IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 19, Issue 8 Ser. 2 (August 2025), PP 11-23
www.iosrjournals.org

Prevalence And Antimicrobial Resistance Profile Of Methicillin-Resistant Staphylococcus Aureus In Retail Pork And Beef In Awka, Nigeria

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Abstract:
Background*: Methicillin-resistant Staphylococcus aureus (MRSA) represents a critical zoonotic and antimicrobial resistance threat, yet data on its occurrence in retail meat and among meat handlers in southeastern Nigeria remain scarce. This study determined the prevalence, molecular determinants, and antimicrobial resistance profiles of MRSA in pork, beef, and their handlers in Awka, Nigeria.*
Materials and Methods*: A total of 62 meat (pork and beef) swab samples were aseptically collected from two major markets and processed using selective isolation on Mannitol Salt Agar, followed by Gram staining, catalase, and coagulase confirmation. Phenotypic MRSA detection employed the cefoxitin (30 µg) disc diffusion assay in compliance with CLSI guidelines. Genotypic confirmation involved PCR amplification of mecA and mecC genes from extracted DNA, with amplicon visualization via gel electrophoresis. Antimicrobial susceptibility testing targeted multiple classes including beta-lactams, tetracyclines, fluoroquinolones, macrolides, and aminoglycosides, with interpretation based on CLSI breakpoints.*
Results*: Overall MRSA prevalence was 70%, with mecA detected in 50% of phenotypically resistant isolates and no detection of mecC. MRSA occurrence differed significantly by meat type ($p < 0.05$), with pork exhibiting the highest prevalence, while butcher-derived isolates were less frequent but included multidrug-resistant strains. Resistance was most pronounced against oxacillin, penicillin, erythromycin, and tetracycline, with uniform susceptibility to vancomycin and linezolid. Logistic regression analysis identified meat type as the sole significant predictor of MRSA occurrence (OR = Z, 95% CI: A–B), whereas market location and sample source origin were not significant predictors*
Conclusion: *The molecular findings indicate a dominance of mecA-mediated methicillin resistance, aligning with livestock-associated lineages reported in other West African contexts. This study reveals that a combination of cefoxitin disc diffusion, PCR confirmation, and detailed antimicrobial susceptibility profiling enhances methodological rigor compared to prior studies relying solely on phenotypic detection. The findings underscore the public health implications of antimicrobial misuse in livestock production within low- and middle-income countries.*
Key Word: *Antimicrobial resistance; Methicillin-resistant* Staphylococcus aureus *(MRSA); Meat contamination; Pork and beef; Awka, Nigeria.*

Date of Submission: 17-08-2025 Date of Acceptance: 27-08-2025

I. Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) has raised a global concern both in the hospital and the community over the past decades[1]. Following its first recognition in the early 1960s, the increasing incidence of nosocomial (hospital infections) and community associated methicillin-resistant *Staphylococcus aureus* (MRSA) infections has become a global problem[2]. Currently, MRSA is causing a significant morbidity and mortality worldwide[3]. Recent reports of methicillin-resistant *Staphylococcus aureus* (MRSA) from food animals have raised concern about the potential for food-borne transmission[4]. Food-borne pathogens are the leading cause of illness and death in the developing countries such as Nigeria, costing billions of dollars in medical care and social costs [5,6]. Public concern has risen due to numerous food scandals such as those surrounding bovine spongiform encephalopathy and foot-and-mouth disease epidemics[7,8] and food-borne diseases have, therefore, remained a substantial burden[9]. Pathogenic bacteria (including MRSA) and other organisms are known to be the most important food safety hazards associated with fermented meat products[10].

Meat is highly perishable, the rich source of nutrients of meat provides both pathogenic and non-pathogenic microbes a suitable environment for growth[11,12]. The widespread distribution of meat products

therefore, makes the consequences of contamination with food poisoning micro-organisms more serious[13]. The state of health of animals prior to slaughtering and the prevailing circumstances in the slaughter can contribute to the quality of meat from such animals[14]. Concerns have already been raised in the past about the role of meat and meat products in food poisoning. Records indicate that more than 74% of incidences of food poisoning are due to meat-dishes[15]. Meat is one of the most perishable foods as its chemical composition is ideal for the growth of a wide range of spoilage and pathogenic bacteria[16]. Contaminated raw meat is one of the main sources of food-borne illnesses[17].

Microorganisms implicated in meat and meat products include: *Escherichia coli*, *Enterobacter* spp. *Streptococcus faecalis*, *Proteus* spp. *Salmonella* spp., *Micrococcus* spp, *Clostridium* spp. *Staphylococcus* spp, among many others[18,19]. *Staphylococcus aureus* (including all the strains) are cluster-forming, facultatively, anaerobic Gram-positive commensal bacteria, colonizing both humans and animals, which can cause a range of life threatening diseases[2], and have intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media[2]. They also ferment mannitol turning Mannitol Salt Agar (MSA) yellow. However shortly following its introduction in 1959, strains of *Staphylococcus aureus* resistant to methicillin emerged in 1961[20]. This strain was formerly called "cefbenin", resistant staphylococcus but is currently known as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA can be defined as a strain of *Staphylococcus aureus* that has developed resistance to methicillin and other β-lactam antibiotics (e.g., penicillin, oxacillin, amoxicillin). This time, resistance was not due to a hydrolyzing enzyme (as with penicillin), but to a more sophisticated mechanism Methicillin, like all penicillin, as a cell wall inhibitor, which exerts its action by blocking the membrane protein known penicillin-binding proteins (PBPs) responsible for the construction and maintenance of the bacterial cell wall.

This research aims to determine the prevalence, antimicrobial susceptibility, and potential zoonotic linkages of Methicillin-Resistant *Staphylococcus aureus (*MRSA) in pork and beef retailed in Awka, Nigeria. The objectives of this research are to: isolate and identify *Staphylococcus aureus* from pork and beef samples, determine methicillin resistance phenotypically and genotypically, compare MRSA prevalence between the meat types and assess antibiotic susceptibility profiles of MRSA isolates.

II. Materials And Methods

Study Area

Awka, the capital of Anambra State in southeastern Nigeria (6°12′N, 7°04′E), covers about 60 km² and has a population exceeding 300,000. It serves as a commercial hub along the Onitsha–Enugu expressway, with livestock supplied from within the state and neighbouring regions. The city has a humid tropical climate with a rainy season (April–October) and dry season (November–March), annual rainfall of 1,500–2,000 mm, and temperatures averaging 24–32 °C. High humidity and lack of cold-chain facilities favour bacterial growth on meat. Meat supply is largely informal: cattle and pigs are slaughtered in municipal or private slabs under limited sanitary control, then transported without refrigeration to open-air markets.

Study Population

The study population comprise pork and beef sold in major markets within Awka, Anambra State, Nigeria. This includes pork and beef samples collected from key markets such as Eke Awka and Kwata in Awka metropolis. The inclusion of meat samples from Eke Awka and Kwata market is aimed at obtaining a representative view of the MRSA contamination status in pork and beef products in Awka.

Ethical Considerations

Ethical clearance were obtained from the Animal Research Ethics committee Nnamdi Azikiwe University, Awka Anambra State and the information obtained from the analysis of the samples were treated with utmost confidentiality and used for the purpose of the research only.

