

ANTIBIOGRAM OF *E COLI* ISOLATED FROM FISH (Salmon fish)/MEAT (Beef).

ABSTRACT

Meat and fish are cherished food delicacies in Nigeria, but can be a source of dissemination of Multi-drug Resistant (MDR) bacteria. Moreover, there are limited studies on these MDR bacteria from Awka metropolies. Therefore, this study examined the bacteriological qualities and antibiogram profiles of *E coli* isolated from meat and fish. Twenty samples of different meat and fish were collected from the study areas and microbiologically analyzed. Total viable count, coliform count, characterization and identification of bacteria were carried out by standard microbiological techniques. Findings revealed that "meat" samples possessed the highest total viable bacteria count (3.4×10^5 to 7.7×10^5 cfu/g) and coliform count (2.1×10^5 to 6.2×10^5 cfu/g). A total of 85 and 78 bacteria were isolated from meat and smoked fish samples respectively. Antibiotic sensitivity pattern was carried out by disc diffusion method. The sensitivity pattern of *E coli* to the following antibiotics; Gentamicin, Amoxycillin/clavulanate, Streptomycin, Cloxacillin, Erythromycin, Chloramphenicol, Cotrimoxazole, Tetracycline, Penicillin, Ciprofloxacin, Ofloxacin, Levofloxacin, Ceftriaxone, Amoxycillin and vancomycin were 92.4%, 63.0%, 44.2%, 35.8%, 52.4%, 61.9%, 15.5%, 31.2%, 7.1%, 78.9%, 76.6%, 100%, 71.4%, 30.7% and 100% respectively. The results of the present study show that the fluoroquinolones are effective in the management of *E coli* infections including methicillin resistant strains in this environment.

Keywords: Antibiotics, Bacteria, Fish, Meat

1. INTRODUCTION

Meat is the flesh of animals which serves as food; it is obtained from sheep, cattle, goat and swine (Ducel, *et al.*, 2022). Fish is one of the most important animal protein and other vital nutrients sources that are widely consumed by all races and classes of people (Collignon *et al.*, 2015). Fish meat contains significantly low lipids and high water compared to that of beef or chicken and is favored over other white or red meats (Nestel, 2020). Meat and fishes are major source of protein and an important source of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of body cells which is necessary for our everyday activities (Aarestrup *et al.*, 2021).

The protein profile of meat and fishes has been described as excellent due to the presence of all the essential amino acids required by the body. The protein and vitamins especially vitamin A and B12 in meat is not available in plant sources (Christianend *et al.*, 2012). Meat and fish market makes an important contribution to the well-being of people but this is not without its health hazards (Boucher, *et al.*, 2017).

There is considerably high food related infections such as diarrhea, typhoid fever and cholera recorded in hospitals and clinics worldwide. In the past people have expressed worry about the role of meat and meat products in food poisoning but available records show that more than 74% of cases of food poisoning worldwide are due to meat dishes. Meat is highly prone to microbial contamination due to its rich source of nutrients which provide a suitable environment for growth of microbes.

The microbial growth can lead to meat spoilage and food borne infections in human, resulting in economic losses (Blaser, 2020).

Dirty environment and unhygienic food handling influence wide spread of bacterial food poisoning (Bauer *et al.*, 2016). Major bacterial pathogens found in meat include *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*. Contamination could come from unhygienic slaughtering, handling and processing conditions or from inherent microflora in normal tissues of animals, air and environment (Bauer *et al.*, 2012)

Antibiograms are tables showing how susceptible a series of organisms are to different antimicrobials. It summarise the cumulative proportions of pathogenic organisms that are susceptible to particular antimicrobials. This provides a profile of the susceptibilities of specific pathogenic bacteria to antimicrobial agents as tested in routine clinical microbiology practice.

The wide spread emergence of antibiotics resistance among pathogenic microorganisms had become serious challenge in clinical therapy (Li *et al.*, 2008). The mechanism by which these microorganisms exhibited resistance includes modification or alteration of target site and alteration of metabolic pathway (Bartoloni, *et al.*, 2019).

Frost *et al.*, (2005) highlighted the means of acquiring resistance to be transferred of gene between bacteria strains, which could be facilitated by mobile genetic elements such as plasmid, transposons, interferon, bacteriophages and insertion elements. However, the co-existence of resistance microorganisms in restaurant wastes that are directly discharged into environment would definitely lead to rapid spread of antibiotics resistance gene among other organisms in ecosystem (Austin, *et al.*, 2020).

