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JOURNAL OF
Clinical Microbiology and Biochemical Technology

ISSN: 2581-527X DOI: https://dx.doi.org/10.17352/jcmbt

Research Article

Assessment of the bacteriological qualities of meat and contact surfaces in markets in Abia State, Nigeria

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Received: 13 October, 2023
Accepted: 19 October, 2023
Published: 20 October, 2023

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Keywords: Meat; E-coli; Bacteriological quality; Meat hygiene; Market sanitation; Hygiene practices

Abstract

Background: Microbial contamination of meat comes from external sources during cutting, handling, and processing of the meat. This study was carried out to assess the bacteriological qualities of meat and contact surfaces in markets in Abia State, Nigeria.

Methods: This research involved the use of a Hazard Analysis Critical Control Point (HACCP) checklist to investigate the sanitation and hygiene practices of meat sellers and a laboratory study of red and white meat, water, and meat-contact surface samples. A total of 425 meat samples collected from 425 meat sellers from some randomly selected markets in Abia State were used for the study. There were also 20 water samples, 22 samples from table surfaces, 22 samples from knife surfaces, and 14 samples from transport vehicles. The multistage simple random sampling technique through balloting was employed to determine communities/markets for the study. Samples for the study were collected and analyzed using standard microbiological techniques such as culturing and the bacteria were enumerated and identified using biochemical and chemical tests.

Results: The prevalent bacterial isolates include *Staphylococcus* sp (78.80%), *Bacillus* sp (73.17%), *Enterococcus* sp (64.00%), *Escherichia coli* (62.11%), *Salmonella* sp (62.11%), *Klebsiella* sp (51.29%), *Micrococcus sp* (44.94%) and *Campylobacter* sp (43.52%). SPSS analysis using the one-way ANOVA showed no significant difference ($p > 0.05$) in bacteria isolated from markets in the three Senatorial Zones of the State. *Staphylococcus* sp was isolated in 61.11% of the tables, 50.00% of vehicles, 41.67% of knives and 46.32% of water; *Salmonella* sp was isolated in 47.22% of the tables, 36.11% of vehicles, 30.56% of knives and 43.85% of water; *Bacillus* sp was isolated in 41.67% of the tables, 44.44% of vehicles, 33.33% of knives and 23.70% of water; *Campylobacter* sp was isolated in 27.78% of the tables, 25.00% of vehicles, 30.56% of knives and none in water. There was no significant difference ($p > 0.05$) in bacteria isolated from the contact surfaces and water from the markets in the three zones of the State.

Conclusion: The bacteriological quality of meat in markets in Abia State could be said to be poor due to the isolation of Indicator bacteria such as *E, coli, Salmonella,* and *Campylobacter* from the studied meat samples. The presence of *E. coli* in the studied meat samples is an indicator of feacal contamination and a red alert for the Public health sector. It is recommended that meat sellers undergo proper training and regularly update their knowledge of meat safety.

Introduction

Meat is a key component of food and it is rich in protein, minerals, vitamins, and oil [1,2]. Meat could be defined as the various tissues of animal origin and include beef from cattle, pork from pigs, mutton from sheep, poultry from chickens, ducks, and turkey [1]. Fish, seafood, insects, and snails are excluded here [3]. Meat is animal flesh that is eaten as food [4]. It is a good source of protein and amino acids (consisting of about 15 to 20 percent of protein); iron; fat, zinc, B vitamins, phosphorus, etc. Meat supply protein which is of paramount importance as it is connected with the immune mechanism of the body; needed for building, repair, and maintenance of body tissues; maintenance of osmotic pressure; and the synthesis

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of certain substances like antibodies, plasma proteins, haemoglobin, enzymes, hormones and coagulation factors [5].

However, the high nutrient, mineral, and water contents of meat amongst other factors, predispose meat and meat products to microbial proliferations; resulting in their quick spoilage and contamination by microorganisms [6]. With a high water content of about 75%, Fresh meats are among the most perishable foods [7]. Meat is one of the most perishable foods and is a good medium for microbial growth due to its high nutrient and water contents, moderate pH, and inherent chemical and enzymatic activities [8-10].

Thus, in order to ensure the wholesomeness, safety, and quality of meat being sold to the public, various management procedures and guidelines for food and meat safety regulations nationally and internationally have been introduced. This is of paramount public health concern considering the continued global emergence and re-emergence of food-borne diseases. Some of these internationally recommended meat/food safety protocols are the Codex Alimentarius Commission CAC - Good Hygiene Practices (GHPs) and Hazard Analysis Critical Control Point (HACCP) - based Standard Operating Procedures (SOPs). The HACCP approach is used to investigate the processes and procedures/management practices that contribute to bacterial contamination, growth, and survival; and to identify points where control measures could be applied to prevent or eliminate the bacteriological hazards or reduce them to acceptable levels [11]. Meat handlers in Nigeria like their counterparts in other developing countries are yet to come to terms with these meat safety management protocols. Animal slaughtering and carcass handling in Nigeria also fall short of acceptable international standards, and fresh meat sold to the public is contaminated from contact surfaces such as retail and slaughter slabs; dirty wheelbarrows and car boots during transportation; openly displayed in the market and are examined with dirty hands by meat sellers and buyers with flies perching on them [12,13].

Meat gets contaminated by microorganisms from external sources during the cutting, handling, and processing of the meat - mainly from the skin and the intestinal tract of the animal [14]. Meat contamination could also occur during refrigeration if the proper cooling temperature is not maintained [15]. Some of the important bacteria that have been implicated in meat contamination and spoilage include *Salmonella, Staphylococcus, Campylobacter, Escherichia coli, Enterobacter, Micrococcus, Bacillus, Clostridium, Streptomyces*, etc [16].

Foodborne illness poses significant public health challenges, and the prevention of foodborne disease is an essential function of both public and environmental health [17]. Given the widespread impact of foodborne illness on people's health, economies, and food systems amongst others; researchers from all over the world are committed to figuring out ways to increase food safety on a variety of levels. This has resulted in research on food safety practices and food handling lately [18]. The HACCP-Good Hygiene Practices (GHPs) protocol for the assessment of meat hygiene and safety was intended for use and then carry out bacteriological qualities assessment of meat and meat contact surfaces in markets in Abia State.