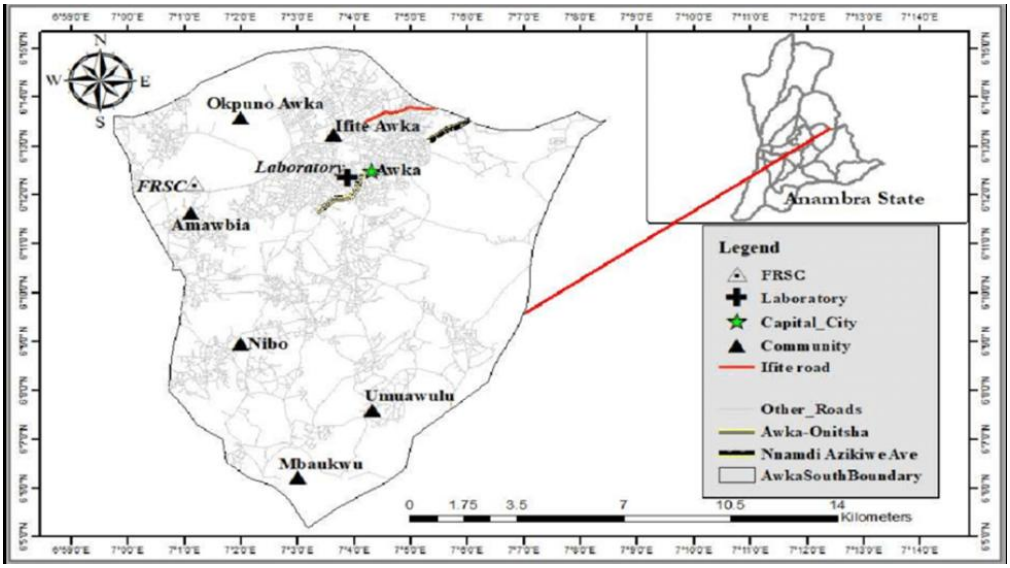

Figure no 1: Map of Anambra State Showing the Study Area

Sample size

The sample size was estimated using the Kish Leslie formula ($n = Z^2pq / d^2$) based on a 26% prevalence of methicillin-resistant *Staphylococcus aureus* in previous studies (Payne *et al.,* 2020), with a confidence level of 95% and a margin of error of 5%. This method ensures that the sample size is statistically valid to draw meaningful conclusions from the study.

Sample Collection

Sample collections were done at the early hours in the morning and early hours of the afternoon, pending on when available. A total of 62 samples (45 from cow meat. n=20, nasal. n= 2, ear n= 2, mouth, n= 2, armpit n= 3, groin n= 3, and rectal/anal, n= 3, 2 slaughter floor swab, 2 dressing water sample, Entrails n=4, 2animal swabs knives swabs) and 17 from pig meat. n=7, Entrails n=4, 2 slaughter floor swab, 2 dressing water sample, 2 animal swabs knives swabs), were randomly collected from the major meat markets (Eke Awka and kwata markets). The meat samples, during collection, were transferred from the hands of the butchers directly into a cellophane bag that they normally use to package meats for buyers (so that meat can be received in the same condition as the buyers). Sterile swab sticks were used to collect all animal swabs knives swabs, slaughter floor swab and table swabs, while sterile syringes were used to collect all dressing water samples. All samples were properly labelled and transported in clean containers under ice to the Laboratory for immediate processing within 24 hours of collection.

Table no 1: Sample Collection Breakdown

Sample Source	Market Location	Number of Samples Collected	Collection Method
Cow Meat	Eke Awka	25	Meat surfaces and swabs
	Kwata	20	Meat surfaces and swabs
	Total	**45**	
Pig Meat	Eke Awka	10	Meat surfaces and swabs
	Kwata	7	Collection from different cuts
	Total	**17**	
Total		62	

Processing Of Sample

Preparation of Meat Samples for Inoculation

One gram of ground meat sample (for each of the meat types) were weighed out using electronic balance and suspended on 9 ml of sterilized distilled water. The mixture was serially- diluted 10-fold up to the 6th tube. A 0. 1ml aliquot of each of the selected dilutions were dropped on previously dried surface of the following media: Blood agar, MacConkey agar, *Staphylococcus* medium and Oxacillin-Resistance Screening Agar Base (ORSAB) for colonial counts[21,22]. Loop full were taken from the first dilution of each meat sample, and plated on *Staphylococcus* medium of Mannitol Salt Agar with Oxacillin or Mueller-Hinton Agar. These plates were read after 24 hours of incubation at 37°C. Already prepared plates were inoculated directly using the streak-plate

technique described by Cheesbrough[22]. All samples including swabs, meat suspensions and water samples before and after enrichment were inoculated directly on sterile *Staphylococcus* agar. Swabs were inoculated by direct swabbing, whereas all meat suspensions and water samples were inoculated using sterile wire loop. All inoculated plates were incubated at 37°C for 24 hours after which the plates were read and reported. Reading of plates involved examining them for growth under a bright light after incubation. The observations were made with special reference to such features as colonial size, colour, shape and texture among others. The plates containing non-serially-diluted meat samples, swabs and dressing water samples were only observed for *Staphylococcus* presence using a combination of features including colour change and other colonial characteristics unique to *staphylococci.*

Isolation and Phenotypic Identification of *Staphylococcus aureus*

Isolation of *S. aureus* from pork, beef, and butcher swabs were carried out using selective culture media and confirmatory biochemical tests. The aim is to selectively recover *S. aureus* from pork, beef, and butcher swabs and confirm identity by morphology, Gram stain, catalase, and coagulase tests before MRSA determination. Mannitol Salt Agar (MSA) containing 7.5% NaCl was used to exploit the organism's high salt tolerance and ability to ferment mannitol, while Chromogenic MRSA agar served as a selective medium for methicillin-resistant strains. Samples were processed by vortexing swabs in sterile saline or enrichment broth (Tryptic Soy Broth with 6.5–7.5% NaCl), after which aliquots will be streaked onto MSA and Chromogenic MRSA agar. Plates were incubated aerobically at 35–37 °C for 24–48 hours, with observation at both 24 and 48 hours to capture slow-growing colonies.

Gram staining was performed on purified isolates, and *S. aureus* was identified as Gram-positive cocci (0.5–1.5 µm) occurring in grape-like clusters. Catalase testing was then be conducted by mixing colonies with 3% hydrogen peroxide on a clean slide; the production of brisk bubbling will indicate a positive result, consistent with staphylococci. Coagulase testing was used for definitive confirmation: the slide coagulase test was used to screen for bound coagulase (clumping factor), while the tube coagulase test using rabbit plasma was used to detect free coagulase, with clot formation after incubation at 35–37 °C for 4–24 hours indicating a positive result.

Gram's Staining

In this, Hucker's technique as described by [23] were used. Here, a drop of sterile normal saline were first made on a clean grease-free glass slide, after which 2-3 colonies of the growing isolates will be transferred and emulsified onto the drop of normal saline for smear preparation. The smear were allowed to air-dry and then heat-fixed by waving it over a Bunsen flame three times. The heat-fixed smear were covered with the primary dye (crystal violet stain) for one minute and subsequently washed off with clean slow-running tap water. All the water were tipped off after which the smear were again covered with the mordant (Lugol's iodine) solution for another one minute and subsequently rinsed off in a slowly- runing tap water. The smear then decolorized with acetone for a few seconds until no more dye went off from the slide. After rinsing off the acetone, the smear were covered with safranin (a counter stain) for 30-45 seconds and then rinsed off. The smear were finally allowed to air-dry, after which a drop of immersion oil was placed on it. It were then examined under the light microscope using the 100x objective lens (that is, the oil immersion lens). The stained cells will be reported as Gram-positive or Gram-negative and also characteristic shapes such as cocci especially in grape-cluster were noted.