Thus, the apparent increase of the occurrence of antibiotics resistance among microorganisms from various areas such as clinical, foods, water and its possible implications require adequate surveillance to detect and proffer solution to the emergence of antimicrobial resistance mechanisms.

Statement of the problem

Food contamination with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades, possibly as the result of selection pressure created by the use of antimicrobials in food-producing animals (Ameh and Amadusa 2016).

Outbreaks of infections somehow related with poor hygiene and consumption of contaminated food have been on the increase especially in developing countries like Nigeria, and some were caused by *E coli*. Moreover, antibiotic resistance levels are also elevated among food-borne pathogens such as *e coli*. Although, it is difficult to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, the presence of such bacteria in food items could play a role in the spread of antimicrobial resistance amongst food-borne pathogens (Alkekruse *et al.*, 2014).

Thus, adequate information should be gathered to develop an effective strategy to reduce the outbreak of food borne illnesses and resistance burden in the community.

Objective of the study

The aim of this study is therefore, to assess the antibiogram of *E coli* isolated from meat and fishes sold within and around Awka metropolies.

Specific objectives

1. To isolate and identify bacterial species associated with meat and fish contamination.
2. To determine the *E coli* load of isolated bacteria.
3. To determine the antibiotic susceptibility pattern of the isolated *E coli*

SCOPE OF STUDY

The scope entails collection of fish and meat samples within Awka metropolies and determination of antibiotic susceptibility pattern of microbes isolated from them.

SIGNIFICANCE OF STUDY

This study is significant as it will help to reduce contamination and illness caused by food pathogeninfection.

It was envisaged to lay down the groundwork for enhancing traditional knowledge and practices through modern approaches of drug development.

Materials and methods

2.1 MATERIALS

The media used in this work include Nutrient agar, MacConkey agar, Peptone water, Petri dish, autoclave, inoculating wire loop, forceps, Bunsen burner, Conical flask, Antibiotic discs, Weighing balance, Test tube rack, plastic pipette, wire loop, Microscope, Incubator, beakers, glass slide, sterile cotton wool, test tube rack, universal container. The composition of the media, their method of production is presented in the appendix.

2.2 SAMPLE COLLECTION

The fish and meat samples fish and meat samples from ten (10) restaurants within Awkametropolies. The samples were brought to the laboratory and analyses within 6hours of collection.

2.3 ANALYSIS OF SAMPLE

Each sample was serially diluted using sterile distilled water as diluents (Aarestrup *et al.*, 2021). 9ml of distilled water was measured out into test tubes, using separate sterile pipettes, 1g of sample was measured out into the first test tube properly mixed. using a different sterile pipette, 1ml from the first test tube was pipette into the second test tube already containing 9ml of distilled water, this continued following the same procedure till the last dilution (ie the last test tube).using the pour plate method 1ml each of each sample unit from the test tubes was pipetted into the sterile Petri dishes (using separate sterile pipettes per sample) with their duplicates, then into each Petri dish the prepared MacConkey agar was poured aseptically and mixed by movement of the plate while flat on the bench. This was also carried out on Nutrient agar media was used. The plates were incubated at 37°C for the 24hr.

After incubation the representative colonies on the plates were subcultured on fresh nutrients agar to obtain pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for biochemical identification.

MICROBIAL LOAD ASSAY

Total aerobic Counts (TAC), Total fungal Counts (TFC), Total Staphylococcus count (TSC), Total feacalcoliform count (TFC), Total feacalstrptococci count (TSC) and Total Coliform Counts (TCC) were analysed using the pour plate method measured in Colony Forming Unit (cfu/ml) as described by Adegoke (2004). The pour plate method was done in triplicates for each sample of a serial dilution and the average microbial growth was found and recorded thus given as

$$n = \frac{a+b+c}{3} \quad \text{—————}$$

Also, the colony forming unit is given as :

$$C = \frac{n}{Vd}$$

where, n = number of colony

V= volume of diluents

d = dilution factor (equivalent to 1 ml)

Therefore, the ratio of 1 ml:10 ml of aliquote (sample solution) = 0.1 ml.

3.4 Identification of Isolates

The bacteria were identified on the basis of their morphological, biochemical and fermentation tests as mentioned by Cheesbrough (2006).