It is generally believed that microbial contamination of meat comes from external sources during the cutting, handling, and processing of the meat. According to several publications, poor meat handling and management practices (in the storage, transportation, and processing, etc) at variance with internationally recognized standards as observed in some States in Nigeria have been implicated in meat contamination resulting in food poisoning and food-borne diseases outbreaks; and other debilitating conditions such as kidney disorder (resulting from toxins produced by microorganisms in meat) [17-22]. Some of these practices include non-adherence to internationally recommended standards such as the CAC - Good Hygiene Practices (GHPs) and HACCP - based Standard Operating Procedures (SOPs). It is reported that about 75 million cases of food poisoning and 5000 deaths occur annually in the USA; with contaminated animal flesh accounting for 70% of the food poisoning [23-25]. Nigeria like other developing countries does not have accurate information on the prevalence and impact of food-borne diseases; however, it is an established fact that diarrhea – the most common manifestation of food-borne diseases is a major cause of sickness and death in the country. Contaminated meat/food is an important cause of illness, disability, and death globally; and food-borne diseases impede socioeconomic development by straining healthcare systems; contributing to a decrease in workers' productivity; loss in school days; reducing family income as huge sums of money are spent on medical bills; causing pains and suffering and early death [22,26]. These grave public health implications occasioned by increased mortality, morbidity, and disability resulting from meat/food-borne diseases could be averted if internationally recognized food safety systems such as HACCP-SOPs and GHP are incorporated in the meat management by meat handlers in Nigeria. I undertook this project due to the magnitude of the problem of poor hygiene practices and carcass handling in Nigeria which fall short of acceptable international standards with the resultant contamination of fresh meat sold to the public and the attendant problems. The general objective of this study is to assess the bacteriological qualities of meat and contact surfaces in markets in Abia State, Nigeria.

Methods

Study design

This research design was an experimental study involving laboratory tests/analysis to assess the bacteriological qualities of meat and meat contact surfaces in markets in Abia State.

Study setting

Abia state was created from part of Imo state on 27th August 1991. The geographical coordinates of Abia state are 5.4309°N 7.5247°E. As at the 2006 census, the population of Abia state was put at 2,833,999. Its capital city is Umuahia and the major commercial city is Aba. English is widely spoken and serves as the official language in governance and business. Christianity is the predominant religion of the Abia people. Abia state has 3 senatorial zones with 17 Local Government Areas (LGAs). The senatorial zones are Abia Central, Abia North, and Abia South. The LGAs include Aba North, Aba South, Arochukwu, Bende,

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Ikwuano, Isiala Ngwa North, Isiala Ngwa South, Isuikwuato, Obi Ngwa, Ohafia, Osisioma Ngwa, Ugwunagbo, Ukwa East, Ukwa West, Umuahia North, Umuahia South and Umu Nneochi. Figure 1 shows the 3 senatorial zones and the LGAs in each zone of Abia state.

Study population

Meat handlers include meat handlers in abattoirs/ slaughterhouses; meat handlers in the markers (meat sellers) and meat handlers in transit from abattoirs to markets.

The study population here is meat (red and white) sellers in markets in Abia State, Nigeria. According to the information from the meat sellers Associations in Abia State, there are about three thousand one hundred (3100) meat sellers across the various markets in Abia State. Ten (10) Local Government Areas (LGAs) out of the Seventeen (17) LGAs from the three Senatorial Zones in Abia State were randomly through balloting selected for this study.

Sample size and sampling technique

Sample size: The sample size calculation of the population of meat sellers in the markets for this study was determined using Taro Yamane's (1967) formula:

$N = N/1+N(e)^2$

Where n = sample size; N = Population size; e = Level of precision (5%)

$n = 3100/1+3100 \times (0.05)^2$

$= 3100/1+3100 \times .0025$

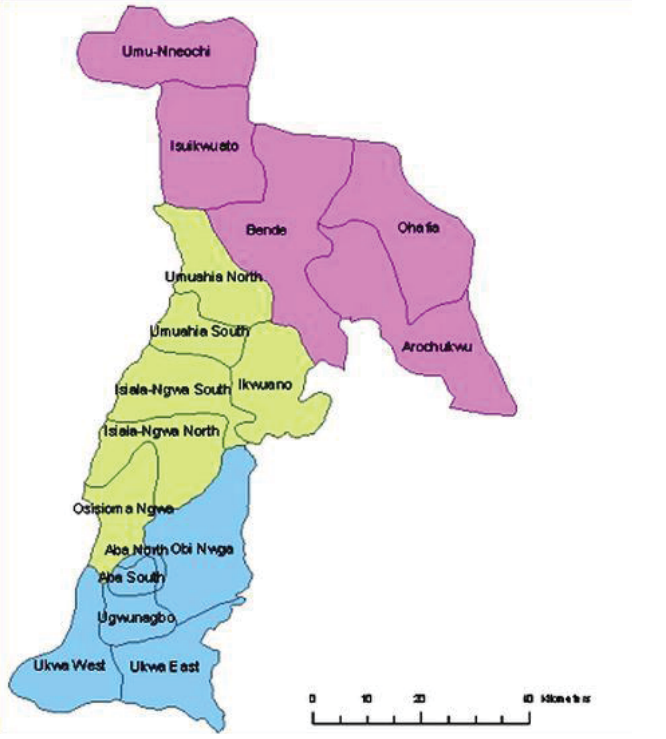

Figure 1: Geographical Map of the Study Area- Abia State showing the three (3) Senatorial Zones and LGAs (Source: Nigerian Muse, 2010).

$=4250/1 + 7.75$

$=3100/8.75$

$= 354.28$ approximately 354

Adding 20% to account for attrition, then the 20% of 354 = $0.20 \times 354 = 70.85$ approximately 71

Therefore, the total sample size for this study is 354 + 71 = 425 meat sellers

Sampling technique: A multistage simple random sampling technique was adopted for this study.