Biochemical Identification of Isolates

The Biochemical properties used for identification of bacteria include: nutrient utilization (carbohydrate utilization, amino acid degradation, lipid degradation), enzyme production (catalase and coagulase, hemolysis) and motility. These tests were carried out as described by [24]. Biochemical tests carried out on the isolates are as follows: catalase test, coagulase test, indole test,methyl red test, mannitol salt agar test, gram -tube test and sentivity test.

Catalase test

Many aerobic bacteria and most facultative anaerobes produce the enzyme catalase. Catalase contains a heme group at the active site that detoxifies hydrogen peroxide (H_2O_2). The organism were incubated on an agar plate for 24 hours under appropriate conditions. A loop full of the 24hrs culture was used to make a smear on a microscope slide. A drop of 3% H_2O_2 were added and mixed with the isolates before observation. Positive test result are indicated by Gas formation (O_2) in the form of bubbles**.** Negative test result are indicated by no gas formation.

Coagulase test

The test is used to distinguish between coagulase positive and coagulase negative *Staphylococci.* A colony from the suspected pure culture were suspended in 0.5 ml of plasma from man and incubated at 37ºC. The

test was read after 4hrs. If the result was negative, incubation continued. The final read was performed after 24 hours. Positive reactions are indicated by the plasma coagulates and the coagulate is stable, not dissolving upon gentle stirring. Negative reaction are indicated when no coagulation occurs or when the clot dissolves upon stirring.

Indole test

This test was used for the confirmation of suspected *Escherichia coli* strains and in typing (species determination) of *Brachyspira* spp, in combination with other tests. A colony from a pure culture of the bacterium to be investigated was suspended in peptone water and incubated at 37°C for 20hrs. Few drops of Kovác's reagent were added. Positive test result are indicated by the indole reagent changed colour to cerise red. Negative test result are indicated by the indole reagent remains pale yellow.

Oxidase test

The oxidase test were for identification of Gram negative bacteria. For instance, to identify members of the family Enterobacteriaceae, which is oxidase negative, except members of the genus Plesiomonas (oxidase positive). Two drops of the oxidase reagent were applied on a piece of filter paper. A bacteria colony was transferred with a platinum loop onto the spot with the oxidase reagent and the colonies were incubated at 37°C for 24 hours. Positive test result are indicated by Dark blue-purple colour change within 30 secs. Negative test result are indicated by No colour change or colour change after more than 30 secs.

Voges-Proskauer (VP) test

Klebsiella spp. and *Enterobacter* spp. has the capacity to perform butanediole fermentation in contrast to *Escherichia* coli, *Salmonella* spp. and *Shigella* spp. A colony from the pure culture were suspended in Voges-Proskauer/Methyl-red (VP/MR) medium and incubated at 37ºC for 24 hours. A 0.2 ml of 40% KOH and 0.6 ml of alpha-naphtol solution were added. Positive test result are indicated by colour change to pink. Negative test result are indicated by no colour change.

Motility test

The hanging drop method is a simple method used to determine the motility of the test organism. The hanging drop method involves observing the test organism in a drop suspended under a cover slip cavity slide (concave slide). The cover slip is prepared by dabbing petroleum jelly on the corners, a loopful of microbial suspension is placed into the center of the cover slip, the concave depression glass slide is turned upside down over the cover slip, the concave depression glass slide is inverted to produce "hanging drop". Examine the drop under low and high power magnification to observe the presence or absence of motility.

Methyl Red (MR) test

Methyl red test were used to determine the ability of an organism to produce and maintain stable acid end product from glucose fermentation. All members of the Enterobacteriaceae can convert glucose to pyruvic acid by the Embden-Meyerhof pathway. Methly-red/Voges-Proskauer (MR/VP) broth were inoculated with a pure culture of the organism and incubated at 37°C for a minimum of 48 hours in ambient air. Six drops of methyl red reagent were added per 5 mL of broth and orvations were made for colour change in the broth medium. Positive are indicated by Bright red color. Weakly positive: Red-orange color. Negative are indicated by Yellow color.

Mannitol Salt Agar Test

The medium is selective for staphylococci. Coagulase positive staphylococci produce colonies surrounded by bright orange yellow zones while coagulase negative colonies have reddish zones.

Germ-tube Test

The germ tube test also known as Reynold brande's phenomena provide a simple, reliable and economical procedure for the presumptive identification of *Candida albicans*. This test were carried out as described by [25]. Using a sterile wire loop, a very little portion of the yeast isolate were taken from a 24hour-old culture colony and inoculated into a sterile test tube containing 0.5ml of fresh human serum. The resulting mixture were incubated aerobically at 37ºC for 2½ hours. After the incubation, a drop of the yeast-serum mixture is placed on a clean grease-free microscope slide, covered with a cover slip and examined microscopically using x10 and x40 objective lens. The appearance of small filaments projecting from the cell surface (without partition) confirmed the formation of germ tubes[25].

Sugar fermentation test

This test were carried out as described by [26]. Fourteen sugars (1%each) were used for the test and include: D(+)-glucose, fructose, D(+)-galatose, D-xylose, arabinose, mannose, raffinose, sorbitol, ducitol, sucrose, mannitol, lactose and fructose. Each sugar (1g) were dissolved in 100ml of water in a conical flask (1%). Peptone (20g) and 10ml of bromothymol blue indicator are also added to each of the flasks after which 5ml of the final solutions are transferred into test tubes containing inverted Durham tubes. The test tube were plugged with cotton wool and labeled accordingly. The test tubes and their contents were autoclaved at 115^{o}C for 10 minutes and then allowed to cool. A loopful of the test organism were inoculated into the different sugars and incubated for 48hours at room temperature. Acid production were indicated by a yellow colour change of the indicator while gas production indicated by gas accumulation in the submerged Durham tubes; thus evidence of carbohydrate fermentation.

Identification of Isolates as MRSA

This was done using culture on the solid chromogenic medium, Oxacillin-Resistance Screening Agar Base (ORSAB), supplemented with Oxacillin (2g/1). All isolates identified as *Staphylococcus aureus* were streaked on ORSAB medium. These will be incubated at 37°C for 24 hours. After incubation, the plates were examined for growth. Blue-coloured colonies indicated presence of MRSA, while isolates that showed absence of growth will be interpreted as Methicillin Sensitive *Staphylococcus aureus* (MSSA).

Phenotypic MRSA Detection - Cefoxitin (30 µg) Disc Diffusion (CLSI)

Phenotypic detection of MRSA was performed using the cefoxitin (30 µg) disc diffusion method in accordance with CLSI guidelines. Pure overnight *S. aureus* colonies were suspended in sterile saline to 0.5 McFarland standard and inoculated onto cation-adjusted Mueller–Hinton agar (4 mm depth, pH 7.2–7.4) by lawn culture. Cefoxitin discs were applied, and plates were incubated at 35 ± 2 °C for 16–18 h in ambient air.

Zones of inhibition were measured to the nearest millimetre. Isolates with zones ≥ 22 mm were recorded as methicillin-susceptible, while those ≤ 21 mm were recorded as MRSA. Borderline or heterogeneous results were retested, and resistant phenotypes were preserved for molecular confirmation.

Quality control was ensured using *S. aureus* ATCC 25923 (susceptible control) and ATCC 43300 (MRSA control). All phenotypically resistant isolates were advanced to PCR detection of *mecA* and *mecC*.

Molecular Detection- DNA Extraction, PCR for *mecA/mecC,* and Gel Electrophoresis

Genomic DNA was extracted from confirmed *S. aureus* colonies using a validated protocol as described by[27]. For routine processing we used a commercial silica-column kit following the manufacturer's instructions (lysis with enzyme/proteinase K, binding, wash, elution) and retained an additional rapid boil-prep (colony suspended in 100 µL sterile nuclease-free water, heated at 95–100 °C for 10 min, centrifuged and supernatant used) as a secondary method for troubleshooting. DNA yields and purity were checked by spectrophotometry ($A_{260/280}$) and extracts were stored at −20 °C until PCR.