Bacteria Identification

Gram Staining

The method used was that described by carpenter (1977) and Thomas (1973). Smears of the isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with a gentle running tap water. The slides were flooded with dilute Gram's iodine solution. This was washed off with water and the smears were decolorized with 95% alcohol till the blue colour no more dripped out (about 30 seconds).The smears were then counter stained with saffranin solution for about 10 seconds. Finally, the slides were washed with tap water, air dried and observed under oil immersion objectives.

Motility Test

This test was used to determine which of the isolates were motile. Motility test is usually used to differentiate motile organisms from non-motile ones. For this test, the hanging drop technique was employed and the technique was carried out as described by Kirk *et al.*, (1975). A little Vaseline jelly was rubbed around the cavity of a hanging drop slide. A drop of peptone water containing the pure culture was placed on a cover slip. The hanging drops slide was then placed over the drop of peptone water in such a

way that the center of the depression lies over the drop. The slide was quickly inverted and viewed under the microscope, using oil immersion objective.

BIOCHEMICAL TESTS

Urease Test

This test was used to demonstrate the ability of the isolates to produce the enzyme urease which splits urea forming ammonia. The test is usually used to differentiate organisms like proteus from other non urease positive organisms, (Baker and Breach 1974). The method used was that described by speck (1971). A loop full of the isolates was used to inoculate a tube of urea-agar. The tubes were incubated at 37°C. a change in colour from yellow to red confirmed the presence of urease.

Catalase Test

This test was used to demonstrate which of the isolates could produce the enzyme catalase that release oxygen from hydrogen peroxide. This test is usually used as an aid to differentiate *Staphylococci* from *Streptococci* and to differentiate other catalase positive organism from catalase negative (Barker 1976). The method employed here was that described by Speck (1976).

A loopful of the pure colony was transferred into a plane, clean glass slide. The sample was then mixed with a drop of 3% v/v hydrogen peroxide. The reaction was observed immediately Gas production indicated by the production of gas bubbles confirmed the presence of catalase.

Methyl Red Test

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usually used as an aid in the identification and differentiation of the *Enterobacteriaceae*(Baker 1976). This test was carried out as described by Kirk *et al* (1975). Tubes of buffered glucose-peptone broth were lightly inoculated with the isolates. The tubes were

incubated at 37°C for not less than 48 hours. About 5 drops of the methyl red reagent was added into 5 ml of the culture. The production of a bright red colour immediately on the addition of the reagent showed a positive test. Methyl red test indicator consists of 0.1g methyl Red, 300ml of 95% ethyl alcohol.

Voges -Proskeur Test (V.P. test).

This test was used to detect which of the isolates were able to produce a neutral end point acetyl methyl carbinol (acetoin) from glucose fermentation or its reductive product butylene glycerol. The test is usually used to differentiate between Gram negative organisms especially members of the *Enterobacteriaceae*, (Baker, 1976). The test was carried out as described by Kirk *et al* (1975). Tubes of buffered glucose peptone broths were lightly inoculated with a young culture of the isolates. The tubes were incubated at 37°C for not less than 48 hours. Burrits reagent was used for the test. 0.6% v/v of solution A and 0.2ml of solution B were added into 1ml of the culture in turns. The mixtures were shook well after each addition.

Positive reaction was indicated by a pink colour that appears immediately or within 5 minutes at the topmost part of the tube.

Indole Test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test is usually used as an aid in the differentiation of Gram negative, *Bacilli* especially those of the *Enterobacteriaceae* (Baker 1976). The test was carried out as described by Kirk *et al.*, (1975). Tubes of peptone water were inoculated with young culture of the isolates. The tubes were incubated at 37°C for 48 hrs. About 4 drops of Kovac reagent were added into 1ml of each of the culture tubes. Positive test was indicated by a red colour that occurs immediately at upper part of the test tube.

Citrate Utilization Test

This test was used to identify which of the isolates can utilize citrate as the sole source of carbon for metabolism. The test is usually used as an aid in the differentiation of organisms in the *Enterobacteriaceae* and most other genera. (Baker 1976). The medium used for this test was the Simon's citrate agar. Slant tubes of Simon's citrate agar were inoculated with young culture of the isolates. The inoculation was done by stabbing the medium on the tubes using sterile straight inoculating wire containing the culture. The tubes were then incubated at 37°C for about 24 hours. Change in colour from green to blue after about 24 hours of incubation indicated positive result.