Selection of LGAs, markets: A simple random sampling using balloting was used for the selection of ten (10) out of the seventeen (17) Local Government Areas (LGAs) in Abia State for the study thereby giving every LGA in Abia State an equal chance of selection by the researcher. Thereafter, through balloting, markets were selected from enumerated major markets in the selected LGAs and communities for sampling.

Selection of respondents: The respondents together with the meat samples were randomly selected through balloting whereby all respondents present at the time of study who picked even numbers were selected until the minimum sample size of the study was obtained.

Thus, a total of 425 samples of meat and meat sellers were randomly selected from markets in ten (10) LGAs in Abia State, Nigeria for the study.

The sampled markets in Aba, Umuahia, and Ohafia Senatorial Zones have a total number of 340, 250, and 100 meat sellers respectively out of which 200, 160, and 65 randomly selected meat sellers were drawn/participated in this study from the three senatorial zones respectively.

Table 1 below shows the distribution of participating meat sellers in the sampled markets according to the Senatorial Zones in Abia State.

Inclusion and exclusion criteria

All meat sellers in the markets (both males and females from the ages of 18 years and above) who practice their trade in Abia State; and gave their consent for the study were part of this research work. Meat sellers/handlers who did not give informed consent to be part of the study were excluded.

Instrument for data collection

The instruments for data collection were questionnaires and Laboratory equipment for the assessment of the bacteriological qualities of meat samples.

Laboratory equipment and materials for the bacteriological assessment

Glass wares: Test tube, Pipettes (1ml, 2mls, 5mls, 10mls), Conical flasks (100mls. 250mls, 500mls, 1000mls), Beaker (100mls, 250mls, 500mls, 1000mls), Glass spreader, Glass

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Table 1: Distribution of participating meat sellers/ meat samples in the sampled markets according to the Senatorial Zones in Abia State.

Study Area	Frequency	Percentage	Cumulative Percent
Aba Senatorial Zone	200 (R = 120; W = 80)	47.06	47.06
Umuahia Senatorial Zone	160 (R = 62; W = 98)	37.64	84.7
Ohafia Senatorial Zone	65 (R = 42; W = 23)	15.30	100
Total	425 (R = 224; W = 201)	100	

R = Red Meat (Beef), W = White Meat (Chicken)

slides, Coverslips, Bijou bottles, Measuring cylinder (250mls, 500mls, 1000mls), Volumetric flask (100mls, 250mls, 500mls, 1000mls), Centrifuge tubes, Durham tubes.

Personal protective equipment: Hand gloves, Face mask, Nose mask, Laboratory coat.

Reagents and chemicals: Alcohol, Acetone, Kovacs reagents, Alpha naphthol, Indole reagent.

Media and diluents: Distilled water, Peptone water, Normal Saline Solution, Grams reagents, Methyl red, Bromothymol blue, Methylene blue, MR-VP Broth, Glucose, Sucrose, Lactose, Mannitol, Maltose, Fructose, Salmonella Shigella Agar, Eosin Methylene Blue Agar, MacConkey Agar, Nutrient Agar, Nutrient Broth, Campylobacter Blood Free Agar, Salmonella Shigella Agar, Sodium Chloride, Mannitol Salt Agar, Simmon's Citrate agar.

Equipment for laboratory analysis: Colony counter, Microscope, pH meter, Water Bath, Autoclave, Hot Air Oven, Centrifuge, Refrigerator, Laboratory blender, Weighing balance (electronic and manual), Digital Meat Thermometer.

Other materials: Cotton wool, Masking tape, Markers, Blotting paper, Wire loop, Mounting needle/inoculating needle, Gas cylinder, Cold box.

Procedure for samples (data) collection/preparation and analysis

Collection of samples: Four hundred and twenty-five meat samples comprising 224 red meat beef (120 from the Aba zone, 62 from the Umuahia zone, and 42 from the Ohafia zone) and 201 white meat chicken (80 from the Aba zone, 98 from Umuahia zone and 23 from the Ohafia zone) were collected from markets in Abia State. Collections of the meat samples were in sterile containers and collected samples were transported in an ice-packed cooler to the laboratory. Samples were also taken from the water sources (20 samples) in the market and contact surfaces of the meat handlers which included tables (22 samples), knives (22 samples), and transport vehicles (14 samples). Samples from contact surfaces were collected using sterile specimen sponges wetted with 10ml of buffered peptone water (Oxoid) from sterile Whirl-Pak bags (Sponge-Bag, PBI-International) using a template of 100cm^2 surface area. Sponging within the selected area consisted of 5 passes vertically (up and down was considered as one pass) and then 5 passes horizontally (side to side was considered one pass). The sponge was placed into a Stomacher bag, labelled, and delivered in a cold box to the laboratory within 4 hours. All collected samples were properly labelled and taken to the Environmental Health Laboratory, College of Health Sciences, Abia State University Aba for analysis. Copies of the questionnaires were administered to the meat sellers with the help of Field Assistants who had already been trained for that purpose.

Preparation of media and diluents: All bacteriological media were prepared according to the manufacturer's specifications. Nutrient agar was used in the isolation of heterotrophic bacteria, MacConkey Agar for faecal coliform bacteria, Eosin Methylene Blue Agar for *Escherichia coli*, Campylobacter Agar for *Campylobacter* species, Mannitol Salt Agar strictly for *Staphylococcus aureus* and Salmonella Shigella Agar for the isolation of *Salmonella* and *Shigella* species). Physiological saline used as diluents was prepared by dissolving 9.8 g of sodium chloride in 1000ml of distilled and dispensed in 90 ml and 9 ml portions. Both diluents and media were sterilized in an autoclave at 121 °C for 15 minutes.

Sample analysis and tests: preparation of samples and inoculation Ten (10) grams of meat sample was macerated in a sterile laboratory blender containing 90 ml of sterile peptone water. The ten-fold dilution method was used by transferring 1 ml from each tube until the required dilution was obtained. An aliquot portion (0.1 ml) of appropriate dilution was inoculated into the pre-sterilized and surface-dried medium. Inocula were spread evenly to ensure uniform and countable colonies and plated on different types of media for microbial growth and enumeration. Plates were incubated at 28 °C for 48 hours for heterotrophic bacteria.