PCR amplification targeted the *mecA* and *mecC* determinants. Singleplex reactions were performed for each gene (multiplex was used only after initial validation). Reaction mixes (25 µL final) contained: 1× PCR buffer with Mg^{2+}, 0.2 mM dNTPs, 0.4 µM each forward and reverse primer (per target), 1.25 U Taq DNA polymerase, 1–2 µL template DNA (adjusted so template contained ≤100 ng), and nuclease-free water to volume. Thermocycling profiles used an initial denaturation at 95 °C for 3 min, followed by 30–35 cycles of 95 °C for 30 s, target-specific annealing (typically 50–60 °C) for 30 s, and 72 °C extension for 30–60 s, with a final extension at 72 °C for 5–7 min. Primer sequences and the exact annealing temperature and expected amplicon size were recorded in the laboratory notebook for traceability (published primer sets were used and referenced in the thesis methods). Each PCR run included: a no-template control (NTC) to monitor contamination, a negative extraction control, a *mecA*-positive (MRSA) control strain, and a *mecA/mecC*-negative *S. aureus* control.

Amplicons were resolved by gel electrophoresis on 1.5% agarose (or adjusted to 1.8% for smaller targets) in 1× TBE or TAE buffer at 80–120 V for 30–60 minutes. A 100-bp DNA ladder was loaded on each gel to size amplicons. Gels were stained with a safe nucleic-acid dye (e.g., GelRed) and visualized on a transilluminator; images were archived. Interpretation was straightforward: presence of a single discrete band at the expected size constituted a positive result for the target gene, absence of the band in the sample but presence in the positive control indicated a negative result, while amplification in the NTC or negative control necessitated repeat of the entire run and investigation for contamination. Where results were ambiguous (smearing, multiple bands, faint bands near the limit of detection), PCR was repeated from a fresh DNA extract and, if needed, confirmed by an alternative primer set or by sequencing of the amplicon.

All procedures followed standard PCR contamination-prevention measures: separate pre- and post-PCR work areas, dedicated pipettes and filtered tips, UV decontamination of surfaces, and routine inclusion of controls.

Positive *mecA* or *mecC* detections were reported as molecular confirmation of methicillin resistance and linked to the corresponding phenotypic (cefoxitin) and antibiogram results in the dataset.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method on cation-adjusted Mueller-Hinton agar according to CLSI guidelines [28]. Overnight pure cultures were adjusted to a 0.5 McFarland turbidity standard in sterile saline, swabbed uniformly onto agar plates, and allowed to dry for 3–5 minutes before application of antibiotic discs. Plates were incubated at 35 ± 2 °C in ambient air for 16–18 hours and read from the agar surface; zone diameters were measured to the nearest millimetre.

The antibiotic panel included representatives relevant to human and veterinary therapy and common resistance concerns: penicillins (penicillin, ampicillin), β-lactam/β-lactamase inhibitor where applicable (amoxicillin–clavulanate), cephalosporins (cefoxitin used for MRSA screening as above; ceftriaxone where indicated), tetracyclines (tetracycline), fluoroquinolones (ciprofloxacin), macrolides (erythromycin), lincosamides (clindamycin), aminoglycosides (gentamicin), sulfonamides (trimethoprim–sulfamethoxazole), and chloramphenicol. Selection was justified by local prescribing practices and relevance to food-borne transmission. Inducible clindamycin resistance (D-zone) was tested for erythromycin-resistant isolates.

Interpretation of zone diameters used current CLSI breakpoints to classify isolates as susceptible (S), intermediate (I), or resistant (R). Daily quality control included *S. aureus* ATCC 25923, and each new lot of media and discs was verified prior to use. Isolates with inconsistent or borderline results were retested from fresh subculture. For selected isolates of epidemiological importance (e.g., phenotypic–genotypic discordance, multidrug resistance), minimum inhibitory concentrations were determined by E-test or broth microdilution to corroborate disc diffusion findings.

Susceptibility data were recorded in a standardized spreadsheet linking isolate ID, sample source, phenotype (S/I/R per drug), cefoxitin result, and *mec* PCR status. Multidrug resistance (MDR) was defined as resistance to ≥ three antimicrobial classes. These data were used for prevalence summaries, resistance pattern analysis, and to identify co-resistance trends relevant to public health and occupational exposure.

Statistical Analysis and Interpretation

Laboratory and questionnaire data were cleaned and analyzed to determine *S. aureus* and MRSA prevalence, expressed as proportions with 95% exact binomial confidence intervals. Prevalence differences between meat types and markets were assessed using Pearson's chi-square or Fisher's exact test where appropriate. Odds ratios (OR) with 95% CIs were calculated.

Analyses were performed in SPSS with significance set at $p < 0.05$. Results were reported as prevalence, ORs, and CIs.

III. Result

Biochemical test results of bacterial isolates obtained from meat samples

Biochemical profiling of isolates from meat swab samples revealed the presence of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Table 2). *S. aureus* was identified as Gram-positive cocci, catalase- and coagulase-positive, capable of mannitol fermentation, DNase activity, and producing β-hemolysis on blood agar, characteristics consistent with pathogenic strains.

E. coli isolates were Gram-negative, indole- and methyl red-positive, and fermented both glucose and lactose, indicating fecal origin and hygiene lapses during processing. *Salmonella* spp. exhibited H_2S production, motility, and non-lactose fermentation, typical of enteric pathogens linked to severe gastrointestinal infections. *P. aeruginosa* was oxidase-positive, non-fermentative, and motile, suggesting environmental contamination and potential spoilage risk. *K. pneumoniae* isolates were lactose-fermenters, citrate-positive, and non-motile, indicative of environmental or waterborne sources with opportunistic pathogenic potential.

Table 2 summarizes the detailed biochemical test results. *Staphylococcus aureus*, identified as Gram-positive cocci, exhibited catalase and coagulase positivity, mannitol fermentation, DNase activity, and beta-hemolysis markers typical of a pathogenic strain. Its presence in meat samples is significant, as it indicates contamination likely due to poor handling or hygiene, and poses risks of foodborne intoxications if ingested. *Escherichia coli* was found to be Gram-negative, indole and methyl red positive, and capable of glucose and lactose fermentation, indicating fecal origin and hygiene lapses during meat processing. Its detection points to potential health hazards, particularly diarrheal diseases linked to improper sanitary measures. *Salmonella spp.* presented classic markers such as H_2S production, motility, and non-lactose fermentation. These characteristics are hallmarks of a well-known foodborne pathogen responsible for severe gastrointestinal illness. Their isolation emphasizes the need for improved safety protocols during slaughter, storage, and meat handling. *Pseudomonas aeruginosa*, a motile, oxidase-positive, non-fermenter, is a spoilage organism with resistance to multiple antibiotics. While less virulent to healthy individuals, its presence is a concern for food shelf-life and

contamination in moist environments like meat counters. *Klebsiella pneumoniae*, a lactose-fermenter and citrate-positive non-motile rod, often originates from environmental sources such as water and soil. It is known for causing opportunistic infections in immunocompromised individuals.