Antimicrobial screening tests

The susceptibility of the isolates to selected antibiotics agent was determined by the disc diffusion method (Bauer *et al*, 1966) using antibiotic impregnated paper disc(s). The following Gram- negative antibiotics disc were used: Ciprofloxacin (CIP) (5µg), Tetracycline (TET) (50µg), Norfloxacin (NOR) (10µg), Amoxicillin (AMX) (30µg), Ofloxacin (OFL) (5µg), Chloramphenicol (CHL) (10µg), Cefuroxime (CEF) (30µg), Ampicillin (AMP) (30µg), Gentamycin (GEN) (10 µg). The gram- positive antibiotic disc(s) include: Ciprofloxacin (CPX) (10µg), Norfloxacin (NOR) (10µg), Gentamicin (GEN) (10µg), Amoxicillin (AMX) (30µg), Streptomycin (STX) (30µg), Rifampicin (RIF) (20µg), Erythromycin (ERY) (30µg), Chloramphenicol (CHL) (30µg), Ampiclox (AMP) (20µg) and Levofloxacin (LEV) (20µg).

Standardization of the inoculum

The inoculum will be prepared by inoculating colonies of fresh test cultures into sterile distilled water. The turbidity will be compared to 0.5 McFarland standard prepared according to method of Cheesbrough, (2004).

Screening test

The prepared Mueller-Hinton agar will be poured into plates and allowed to solidify. By means of a 5mm cork borer, five holes will be bored and well separated from one another in the agar. Then a 0.2ml aliquot of the antibiotics was deposited into a 5-mm well made with a sterile cork-borer into Mueller-Hinton and the plates will be incubated aerobically at 37⁰C for 24 hours. The plates will then be examined for any zone of inhibition. The diameter of the zones of growth inhibition will be measured with the use of a ruler from underside of the covered plates. The diameter will be taken.

3. RESULT

The result of the proximate composition of all the fish and meat samples used in the study are presented in table 1.

Table 1: Nutritional composition of fish and meat samples

SAMPLE	MOISTURE	ASH	FIBER	PROTEIN	FATS	CARBOHYDRATE
BEEF	16.00± 0.30	4.30± 0.31	13.70± 0.10	21.80± 0.35	3.00± 0.33	41.20± 0.11

CHICKEN	13.70± 0.11	5.10± 0.30	9.80± 0.50	25.00± 0.31	12.70± 0.17	33.70± 0.33
GOAT MEAT	10.50± 0.00	4.75± 0.20	18.00± 0.30	20.80± 0.33	2.24± 0.00	43.71± 0.30
MACKEREL	45.80± 0.35	3.50± 0.11	9.70± 0.36	14.00± 0.30	10.17± 0.37	16.83± 0.35
CATFISH	40.00± 0.20	4.41± 0.30	7.80± 0.31	13.70± 0.33	5.11± 0.33	28.98± 0.33
MANGALA	9.80± 0.30	6.30± 0.31	11.00± 0.30	12.10± 0.00	4.68± 0.00	56.12± 0.20