Test tubes containing swabs were shaken on a vortex mixer for 30 seconds for uniform distribution of bacteria. Tenfold serial dilution of all samples was prepared using sterile normal saline solution (NSS) 0.1ml of each sample was pipetted into an agar plate and incubated at 37 °C for 42 - 48 hours for total viable bacteria count.

For Total Aerobic Mesophilic Count (TAMC), on an agar media plate, 0.1 ml of each sample was pipetted and spread. The inoculated plate was incubated at 32 °C for 48 - 72 hours.

For total coliforms and fecal coliform count, 0.1 ml of each sample was pipetted and spread on violet-red bile agar. The inoculated plate was incubated at 32 °C for 18 - 24 hours to determine the total coliforms, and at 44.5 °C for 18 - 24 hours to determine the fecal coliform.

For Enterobacteriaceae count, 0.1 ml of each sample was pipetted and spread on MacConkey agar supplemented with glucose. The inoculated plate was incubated at 35 °C for 24 hours. All reddish purple/pink colonies were counted as members of the Enterobacteriaceae.

For aerobic spore former bacterial count, meat sample suspension was first heated at 80 °C in the water bath for ten minutes to kill the vegetative cells. Then, 0.1 ml of each sample was pipetted and spread on a Plate Count Agar (PCA) plate. The inoculated plate was incubated at 35 °C for 36 - 72 hours.

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Determination of microbial population: After incubation, plates with colonies between 30 and 300 were counted using the Colony counter and the result was expressed as colony-forming units per gram (CFU/g) to obtain the total population.

Characterization and identification of microbial isolates: After incubation of the various inoculated plates, the predominant bacterial colonies were picked randomly from countable plates and inoculated into test tubes containing about 5ml nutrient broth. The bacterial cultures were purified by repeated streak plating and characterization. The predominant bacterial isolates were characterized based on cultural (colonial), microscopic, and biochemical methods with reference to standard manuals. The identities of the isolates were cross-matched in reference to standard manuals for the identification of bacteria [2].

Microscopic characterization

I. **Gram Staining Test:** The Gram staining technique was used for the bacterial isolates as described by Cheesbrough [2]. A smear of the isolate was made on a grease-free glass slide with a drop of water and allowed to dry. The smear was fixed by mild heating, flooded with crystal violet, and allowed to stand for 30 seconds. The crystal violet was rinsed off with water. Lugol's iodine was added and allowed to stand for 30 seconds. This was washed off with water and acid alcohol, till discoloration. It was counter-stained with Safranin for 10 seconds and rinsed with water. The wet slide was allowed to air dry. A drop of oil immersion was added to the slide and viewed using the X100 objective lens of the microscope.

II. **Spore Staining Test:** The spore stain was used to confirm the presence of spores when indicated in the Gram stain. Isolates were heat-fixed on a slide and flooded with 5% malachite green. It was steamed for 3 minutes (without allowing it to boil), dried, and cooled. It was then rinsed off and stained with Safranin for 30 seconds. This was rinsed, dried with filter paper, and viewed under the microscope using oil immersion tens. The positive spores showed green while the negative cells were stained pink.

III. **Motility Test:** This test was used to determine the motility of the bacteria isolated. The test was carried out on a semi-solid agar medium in which motile bacteria swarm and gave a diffuse spreading growth. The medium was dispensed into test tubes, sterilized, and allowed to be set in an upright position. It was then inoculated using an inoculation needle by stabbing it into the medium in the test tube. This was incubated at 37 °C for 24 hours. Diffuse growth from the straight line of inoculation was recorded as a positive result [2].

Biochemical characterization of bacteria isolates

Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to a few biochemical tests described by Cheesbrough [2].

i. **Catalase test:** The enzyme catalase is present in most cytochrome-containing aerobic and facultative anaerobic bacteria. Catalase has one of the highest turnover numbers of all enzymes such that one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen in a second. Catalase activity can be detected by adding the substrate H_2O_2 to an appropriately incubated (18 - 24 hours) tryptic soy agar slant culture. Organisms that produce the enzyme break down the hydrogen and the resulting O_2 production produces bubbles in the reagent drop indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to break down peroxide into O_2 and water and are catalase-negative.

ii. **Coagulase test**: Coagulase is an enzyme that clots blood plasma by a mechanism that is similar to normal clotting. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma component of blood. The only significant disease-causing bacteria of humans that produce coagulase are *Staphylococcus aureus.* Thus, this enzyme is a good indicator of *S. aureus.* In the test, the sample is added to rabbit plasma and held at 37 °C for a specified period of time. Formation of clot within four hours is indicated as a positive result and indicative of a virulent *Staphylococcus aureus* strain. The absence of coagulation after 24 hours of incubation is a negative result indicative of a virulent strain.

iii. **Oxidase test:** The oxidase test is an important differential procedure that should be performed on all gram-negative bacteria for their rapid identification. The test depends on the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and α-naphthol. This method uses N, N-dimethyl-p-phenylenediamine oxalate in which all *Staphylococci* are oxidase negative. In the presence of the enzyme cytochrome oxidase (gram-negative bacteria) the N, N-dimethhyl-p-phenylenediamine oxalate, and α-naphthol react to indophenol blue. *Pseudomonas aeruginosa* is an oxidase positive organism.

iv. **Sugar fermentation/oxidation:** This test is used to differentiate between bacteria groups that oxidize carbohydrates such as members of Enterobacteriaceae. One milliliter (1 ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose was separately under aseptic conditions and transferred into duplicate tubes containing 9 ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. The tubes were stab-inoculated in duplicates while two un-inoculated tubes served as control. Vaseline was used to cover one set of the duplicate tubes, and one control to discourage oxidative utilization of sugar. All tubes were incubated at 37 °C for 48 hours. After the incubation, they were observed for acid production in the culture. Yellow coloration indicates acid production

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in the open tubes only suggesting oxidative utilization of the sugar while acid production in the sealed tubes suggests a fermentative reaction.