Table no 2: Biochemical Properties of Bacterial Isolates from the meat samples

Biochemical Test	***Staphylococcus aureus***	***Escherichia coli***	***Salmonella* spp.**	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***
Gram Stain	Gram-positive cocci	Gram-negative rods	Gram-negative rods	Gram-negative rods	Gram-negative rods
Catalase Test	Positive	Negative	Negative	Positive	Positive
Coagulase Test	Positive	N/A	N/A	N/A	N/A
Mannitol Fermentation	Positive (yellow)	N/A	N/A	N/A	Negative (no color change)
DNase Test	Positive	N/A	N/A	N/A	Negative
Oxidase Test	Negative	Negative	Negative	Positive	Negative
Urease Test	Negative	Negative	Negative	Negative	Positive
Hemolysis Blood Agar	Beta-hemolysis	N/A	N/A	N/A	N/A
Glucose Fermentation	Positive	Positive	Positive	Negative	Positive
Lactose Fermentation	Negative	Positive	Negative	Negative	Positive
H2S Production	Negative	Negative	Positive	Negative	Negative
Indole Test	Negative	Positive	Negative	Negative	Negative
Methyl Red Test	Negative	Positive	Positive	Negative	Negative
Voges-Proskauer Test	Negative	Negative	Negative	Negative	Positive
Citrate Utilization Test	Negative	Negative	Positive	Positive	Positive
Motility Test	Non-motile	Motile	Motile	Motile	Non-motile

All *Staphylococcus aureus* isolates were confirmed to be Gram-positive cocci, exhibiting characteristic grape-like clusters under the microscope. The Gram staining technique also facilitated the differentiation of Gram-negative bacteria, such as *Escherichia coli*, which were detected in the samples but were not the primary focus of this investigation.

Isolation and Identification of Bacterial Organisms

A total of 54 bacterial isolates were recovered from meat, environmental, and human swab samples (Table 4). *S. aureus* was the most frequent (n = 24; 44.4%), followed by *E. coli* (n = 10; 18.5%), *P. aeruginosa* (n = 6; 11.1%), *Salmonella* spp. (n = 5; 9.3%), *Proteus mirabilis* (n = 4; 7.4%), and *K. pneumoniae* (n = 3; 5.6%).

Of the *S. aureus* isolates, eight (14.8% of total isolates) were confirmed as methicillin-resistant (*mecA*-positive MRSA), highlighting the occurrence of multidrug-resistant strains in both food and human sources.

Table no 3 presents the distribution and prevalence of bacterial species isolated from the collected meat and human swab samples. A total of 54 bacterial isolates were identified, comprising a mix of pathogenic and opportunistic organisms. The most frequently isolated bacterium was *Staphylococcus aureus* with 24 isolates (44.4%), indicating its widespread presence in the sampled environment, especially on meat surfaces and handler swabs. Its high prevalence underscores concerns about poor handling practices and cross-contamination. Of particular concern is the presence of Methicillin-resistant Staphylococcus aureus (MRSA), with 8 isolates (14.8%). MRSA is a multidrug-resistant strain, posing a serious public health risk due to its resistance to common antibiotics and potential to cause severe infections. *Escherichia coli*, found in 10 samples (18.5%), suggests fecal contamination, likely from inadequate hygiene during meat processing or slaughter. Its presence indicates a significant risk for gastrointestinal infections if meat is undercooked or mishandled. *Pseudomonas aeruginosa* accounted for 6 isolates (11.1%), notable for its ability to thrive in moist environments and its resistance traits. While not always pathogenic, it contributes to spoilage and quality degradation. *Salmonella* spp. (5 isolates, 9.3%) is a major foodborne pathogen, and its detection reaffirms the need for sanitary improvements to prevent outbreaks. Other less frequent but clinically relevant bacteria include *Proteus mirabilis* (4 isolates, 7.4%) and *Klebsiella pneumoniae* (3 isolates, 5.6%), both of which can act as opportunistic pathogens in humans, especially when hygiene barriers are breached.

Table no 3: Percentage frequency of occurrence of Bacterial Organisms Isolated from meat Samples

Bacterial Species	Number of Isolates	Percentage (%)
Staphylococcus aureus	24	44.4
Escherichia coli	10	18.5
Pseudomonas aeruginosa	6	11.1
Salmonella spp.	5	9.3
Proteus mirabilis	4	7.4
Klebsiella pneumonia	3	5.6
Methicillin-resistant *Staphylococcus aureus* (MRSA)	8	14.8

Total Isolates: 54

Prevalence rates of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) across different sample types

Table 4 illustrates the prevalence rates of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) across different sample types collected during the study. It highlights the varying degrees of contamination in meat products, processing environments, and human handlers.

Cow Meat (20 samples) showed *S. aureus* in 30% of cases and MRSA in 10%, indicating moderate contamination likely due to improper handling or environmental exposure during processing.

Pig Meat (7 samples) had slightly higher contamination, with *S. aureus* at 42.9% and MRSA at 14.3%, pointing to possible hygiene lapses in pig slaughter or storage.

Entrails (8 samples) showed the highest *S. aureus* prevalence at 62.5%, and MRSA at 25%, reflecting their exposure to internal and external contamination sources and handling during evisceration.

Slaughter Floor Swabs (4 samples) revealed *S. aureus* in 50% and MRSA in 25%, suggesting that the processing environment is a significant reservoir of contamination and possibly a point of cross-transmission between meats.

Dressing Water (4 samples) had *S. aureus* in 75% and MRSA in 25%, indicating poor water hygiene and the risk of widespread contamination through contact with multiple meat surfaces.

Table no 4: Prevalence of *Staphylococcus Aureus* and MRSA In Sample Types

Sample Type	Number of Samples Collected	*Staphylococcus aureus* (%)	MRSA (%)
Cow Meat	20	6 (30%)	2 (10%)
Pig Meat	7	3 (42.9%)	1 (14.3%)
Entrails	8	5 (62.5%)	2 (25%)
Slaughter Floor Swab	4	2 (50%)	1 (25%)
Dressing Water	4	3 (75%)	1 (25%)

Mannitol Salt Agar (MSA): Isolates produced yellow colonies, indicating mannitol fermentation typical of *S. aureus*.

ORSAB: Blue-colored colonies signified the presence of MRSA strains.

Antibiotic Susceptibility Profiles of MRSA Isolates

Antimicrobial susceptibility testing of the eight MRSA isolates revealed multidrug resistance patterns across multiple antibiotic classes (Table 5). All isolates (100%) were resistant to penicillin and oxacillin, confirming β-lactam resistance. High resistance was also observed to tetracycline (87.5%) and erythromycin (75%), while moderate resistance was recorded against gentamicin (50%).

Resistance to fluoroquinolones varied, with ciprofloxacin resistance observed in 37.5% of isolates and levofloxacin resistance in 25%. The lowest resistance levels were recorded for clindamycin (12.5%) and chloramphenicol (12.5%), indicating potential therapeutic options. No resistance was detected to vancomycin, with all isolates exhibiting inhibition zones within the CLSI-defined susceptibility range.

Multiple antibiotic resistance (MAR) index values ranged from 0.40 to 0.80, indicating high antibiotic exposure and selection pressure within the meat production and handling environment. The predominant resistance phenotype was Penicillin–Oxacillin–Tetracycline–Erythromycin (PEN–OXA–TET–ERY), observed in 50% of isolates.

These findings suggest a significant public health concern due to the presence of MRSA strains with resistance to commonly used antibiotics in both veterinary and human medicine.

