics in the food production industry. In Nigeria, the use of antibiotics in livestock production is largely unregulated, leading to the indiscriminate administration of these drugs to promote growth or prevent disease. Such practices provide a selective pressure that fosters the development of resistant strains.

The study also observed a variation in the prevalence of *S. aureus* among different types of meat. Beef samples showed the highest prevalence (41.7%), followed by chicken (33.3%) and pork (25%). This finding is consistent with other research that has shown that beef is more likely to be contaminated with *S. aureus* compared to other meats. For example, Akinmoladun *et al.* (2021) found that beef had the highest contamination rate of *S. aureus* compared to chicken and pork in their study of retail meat samples from Nigeria. This difference could be due to various factors, including differences in slaughter practices, meat handling, and storage conditions. Beef is often more prone to contamination during slaughter and handling due to the larger surface area and higher fat content, which can provide a more favorable environment for bacterial growth. Moreover, beef is typically stored and transported for longer periods before it reaches consumers, increasing the chances of bacterial proliferation.

The present study investigated the antibiotic resistance profile of *Staphylococcus aureus* isolates from retailed meat sold in Owerri, Nigeria. The high prevalence of *S. aureus* observed in this study raises significant public health concerns, particularly due to the associated multi-drug resistance patterns, which pose a threat to both food safety and treatment efficacy.

The prevalence rate of *S. aureus* was highest in beef (41.7%), followed by chicken (33.3%) and pork (25%). The Chi-Square test revealed statistically significant associations between meat type and bacterial prevalence for beef ($\chi^2 = 7.45$, $p = 0.024$) and chicken ($\chi^2 = 4.35$, $p = 0.038$), but not

pork (χ^2 = 2.10, p = 0.150) as shown in Table 4. These findings agree with previous reports by Momoh *et al.* (2020) and Okoli *et al.* (2021), who noted that beef, often exposed longer in open market settings, tends to harbor higher levels of microbial contamination. Conversely, pork was found to carry a lower burden of *S. aureus* in our study, possibly due to shorter market exposure or differences in post-slaughter handling and packaging.

The correlation analysis between meat type and resistance patterns revealed that beef had the strongest association with antibiotic resistance, with correlation coefficients ranging from ρ = 0.55 to 0.62 as revealed in Table 5. The highest correlation was with Ciprofloxacin (ρ = 0.62), a fluoroquinolone widely used both in human medicine and veterinary practice. Chicken and pork exhibited weaker correlations (ρ values from 0.40 to 0.50), indicating relatively less resistance associated with these meat types. These findings are comparable to the report by Sani *et al.* (2019), who observed higher rates of quinolone resistance in *S. aureus* isolated from cattle meat in northern Nigeria, likely due to heavy prophylactic antibiotic use in large-scale cattle farming.

The high MDR rates observed in the current study are consistent with global warnings about the spread of antimicrobial-resistant foodborne pathogens, particularly in low- and middle-income countries (WHO, 2021). The evidence suggests that antibiotic-resistant *S. aureus* strains from meat may act as reservoirs of resistance genes, which can be transmitted to humans via direct consumption or cross-contamination. Notably, the World Health Organization (2019) has identified *S. aureus*, especially methicillin-resistant strains (MRSA), as a high-priority pathogen for research and mitigation due to its clinical significance and limited treatment options.

In addition to public health concerns, the resistance profile observed characterized by reduced susceptibility to commonly used antibiotics such as Rifampicin, Azithromycin, Ciprofloxacin, and Cefuroxime raises treatment challenges. The presence of MDR strains in food means that even minor foodborne infections may become complicated, requiring higher-tier antibiotics or combination therapies that may not be readily available or affordable in resource-limited settings.

Given these findings, it is evident that *S. aureus* contamination in retailed meat in Owerri is not only prevalent but also associated with significant levels of resistance, particularly in beef. This reflects broader trends in Nigeria and across Sub-Saharan Africa, where the misuse of antibiotics in animal husbandry remains poorly regulated, and hygiene standards in meat processing and retail are often compromised (Nworie *et al.* 2020; Anigbogu *et al.* 2022).

In addition to the findings regarding antibiotic resistance, this study also contributes to the growing body of literature on the public health implications of antibiotic-resistant bacteria in the food chain. The consumption of contaminated meat, particularly when it is not cooked thoroughly, represents a potential pathway for the transmission of resistant *S. aureus* to humans. Studies such as that by Akinmoladun *et al.* (2020) emphasize the need for better meat handling and storage practices to reduce the risk of contamination. Furthermore, public health campaigns should be implemented to

educate consumers about the risks associated with consuming undercooked meat and the importance of practicing good hygiene when handling food. Additionally, there is a need for regulatory oversight to control the use of antibiotics in food animals and reduce the potential for the emergence and spread of resistant pathogens.

The overall findings from this study are alarming, given the high prevalence of resistance to commonly used antibiotics and the widespread occurrence of multi-drug resistance in *S. aureus* isolates from retailed meat in Owerri. These results highlight the urgent need for more stringent policies regulating the use of antibiotics in agriculture, better hygiene practices in the meat production industry, and public health interventions aimed at controlling the spread of antimicrobial resistance.

Conclusion

The findings of this study highlight the significant presence of *Staphylococcus aureus*, including multi-drug-resistant strains, in retailed meat sold in major markets across Owerri, Nigeria. Beef samples showed the highest contamination rate, followed by chicken and pork, with statistically significant associations between meat type and *S. aureus* prevalence. More importantly, a strong correlation was observed between individual antibiotic resistance—particularly to Rifampicin, Cefuroxime, and Azithromycin and the overall multi-drug resistance pattern.

These results reflect a growing public health threat, underscoring the role of retail meat as a potential reservoir and transmission route for antibiotic-resistant bacteria to humans. The widespread resistance to multiple classes of antibiotics suggests a possible overuse or misuse of these drugs in animal husbandry, compounded by poor hygiene practices during slaughtering, processing, and marketing.

The emergence and spread of multi-drug-resistant *S. aureus* from food sources reinforce the urgent need for stricter regulation of antibiotic use in livestock, improved hygiene standards in meat handling, and routine surveillance of antimicrobial resistance in the food supply chain. Without such interventions, the effectiveness of commonly used antibiotics in both human and veterinary medicine may continue to decline, with serious implications for disease management and food safety.

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