v. **Hydrogen sulphide production (H_2S) test:** The test isolates were aseptically inoculated into a tube containing triple sugar iron agar starting by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37 °C for 72 hours and was examined daily. Black precipitation and yellow coloration were checked for. Black precipitate indicates H_2S production and yellow coloration for sucrose, lactose, and glucose fermentation.

vi. **Urease test:** The Urease Agar slant in the McCartney bottle was inoculated with the bacteria isolate at 30 °C for 4 hours and then overnight. A pink color in the medium indicated a positive result.

vii. **IMViC test:** This test consists of four different tests; they are Indole production, Methyl-Red test, Voges Proskaeur test, and Citrate utilization test. This test is specifically designed to determine the physiological properties of microorganisms. They are especially useful in the differentiation of Gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter-Klebsiella* group.

viii. **Indole test:** This test demonstrates the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacteria isolates were inoculated into the medium and incubated at 37°C for 48 hours. At the end of the incubation period, 3 drops of Kovac's reagents were added and then shaken. A red color ring at the interface of the medium denotes a positive result. Methyl red and Voges-Proskaeur test must be considered together since they are physiologically related. The opposite test is usually obtained from the MR and VP test, that is, MR+, VP-, or MR-, VP+.

A methyl red test was performed to demonstrate the capacity of different organisms to produce acid from the fermentation of sugar (dextrose). Methyl-red-positive organisms produce a red coloration when five drops of a methyl-red indicator are added to a 48-hour-old MR-VP broth culture.

The Voges-Proskaeur test demonstrates the ability of organisms to produce acetone from glucose metabolism. Some organisms metabolize glucose to produce pyruvic acid which is further broken down to yield Butane-diol and acetyl-methyl carbinol as an intermediate product. Into one milliliter of the culture, one milliliter of six percent alcoholic solution of alpha-naphthol was added to one milliliter of 16% KOH and allowed to stand for 15 - 20 minutes. The development of a red-to-pink color was a positive test.

iX. **Citrate utilization test:** This is one of the several techniques used to assist in the identification of Enterobacteria. The principle of the test is based on the ability of an organism to use citrate as its only source of carbon. The test was carried out using Simmon's citrate agar. The slopes of the media were prepared in bijou bottles as recommended by the manufacturers. A sterile straight wire was used to the slope with a saline suspension of the test organisms before stabbing the butt. The bottles were incubated at 37 °C for 48 hours. Bright blue colors in the medium mean a positive test while no change in color of the medium indicates a negative citrate test [2]

Method of data analysis

The data from this research work was collated manually by the Researcher; and then entered into the computer by a statistician. The Statistical Package for the Social Sciences (SPSS) software (version 20) was used in the analysis of the data. Results were expressed in percentages, frequencies, and tables. One-way ANOVA and the independent sample T-test were used to test the hypotheses at 95% confidence interval and 0.05 Level of significance.

Ethical clearance/ informed consent

An informed consent was obtained from all meat handlers who participated in the study. The purpose of the research was explained to each respondent and verbal informed consent was obtained from them before inclusion into the study. Also, the anonymity of the respondents was assured and ensured.

Results

A total of four hundred and twenty-five (425) meat samples comprising 224 red meat- beef (120 from Aba zone, 62 from Umuahia zone, and 42 from Ohafia zone) and 201 white meat-chicken (80 from Aba zone, 98 from Umuahia zone and 23 from Ohafia zone) collected from four hundred and twenty-five (425) meat sellers from markets in Abia State were used for this study. There were also twenty (20) water samples, twenty-two (22) samples from table surfaces, twenty-two (22) samples from knife surfaces, and fourteen (14) samples from transport vehicles. The results of the data collected and analyzed are presented in the tables below:

Bacteriological qualities of meat samples and meat contact surfaces

A total of 425 meat samples comprising 224 red meat- beef (120 from the Aba zone, 62 from the Umuahia zone, and 42 from the Ohafia zone) and 201 white meat- chicken (80 from the Aba zone, 98 from the Umuahia zone and 23 from Ohafia zone) were collected and analysed. There were also twenty (20) water samples, twenty-two (22) samples from table surfaces, twenty-two (22) samples from knife surfaces, and fourteen (14) samples from transport vehicles.

The results of the predominant bacterial isolates of the meat samples and the meat contact surface from markets in Abia State were as presented in the tables below:

Bacteria isolated from the meat samples (red and white meat) from markets in Abia state

The result of the Bacteria isolated from the 425 meat samples (red and white meat) from markets in Abia State is presented in Table 2 below:

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Table 2 showed that out of the total of 425 meat samples collected and analyzed, *Staphylococcus sp*. was isolated in 335 (78.80%) of the meat samples; *Escherichia coli*, 264 (62.11%); *Micrococcus* sp., 191 (44.94%); *Salmonella* sp., 264 (62.11%); *Bacillus* sp., 311 (73.17%); *Campylobacter* sp., 185 (43.52%); *Klebsiella* sp., 218 (51.29%); *Enterococcus* sp., 272 (64.00%); *Shigella* sp., 106 (24.94%); *Pseudomonas* sp., 64 (15.05%); *Enterobacter* sp., 161 (37.88%).

Bacteria isolated from red meat samples from markets in Abia state

The result of the Bacteria isolated from red meat samples from markets in Abia State is presented in Table 3 below. A total of 224 red meat samples were collected and analysed (120 from the Aba zone, 62 from the Umuahia zone, and 42 from the Ohafia zone).

Table 3 showed that out of the 224 red meat sampled, *Staphylococcus* sp was isolated in 175 (78.12%) of the red meat samples; *Escherichia coli*, 139 (62.05%); *Micrococcus* sp., 100 (44.64%); *Salmonella* sp., 138 (61.60%); *Bacillus* sp., 167 (74.55%); *Campylobacter* sp., 99 (44.19%); *Klebsiella* sp., 116 (51.78%); *Enterococcus* sp., 142 (63.39%); *Shigella* sp., 55 (24.55%); *Pseudomonas* sp., 34 (15.17%); *Enterobacter* sp., 84 (37.50%).