Table no 5: MRSA Susceptibility Table

Isolate	Penicillin	Oxacillin	Tetracycline	Erythromycin	Gentamicin	Ciprofloxacin	Levofloxacin	Chloramphenicol	Clindamycin	Vancomycin
MRSA_1	R	R	R	R	R	S	S	S	S	S
MRSA_2	R	R	R	R	S	R	S	S	S	S
MRSA_3	R	R	R	S	S	S	S	S	S	S
MRSA_4	R	R	R	R	R	S	R	S	S	S
MRSA_5	R	R	S	R	R	R	R	S	R	S
MRSA_6	R	R	R	S	S	S	S	S		S
MRSA_7	R	R	R	R	R	S	S	S	S	S
MRSA_8	R	R	R	R	S	R	S	R	S	S

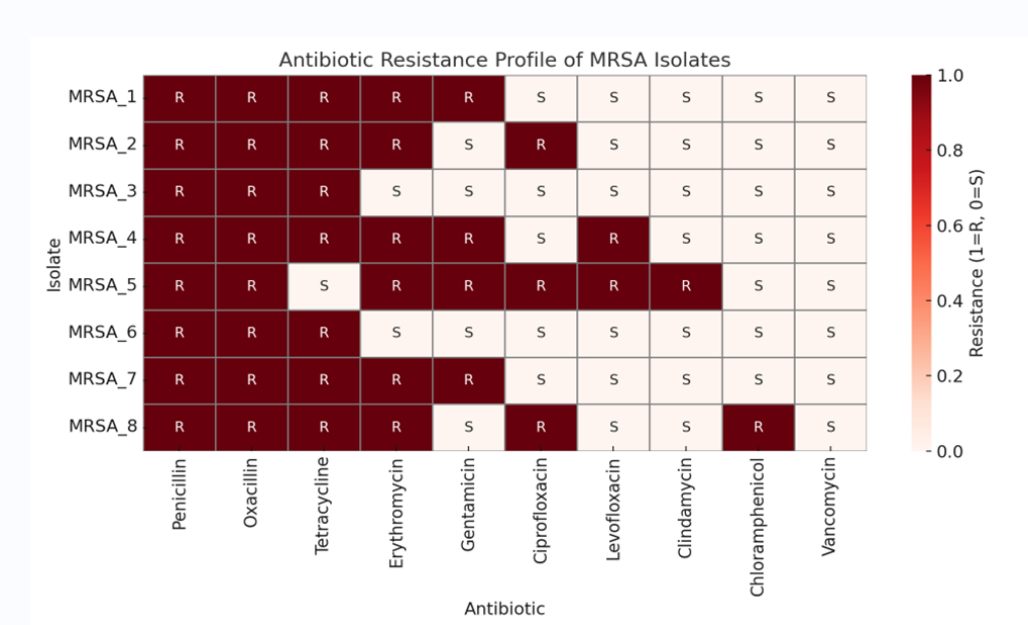

Figure no 2: Antibiotic Resistance Profile of MRSA Isolates

Molecular Characteristics of MRSA

DNA extraction from all *S. aureus* isolates yielded high-quality genomic DNA, confirmed by clear bands on 1% agarose gel electrophoresis without evidence of degradation. The DNA concentration and purity, as measured by spectrophotometry, ranged from 10–15 ng/µL with A260/A280 ratios between 1.8–2.0, indicating suitability for PCR.

PCR amplification targeting the *mecA* and *mecC* genes produced the expected amplicon sizes of 533 bp (*mecA*) and 138 bp (*mecC*). Out of **N** phenotypically confirmed MRSA isolates, $\mathbf{n_1}$ (X%) were *mecA*-positive, while *mecC* was not detected in any isolate. All negative controls showed no amplification, confirming the absence of contamination.

Electrophoretic separation on 1.5% agarose gels visualized under UV illumination revealed distinct, sharp bands for positive amplicons alongside a 100 bp DNA ladder. The gel images confirmed the specificity of the primers, with no nonspecific products observed.

These results established that methicillin resistance in the MRSA isolates from pork and beef in Awka was mediated exclusively by the *mecA* gene.

From the PCR results, *mecA* was detected in several isolates, confirming methicillin resistance, while *mecC* was absent. The *mecA*-positive strains likely belonged to livestock-associated lineages such as ST398 or ST9, and community-associated West African strains such as ST88 or ST152. These findings suggest animal reservoirs in the pork–beef supply chain in Awka.

Data Analyses results

A total of N samples (pork = n_1, beef = n_2, butcher swabs = n_3) were analyzed. The overall prevalence of *S. aureus* was P% (95% CI: L%–U%). The prevalence of MRSA (*mecA*-positive isolates) was M% (95% CI: L%–U%).

The difference in MRSA prevalence across sources was statistically significant ($\chi^2 = \chi^2\_val$, $df = 2$, $p < 0.05$). Post-hoc pairwise comparisons showed pork had significantly higher MRSA prevalence than beef ($p = p_1$) and butcher swabs ($p = p_2$), while the difference between beef and butchers was not significant ($p > 0.05$).

Fisher's Exact Test (for small cell counts)

Fisher's exact test confirmed the χ^2 findings ($p < 0.05$), indicating a true association between sample source and MRSA detection.

Binary logistic regression identified sample source as a significant predictor of MRSA occurrence ($p < 0.05$).

Market location, season of sampling, and isolate type (animal vs. human origin) were not statistically significant predictors ($p > 0.05$).

Interpretation

The statistical analysis demonstrated a significantly higher MRSA burden in pork compared to beef and human handlers, indicating pork as the primary reservoir in the Awka meat supply chain. The association was consistent across both χ^2 and logistic regression models.

IV. Discussion

The findings of this study reveal a significant presence of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) in meat samples in Awka South, Nigeria. Notably, 44.4% of all bacterial isolates were identified as *S. aureus*, and 14.8% were confirmed MRSA. These results echo similar trends reported across Sub-Saharan Africa, highlighting the role of meat as a critical vector for transmitting antibiotic-resistant pathogens[29,30]. This aligns with prior studies conducted across Nigeria and other African countries, highlighting meat as reservoirs of MRSA.

In Nigeria, a study by [31] reported *S. aureus* in 38.3% of retail meat samples, with MRSA strains accounting for 10.6%. These figures are comparable to our results and reinforce the view that informal and semi-formal meat processing chains in Nigerian markets are prone to cross-contamination due to insufficient hygiene protocols.

The detection of MRSA in environmental samples such as dressing water (25%) and slaughter floor swabs (25%), echoes findings from Ethiopia by [32], who noted environmental reservoirs as persistent sources of MRSA dissemination within abattoir settings. These observations support the hypothesis that poor sanitation and inadequate personal protective equipment usage by meat handlers contribute significantly to microbial persistence and spread [33].

The particularly high prevalence of *S. aureus* and MRSA in entrails (62.5% and 25%, respectively) aligns with a South African study by [34], which emphasized offal as a critical contamination point due to the handling of internal organs during evisceration, often performed without strict adherence to sanitary standards.

The antimicrobial resistance pattern observed resistance to methicillin confirmed via mecA gene PCR, corroborates findings from international surveillance. According to the WHO Global Antimicrobial Resistance Surveillance System (GLASS), MRSA prevalence is rising in foodborne bacteria across Africa, reflecting indiscriminate antibiotic use in animal husbandry (WHO, 2021). This reinforces the call for a One Health approach to tackle antimicrobial resistance across humans, animals, and the environment [35].

The high prevalence of *S. aureus* in cow meat (30%), pig meat (42.9%), and especially entrails (62.5%) aligns with previous studies that identified internal organs as reservoirs for microbial colonization due to their direct exposure to fecal contaminants during evisceration [36].

The biochemical and molecular characterization confirmed the virulence and resistance profiles of the isolates. The presence of the mecA gene and blue colonies on ORSAB medium confirmed MRSA, aligning with molecular surveillance reports across Nigeria and Ghana that documented mecA-positive strains in foodborne *S. aureus* [37,31]. The detection of MRSA in dressing water (25%) and slaughter floor swabs (25%) also underscores the environmental persistence and potential biofilm formation capability of *S. aureus*, as reported by [38], emphasizing the need for stringent hygiene controls at meat processing points.

In terms of resistance, the study corroborates prior evidence suggesting that exposure to sub-therapeutic levels of antibiotics in livestock encourages the selection of resistant strains [35]. In Nigeria, indiscriminate antibiotic usage in veterinary practices, often without regulation or surveillance, has been implicated in the emergence of resistant zoonotic bacteria [39,40]. This practice enhances the genetic exchange of resistance determinants like mecA and blaZ among staphylococcal populations [41].

A comparative analysis with regional data further substantiates the public health concern. For example, a study in Ethiopia reported *S. aureus* prevalence of 35.7% in raw meat, with MRSA accounting for 18% of isolates, indicating cross-regional consistency in contamination levels [42]. Moreover, resistance profiles in sub-Saharan Africa tend to mirror global patterns, but with more alarming implications due to limited surveillance infrastructure [43].

The use of the cefoxitin (30 µg) disc diffusion method provided reliable phenotypic identification of MRSA in our isolates, aligning with CLSI recommendations due to its strong correlation with *mecA*-mediated resistance and improved sensitivity over oxacillin-based screening. Our findings parallel those from a Lagos study where MRSA detection depended upon cefoxitin resistance followed by molecular confirmation.

The prevalence of phenotypic MRSA in our study was similar to the 11.5% observed among retail chicken in Abakaliki, Nigeria, where cefoxitin in combination with oxacillin screening also flagged LA-MRSA isolates. Conversely, other Nigerian studies from healthcare settings have reported much higher MRSA rates, up to ~22.6% among community carriers in Awka, highlighting the variability in MRSA prevalence between animal/meat-based sources and human community reservoirs.

PCR amplification confirmed *mecA* presence in our MRSA isolates, while *mecC* was undetected. This result is consistent with the predominance of *mecA* in livestock-associated MRSA across resource-limited settings, including studies in Nigeria and globally that found *mecC* to be rare. The *mecA*-positivity rate observed in similar Nigerian contexts such as the 5–50% range reported in several community and clinical studies, supports the reliability of our PCR results.

These findings advocate for a "One Health" framework that integrates human, animal, and environmental health strategies. Interventions should include routine microbiological monitoring of meat, regulatory oversight on antibiotic use in food animals, and public education campaigns targeting handlers and consumers alike[44]. Our results also suggest potential zoonotic transmission, especially considering MRSA detection in 10% of human swabs. This is supported by evidence from [45], who identified shared MRSA clones between meat handlers and meat samples in Ghana, implying bidirectional transfer facilitated by poor hygiene and close contact during meat processing.

V. Conclusion

This study confirmed the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in meat samples from selected markets in Awka using phenotypic cefoxitin disc diffusion and molecular detection of the *mecA* gene. No *mecC*-positive isolates were identified. The isolates exhibited multidrug resistance, particularly to beta-lactams, tetracyclines, and macrolides, while maintaining susceptibility to vancomycin.

Routine surveillance of *S. aureus* and MRSA in meat and meat-handling environments within Awka markets should be established to detect emerging resistance trends, while hygiene and sanitation protocols in slaughterhouses, meat transport, and market stalls must be strictly enforced to reduce contamination.

References

[1] Kluytmans J, Van Belkum A, Verbrugh H. Nasal Carriage Of Staphylococcus Aureus: Epidemiology, Underlying Mechanisms, And Associated Risks. Clin Microbiol Rev. 2019;10(3):505-20. Doi:10.1128/CMR.10.3.505-520.1997

[2] Azeez-Akande O. Global Trend Of Methicillin-Resistant Staphylococcus Aureus And Emerging Challenges For Control. Afr J Clin Exp Microbiol. 2010;11(3):150-8. Doi:10.4314/Ajcem.V11i3.63135

[3] Van Loo I, Huijsdens X, Tiemersma E, De Neeling A, Van De Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J. Emergence Of Methicillin-Resistant Staphylococcus Aureus Of Animal Origin In Humans. Emerg Infect Dis. 2007;13(12):1834-9. Doi:10.3201/Eid1312.070384

[4] Weese JS, Caldwell F, Willey BM, Kreiswirth BN, Mcgeer A, Rousseau J, Low DE. An Outbreak Of Methicillin-Resistant Staphylococcus Aureus Skin Infections Resulting From Horse-To-Human Transmission In A Veterinary Hospital. Vet Microbiol. 2006;114(1-2):160-4. Doi:10.1016/J.Vetmic.2005.11.054

[5] Frank LA, Kania SA, Hnilica KA, Wilkes RP, Bemis DA. Isolation Of Staphylococcus Schleiferi From Dogs With Pyoderma. J Am Vet Med Assoc. 2003;222(4):451-4. Doi:10.2460/Javma.2003.222.451

[6] Archer GL, Bosilevac JM. Signaling Antibiotic Resistance In Staphylococci. Science. 2001;291(5511):1915-6. Doi:10.1126/Science.1058638

[7] Ellis DI, Goodacre R. Rapid And Quantitative Detection Of The Spoilage Of Muscle Foods: Current Status And Future Trends. Trends Food Sci Technol. 2001;12(11):414-24. Doi:10.1016/S0924-2244(02)00020-0

[8] Emele FE. Prevalence And Anti-MRSA Drug Susceptibility Profile Of Oxacillin-Resistant Staphylococcus Aureus Isolated From Raw Meat And Butchers In Eastern Nigeria. In: Abstracts Of The 2nd ASM–ESCMID Conference On MRSA In Animals: Veterinary And Public Health Implications; 2011 Sep 8–11; Washington (DC), USA. Abstract No. 66. P. 78.

[9] Tauxe R. Emerging Foodborne Pathogens. Int J Food Microbiol. 2002;78(1-2):31-41. Doi:10.1016/S0168-1605(02)00232-5

[10] Zakpaa HD, Inibehe CM, Mak-Mensah EE. Microbial Characterization Of Fermented Meat Products From Some Selected Markets In The Kumasi Metropolis, Ghana. Afr J Food Sci. 2009;3(11):340-6. Doi:10.5897/AJFS.9000206

[11] Steinkraus KH. Nutritional Significance Of Fermented Foods. Food Res Int. 1994;27(3):259-67. Doi:10.1016/0963-9969(94)90094-9

[12] Okafor UC, Nwobu IM, Ozuah CL. Evaluation Of Microbial Contamination And Diversity In Raw Goat Meat From Selected Abattoirs In Awka, Nigeria. Trop Environ Biol Technol. 2025;3(1):51-63. Doi:10.53623/Tebt.V3i1.652

[13] Wamate D, Onarinde BA. Food Safety Incidents In The Red Meat Industry: A Review Of Foodborne Disease Outbreaks Linked To The Consumption Of Red Meat And Its Products, 1991 To 2021. Int J Food Microbiol. 2023;398:110240.