Comparison of bacteria isolated from red meat samples from markets in the Three Senatorial zones in Abia state

The result of the comparison of the Bacteria isolated from 224 red meat-beef samples (120 from the Aba zone, 62 from the Umuahia zone, and 42 from the Ohafia zone) from markets in the three Senatorial Zones in Abia State is presented in Table 4 below:

Table 4 showed that *Staphylococcus* sp was isolated in 81.09% of the red meat in Umuahia, 77.18% in Aba and 77.11% in Ohafia; *Escherichia coli*, 64.12% in Umuahia, 60.00% in Aba and 65.06% in Ohafia; *Micrococcus* sp, 48.10% in Umuahia, 43.55% in Aba and 43.55% in Ohafia; *Salmonella* sp, 65.28% in Umuahia, 59.19% in Aba and 65.14% in Ohafia; *Bacillus* sp, 73.43% in Umuahia, 76.23% in Aba and 71.28% in Ohafia; *Campylobacter* sp, 40.58% in Umuahia, 43.84% in Aba and 49.84% in Ohafia; *Klebsiella* sp., 51.40% in Umuahia, 50.10% in Aba and 58.25% in Ohafia; *Enterococcus* sp., 68.81% in Umuahia, 60.05% in Aba and 64.33% in Ohafia; *Shigella* sp., 25.66% in Umuahia, 24.68% in Aba and 22.61% in Ohafia; *Pseudomonas* sp., 14.92% in Umuahia, 13.91% in Aba and 18.16% in Ohafia; *Enterobacter* sp., 39.70% in Umuahia, 37.60% in Aba and 33.89% in Ohafia.

SPSS analysis using the one-way ANOVA showed that there was no statistically significant difference between bacteria isolated from red meat samples from markets in the Senatorial Zones in Abia State [p = 0.7678) > 0.05].

Bacteria isolated from white meat samples from markets in Abia state

The result of the Bacteria isolated from white meat-chicken samples from markets in Abia State is presented in Table 5.0 below. A total of 201 white meat (chicken) samples were collected and analysed (80 from the Aba zone, 98 from the Umuahia zone, and 23 from the Ohafia zone).

Table 5 showed that out of the total 201 white meat sampled, *Staphylococcus* sp was isolated in 160 (79.60%) of the

Table 2: Bacteria isolated from the meat samples (red and white meat) from markets in Abia State.

Bacterial Isolates	n	%
Staphylococcus sp	335	78.80
Escherichia coli	264	62.11
©*Micrococcus* sp	191	44.94
Salmonella sp	264	62.11
Bacillus sp	311	73.17
Campylobacter sp	185	43.52
Klebsiella sp	218	51.29
Enterococcus sp	272	64.00
Shigella sp	106	24.94
Pseudomonas sp	64	15.05
Enterobacter sp	161	37.88

N = 425

Table 3: Bacteria isolated from red meat samples in markets in Abia state.

Bacterial Isolates	n	%
Staphylococcus sp	175	78.12
Escherichia coli	139	62.05
Micrococcus sp	100	44.64
Salmonella sp	138	61.60
Bacillus sp	167	74.55
Campylobacter sp	99	44.19
Klebsiella sp	116	51.78
Enterococcus sp	142	63.39
Shigella sp	55	24.55
Pseudomonas sp	34	15.17
Enterobacter sp	84	37.50

N = 224

Table 4: Comparison of bacteria isolated from red meat samples from markets in the Senatorial Zones in Abia state.

Bacterial Isolates	Umuahia		Aba		Ohafia	
	n	%	n	%	n	%
Staphylococcus sp	50	81.09	93	77.18	32	77.11
Escherichia coli	40	64.12	72	60.00	27	65.06
Micrococcus sp	30	48.10	52	43.55	18	43.55
Salmonella sp	40	65.28	71	59.19	27	65.14
Bacillus sp	46	73.43	91	76.23	30	71.28
Campylobacter sp	25	40.58	53	43.84	21	49.84
Klebsiella sp	32	51.40	60	50.10	24	58.25
Enterococcus sp	43	68.81	72	60.05	27	64.33
Shigella sp	16	25.66	30	24.68	9	22.61
Pseudomonas sp	9	14.92	17	13.91	8	18.16
Enterobacter sp	25	39.70	45	37.60	14	33.89

ANOVA p value = 0.7678
Decision = NS
NS*- Not Significant; S*- Significant

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white meat samples; *Escherichia coli*, 125 (62.18%); *Micrococcus* sp., 91 (45.27%); *Salmonella* sp., 126 (62.68%); *Bacillus* sp., 144 (71.64%); *Campylobacter* sp., 86 (42.78%); *Klebsiella* sp., 102 (50.74%); *Enterococcus* sp., 130 (64.67%); *Shigella* sp., 51 (25.37%); *Pseudomonas* sp., 30 (14.92%); *Enterobacter* sp., 77 (38.30%).

Comparison of bacteria isolated from white meat samples from markets in the three senatorial zones in Abia state

The result of the comparison of the Bacteria isolated from 201white meat-chicken samples (80 from the Aba zone, 92 from the Umuahia zone, and 23 from the Ohafia zone) from markets in the three Senatorial Zones in Abia State is presented in Table 6 below:

Table 6: showed that *Staphylococcus* sp was isolated in 81.18% of the white meat in Umuahia, 77.17% in Aba, and 76.12% in Ohafia; *Escherichia coli*, 64.12% in Umuahia, 60.05% in Aba and 62.53% in Ohafia; *Micrococcus* sp, 48.10% in Umuahia, 43.52% in Aba and 42.50% in Ohafia; *Salmonella* sp, 65.22% in Umuahia, 59.11% in Aba and 64.17% in Ohafia; *Bacillus* sp, 73.44% in Umuahia, 70.28% in Aba and 71.45% in Ohafia; *Campylobacter* sp, 40.53% in Umuahia, 43.88% in Aba and 47.60% in Ohafia; *Klebsiella* sp., 51.47% in Umuahia, 50.10% in Aba and 51.13% in Ohafia; *Enterococcus* sp., 68.86% in Umuahia, 60.04% in Aba and 64.34% in Ohafia; *Shigella* sp., 25.69% in Umuahia, 24.66% in Aba and 27.70% in Ohafia; *Pseudomonas* sp., 14.90% in Umuahia, 13.98% in Aba and 15.41% in Ohafia; *Enterobacter* sp., 39.73% in Umuahia, 37.67% in Aba and 36.51% in Ohafia.