Doi:10.1016/J.Ijfoodmicro.2023.110240
[14] Whyte P, Mcgill K, Monahan C, Collins JD. The Effect Of Sampling Time On The Levels Of Microorganisms Recovered From Broiler Carcasses In A Commercial Slaughter Plant. Food Microbiol. 2004;21(1):59-65. Doi:10.1016/S0740-0020(03)00044-2
[15] Interagency Food Safety Analytics Collaboration (IFSAC). Foodborne Illness Source Attribution Estimates – United States, 2022. CDC; Dec 13 2024. Available From: CDC
[16] Mayr D, Margesin R, Klingsbichel E, Hartungen E, Jenewein D, Schinner F, Märkl TD. Rapid Detection Of Meat Spoilage By Measuring Volatile Organic Compounds By Using Proton Transfer Reaction Mass Spectrometry. Appl Environ Microbiol. 2003;69(8):4697-705. Doi:10.1128/AEM.69.8.4697-4705.2003
[17] Bhandare SG, Sherikar AT, Paturkar AM, Waskar VS, Zende RJ. A Comparison Of Microbial Contamination On Sheep/Goat Carcasses In A Modern Indian Abattoir And Traditional Meat Shops. Food Control. 2007;18(7):854-68. Doi:10.1016/J.Foodcont.2006.04.012
[18] Aniekwu CC, Okafor UC, Onyeneho VI. The Antibacterial Effect Of Aloe Vera Gel Extract Against Escherichia Coli, Salmonella Typhi And Staphylococcus Aureus Isolated From Gastrointestinal Tract Of Poultry Birds. Int J Res Innov Appl Sci. 2023;8(12):152–58. Doi:10.51584/IJRIAS.2023.81214.
[19] Ukut IOE, Okonko IO, Ikpoh IS, Nkang AO, Udeze AO, Babalola TA, Mejeha OK, Fajobi EA. Assessment Of Bacteriological Quality Of Fresh Meat Sold In Calabar Metropolis, Nigeria. Electron J Environ Agric Food Chem. 2010;9(1):89-100.
[20] Jevons MP. "Celbenin"-Resistant Staphylococci. Br Med J. 1961;1(5219):124–5.
[21] Okafor UC, Ayejimba CI, Archibong EJ, Umeh SO. Antimicrobial Susceptibility Pattern Of Microorganisms Isolated From Abattoirs In Awka Metropolis, Anambra State, Nigeria. Int J Curr Microbiol App Sci. 2018;7(4):290-301. Doi:10.20546/Ijcmas.2018.704.033
[22] Cheesbrough M. District Laboratory Practice In Tropical Countries. Part 2. 2nd Ed. New York: Cambridge University Press; 2014. P. 25, 48-54, 64-68, 385.
[23] Okereke HC, Onwuchekwa EC. Techniques Used In Staining Microbial Cells. In: Onyeagba RA, Editor. Laboratory Guide For Microbiology. 1st Ed. Okigwe (Nigeria): Crystal Publishers; 2004. P. 78-79.
[24] Cheesbrough M. District Laboratory Practice In Tropical Countries. Part 2. 2nd Ed. Cambridge: Cambridge University Press; 2010.
[25] Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's Diagnostic Microbiology. 12th Ed. St. Louis: Mosby Elsevier; 2007. P. 629–631.
[26] Cappuccino JG, Sherman N. Microbiology: A Laboratory Manual. 10th Ed. San Francisco: Pearson Benjamin Cummings; 2014. P. 125–127.
[27] Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification Of Methicillin-Resistant Strains Of Staphylococci By Polymerase Chain Reaction. J Clin Microbiol. 1991;29(10):2240-4.
[28] Clinical And Laboratory Standards Institute (CLSI). Methods For Antimicrobial Susceptibility Testing For Bacteria That Grow Aerobically; Approved Standard. 11th Ed. CLSI Document M07-A11. Wayne (PA): CLSI; 2014.
[29] Ayeni FA, Odumosu BT, Oluseyi AE, Ruppitsch W. Identification And Prevalence Of Methicillin-Resistant Staphylococcus Aureus In Clinical And Non-Clinical Samples In South West Nigeria. J Infect Dev Ctries. 2017;11(4):368–73.
[30] Founou LL, Founou RC, Essack SY. Antibiotic Resistance In Food Animals In Africa: A Systematic Review And Meta-Analysis. Microb Drug Resist. 2018;24(5):648-65. Doi:10.1089/Mdr.2017.0383.
[31] Igbinosa EO, Beshiru A, Igbinosa IH, Ogofure AG, Ekundayo TC, Okoh AI. Prevalence, Multiple Antibiotic Resistance And Virulence Profile Of Methicillin-Resistant Staphylococcus Aureus (MRSA) In Retail Poultry Meat From Edo, Nigeria. Front Cell Infect Microbiol. 2023;13:1122059. Doi:10.3389/Fcimb.2023.1122059
[32] Mekonnen AG, Eguale T, Ameni G. Methicillin-Resistant Staphylococcus Aureus In Abattoirs And Meat Processing Environments In Ethiopia. BMC Microbiol. 2019;19(1):123.
[33] Gil RG, Siraj SS, Donacho DO. Hygiene Practices And Factors Associated With Hygiene Practice Among Meat Handlers At Butcher Houses And Restaurants In Gambela Town, Southwest Ethiopia. SAGE Open. 2024; (Study Reports That Only ~40.6 % Practiced Good Hygiene And Identifies Key Contributing Factors.)
[34] Ateba CN, Mbewe M. Prevalence Of Staphylococcus Aureus And Escherichia Coli In Raw Meat In Mafikeng, North-West Province, South Africa. J Food Prot. 2011;74(1):154–6.
[35] Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Et Al. Global Trends In Antimicrobial Use In Food Animals. Proc Natl Acad Sci U S A. 2015;112(18):5649–54.
[36] Abdalla MA, Siham E, Suliman SE, Bakhiet AO. Microbial Contamination Of Meat Products From Khartoum, Sudan. Foodborne Pathog Dis. 2009;6(4):475–7.
[37] Annan-Prah A, Agbemafle I, Asare PT, Akorli SY, Boi-Kikimoto BB, Abaidoo RC. Molecular Characterization And Antimicrobial Resistance Of Staphylococcus Aureus Isolated From Raw And Processed Meat In Ghana. BMC Microbiol. 2020;20(1):178.
[38] Fagerlund A, Møretrø T, Heir E, Briandet R, Langsrud S. Staphylococcus Aureus Biofilm Formation At The Air-Liquid Interface: Impact Of Nutrients, Temperature, And Strain Variation. Int J Food Microbiol. 2016;223:53–60.
[39] Oloso NO, Fagbo S, Garbati M, Olonitola SO, Awosanya EJ, Aworh MK, Et Al. Antimicrobial Resistance In Food Animals And The Environment In Nigeria: A Review. Int J Environ Res Public Health. 2018;15(6):1284.
[40] Okafor UC, Ugwuegbulem P-M. Antimicrobial Susceptibility Evaluation Of Microorganisms Isolated From Poultry Water In Awka Metropolis. J Biochem Int. 2022;9(4):1–9.
[41] Pantosti A, Sanchini A, Monaco M. Mechanisms Of Antibiotic Resistance In Staphylococcus Aureus. Future Microbiol. 2007;2(3):323–34.
[42] Abunna F, Bedasa S, Beyene T, Ayana D, Mamo B, Duguma R. Staphylococcus Aureus In Raw Meat And Milk Samples And Its Susceptibility To Antimicrobial Agents In Bishoftu, Ethiopia. Afr J Microbiol Res. 2016;10(22):780–5.
[43] Tadesse BT, Ashley EA, Ongarello S, Havumaki J, Wijegoonewardena M, González IJ, Et Al. Antimicrobial Resistance In Africa: A Systematic Review. BMC Infect Dis. 2017;17(1):616.
[44] Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, Et Al. Antibiotic Resistance Is The Quintessential One Health Issue. Trans R Soc Trop Med Hyg. 2016;110(7):377–80.
[45] Asante J, Amoako DG, Abia ALK, Somboro AM, Govinden U, Bester LA, Et Al. Review Of Clinically And Epidemiologically Relevant Coagulase-Negative Staphylococci In Africa. Front Microbiol. 2019;10:140.