SPSS analysis using the one-way ANOVA showed no significant difference [P(0.19)>0.05] between bacteria isolated from white meat samples from markets in the Senatorial Zones in Abia state.

Comparison of bacteria isolated from red and white meat samples from markets in Abia state

The result of the comparison of the Bacteria isolated from the 224 red meat and 201 white meat samples from markets in Abia State is presented in Table 7 below:

Table 7 showed that out of the total 224 red and 201 white meat sampled, *Staphylococcus* sp was isolated in 175 (78.12%) of the red meat samples and 160 (79.60%) of the white meat samples; *Escherichia coli*, 139 (62.05%) red meat and 125 (62.18%) white; *Micrococcus* sp., 100 (44.64%) red meat and 91 (45.27%) white; *Salmonella* sp., 138 (61.60%) red meat and 126 (62.68%) white; *Bacillus* sp., 167 (74.55%) red meat and 144 (71.64%); *Campylobacter* sp., 99 (44.19%) red meat and 86 (42.78%) white; *Klebsiella* sp., 116 (51.78%) red meat and 102 (50.74%) white; *Enterococcus* sp., 142 (63.39%) red meat and 130 (64.67%) white; *Shigella* sp., 55 (24.55%) red meat and 51 (25.37%) white; *Pseudomonas* sp., 34 (15.17%) red meat and 30 (14.92%) white; *Enterobacter* sp., 84 (37.50%) red meat and 77 (38.30%) white.

Table 5: Bacteria isolated from white meat samples in markets in Abia State.

Bacterial Isolates	n	%
Staphylococcus sp	160	79.60
Escherichia coli	125	62.18
Micrococcus sp	91	45.27
Salmonella sp	126	62.68
Bacillus sp	144	71.64
Campylobacter sp	86	42.78
Klebsiella sp	102	50.74
Enterococcus sp	130	64.67
Shigella sp	51	25.37
Pseudomonas sp	30	14.92
Enterobacter sp	77	38.30

N = 201

Table 6: Comparison of bacteria isolated from white meat samples from markets in the Senatorial Zones in Abia state.

Bacterial Isolates	Umuahia		Aba		Ohafia	
	n	%	n	%	n	%
Staphylococcus ssp	80	81.18	62	77.17	18	76.12
Escherichia coli	63	64.12	48	60.05	14	62.53
Micrococcus sp	47	48.10	35	43.52	9	42.50
Salmonella sp	64	65.22	47	59.11	15	64.17
Bacillus sp	72	73.44	56	70.28	16	71.45
Campylobacter sp	40	40.53	35	43.88	11	47.60
Klebsiella sp	50	51.47	40	50.10	12	51.13
Enterococcus sp	67	68.86	48	60.04	15	64.34
Shigella sp	25	25.69	20	24.66	6	27.70
Pseudomonas sp	15	14.90	11	13.98	4	15.41
Enterobacter sp	39	39.73	30	37.67	8	36.51

ANOVA P value = 0.19
Decision = NS
NS*- Not Significant; S*- Significant

Table 7: Comparison of Bacteria isolated from red and white meat samples from Markets in Abia state.

Bacterial Isolates	Red meat (N = 224)		White meat (N = 201)	
	n	%	n	%
Staphylococcus sp	175	78.12	160	79.60
Escherichia coli	139	62.05	125	62.18
Micrococcus sp	100	44.64	91	45.27
Salmonella sp	138	61.60	126	62.68
Bacillus sp	167	74.55	144	71.64
Campylobacter sp	99	44.19	86	42.78
Klebsiella sp	116	51.78	102	50.74
Enterococcus sp	142	63.39	130	64.67
Shigella sp	55	24.55	51	25.37
Pseudomonas sp	34	15.17	30	14.92
Enterobacter sp	84	37.50	77	38.30

ANOVA *p* value = 0.527
Decision = NS
NS*- Not Significant; S*- Significant

SPSS analysis using the one-way ANOVA showed no significant difference [P(0.527)>0.05] in bacteria isolated from red and white meat samples from markets in Abia State.

A total of 224 red meat and 201 white meat samples were collected and analyzed.

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Comparison of bacteria isolated found on meat contact surface samples - tables, knives, water, and transport Vehicles in use in markets in Abia state

The result of the comparison of the Bacteria isolated from 78 meat contact surface samples (comprised of 22 table surfaces, 22 knife surfaces, 14 transport vehicles, and 20 water samples) from markets in Abia State is presented in Table 8 below:

Table 8 below showed that *Staphylococcus* sp was isolated in 13 (61.11%) of the 22 samples from tables, 7 (50.00%) of the 14 samples from vehicles, 9 (41.67%) of the 22 samples from knives, and 9 (46.32%) of the 20 water samples; *Salmonella* sp, in 10 (47.22%) of the tables, 5 (36.11%) of vehicles, 7 (30.56%) of knives and 9 (43.85%) of water samples; *Bacillus* sp., in 9 (41.67%) of the tables, 6 (44.44%) of vehicles, 7 (33.33%) of knives and 5 (23.70%) of water samples; *Campylobacter* sp., in 6 (27.78%) of the tables, 4 (25.00%) of vehicles, 7 (30.56%) of knives and none in water. No other bacteria were isolated from the samples.

SPSS analysis using the one-way ANOVA showed that there was no statistically significant difference between the bacterial isolates found on tables, knives, water, and transport vehicles in use in markets in Abia State (p = 0.8100).

Discussion

The results of this study revealed the presence of various bacterial isolates in the meat samples (both red and white meat), with *Staphylococcus* sp, *Bacillus* sp, *Escherichia coli*, *Enterococcus* sp, *Salmonella* sp, *Klebsiella* sp, *Micrococcus sp*, and *Campylobacter* sp being the prevalent isolates. These findings are of great concern as they indicate the potential for bacterial contamination in the meat sold in the markets. *Staphylococcus* sp was the most prevalent isolate, present in a high percentage (78.80%) of the meat samples. This bacterium is known to be commonly associated with human skin and can be transferred to meat during handling and processing, highlighting the significance of proper hygiene practices among meat handlers. Similarly, *Escherichia coli*, a common indicator of fecal contamination, was found in a substantial proportion (62.11%) of the meat samples, suggesting possible contamination from improper slaughter and processing practices. Other bacterial isolates, such as *Micrococcus* sp, *Salmonella* sp, *Bacillus* sp, and *Campylobacter* sp, were also detected at varying rates. The presence of these organisms on the surface of meat samples and the contact surfaces, such as tables, vehicles, and knives, indicates potential fecal and environmental contamination. The poor personal hygiene and sanitation practices among meat sellers/handlers as observed in this study could have contributed to the contamination of the meat. Most of the predominant bacteria in the meat contact surfaces such as *Staphylococcus* sp., *Salmonella* sp., *Bacillus* sp., and *Campylobacter* sp. were also predominant in the meat samples suggesting a possible cross-contamination of the meat carcasses from the contact surfaces. This contamination can occur during various stages, from slaughter to transportation and display of meat in the markets. The findings of this study are in agreement with previous studies by other researchers including Gutema, et al. [27] who reported and linked the isolation of *Salmonella* sp from contaminated chicken meat to poor sanitary and sanitation conditions; Shimelis, et al. [28] who in their studies isolated *E. coli* and *Salmonella* species as the common bacterial isolates from beef at selected slaughterhouses and attributed their sources of contamination to include equipment, transport vehicle, cutting board and worker's hand.

Also, the statistical analysis showed no significant difference in the bacteria isolated from the various markets (p > 0.05), suggesting that the prevalence of these bacterial isolates is consistent across the studied markets. This finding raises concerns about the overall hygiene and sanitation practices in the meat markets, as the presence of these bacteria on meat surfaces can pose significant health risks to consumers. The high prevalence of these bacterial isolates underscores the importance of implementing stringent hygiene and sanitation measures in meat handling and processing as already suggested by previous researchers including Azuamah, et al. [19] and Tesson, et al. [29].

Meat sellers/handlers should be trained on proper hygiene practices, including handwashing, wearing gloves and aprons, and ensuring the cleanliness of equipment and contact surfaces. Additionally, market authorities should enforce regulations and conduct regular inspections to ensure compliance with hygiene and sanitation standards. The findings of this study are consistent with previous research on meat contamination and highlight the need for continuous monitoring and improvement of meat handling practices to ensure the safety and quality of meat products. By addressing the issues of bacterial contamination in meat markets, public health risks can be minimized, and consumers can have greater confidence in the safety of the meat they purchase and consume.

Table 8: Comparison of bacterial isolates found on tables, knives, water, and transport Vehicles in use in markets in Abia State.

Bacteria	Table		Vehicle		Knife		Water	
	n	%	n	%	n	%	n	%
Staphylococcus sp	13	61.11	7	50.00	9	41.67	9	46.32
Escherichia coli	0	0.00	0	0.00	0	0.00	0	0.00
Micrococcus sp	0	0.00	0	0.00	0	0.00	0	0.00
Salmonella sp	10	47.22	5	36.11	7	30.56	9	43.85
Bacillus sp	9	41.67	6	44.44	7	33.33	5	23.70
Campylobacter sp	6	27.78	4	25.00	7	30.56	0	0.00
Klebsiella sp	0	0.00	0	0.00	0	0.00	0	0.00
Enterococcus sp	0	0.00	0	0.00	0	0.00	0	0.00
Shigella sp	0	0.00	0	0.00	0	0.00	0	0.00
Pseudomonas sp	0	0.00	0	0.00	0	0.00	0	0.00
Enterobacter sp	0	0.00	0	0.00	0	0.00	0	0.00

p value = 0.8100
Decision = NS
NS"- Not Significant; S"- Significant

Conclusion

In conclusion, the evaluation of the bacteriological quality of meat and contact surfaces in markets in Abia State, Nigeria, revealed an alarmingly low level of hygiene and sanitation practices among meat vendors and handlers. The presence of Salmonella spp. and Escherichia coli indicator bacteria on meat samples and contact surfaces, such as tables, vehicles, and

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knives, demonstrates the potential for faecal and environmental contamination. This contamination is likely the result of poor personal hygiene and suboptimal meat processing procedures during slaughter, dressing, and other stages of production. The findings highlight the urgent need for comprehensive education and awareness campaigns to improve meat handling practices among individuals involved in the meat industry in Abia State, thereby protecting public health and ensuring the safety of meat products offered for sale.

Contribution to knowledge

This study has added to existing knowledge that meat sellers/handlers in Abia states, Nigeria failed to meet the basic standards of personal hygiene and sanitation practices during the handling of meat in the sampled markets; and this could have led to the compromised bacterial qualities of the meat being sold. This study successfully isolated, identified, and documented the predominant bacterial isolates on meat sold in markets in Abia State as *Staphylococcus sp., Escherichia sp., Salmonella sp., Bacillus sp., Enterococcus sp.* and *Campylobacter sp.*; while *Staphylococcus sp., Salmonella sp., Bacillus sp., and Campylobacter sp.* were predominant bacteria on the meat contact surfaces. This study has also shown that the contamination of meat could have come from external sources through cross-contamination of the meat carcasses from the various contact surfaces during handling. The bacteriological quality of the meat sold across markets in Abia State indicated that there is a Process hygiene criteria failure in meat handling which does not call for the withdrawal of meat being sold to the public but for corrective measures to be put in place to correct reoccurrence. Meat sellers/handlers in Abia State should be enlightened/trained on proper meat handling standards of operation.

Recommendations

Meat handlers are advised to undergo proper training and regular updates on their knowledge of meat safety especially on proper sanitation and good personal hygiene practices. Meat handlers should be educated on the need to comply with standard operation procedures for the handling of meat such as the wearing of aprons and gloves, proper temperature control, and improved means of transportation. Government and Non-governmental agencies should fabricate a prototype of a customized cold chain chest for storage and transportation of meat; as well as for display of meat in the markets.

Availability of data and materials

The Data set from the study is available to the corresponding author upon request.

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