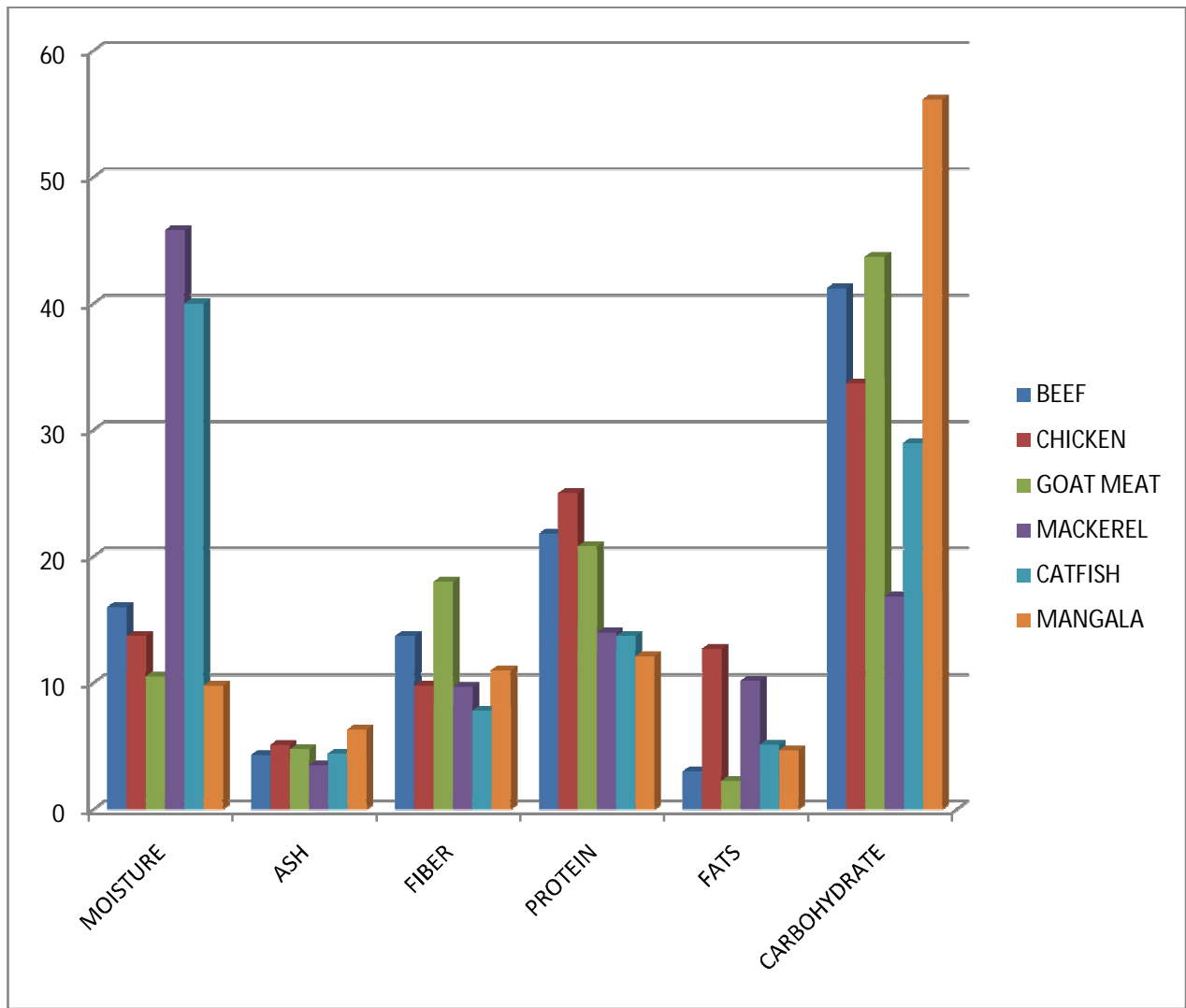


Fig. 1. PROXIMATE COMPOSITION OF DIFFERENT MEAT AND FISH SAMPLES

UNDA

The result of the microbial compositions of all the meat and fish samples are shown in table 2. The study reveals that among the samples collected, samples from mackerel and beef possessed the highest total viable bacteria count while the least total viable count were observed from goat meat and mangala samples.

Table 2: Microbial Analysis of fish and meat samples

SAMPLE	Total viable count(x 10⁴cfu/g)	Total coliform count (x 10⁴cfu/g)	Total fungi count (x 10⁴cfu/g)
BEEF	3.6x10 ²	1.4x10 ²	9.0x10 ²
CHICKEN	2.0x10 ²	1.7 x10 ²	7.7x10 ²
GOAT MEAT	1.8x10 ²	1.0x10 ²	8.8x10 ²
MACKEREL	3.6x10 ²	1.4x10 ²	9.0x10 ²
CATFISH	2.0x10 ²	1.7 x10 ²	7.7x10 ²
MANGALA	1.8x10 ²	1.0x10 ²	8.8x10 ²

*Values are mean scores± Standard deviation of triplicate

*Data in the same column bearing different superscript differ significantly (p < 0.05).

Table 3. Morphological characteristics

Isolate	Colony Characterization			Cell characterization			Organism
	form	elevation	margin	Cell arrangement	Gram reaction		
A	Greenish	Irregular	Flat	Undulate	Small rods	Positive	<i>Bacillus sp,</i>
B	Cream	Circular	Flat	Smooth	Short rods	Negative	<i>Pseudomonas spp</i>
C	pink	Irregular	flat	Smooth	Short rods	Negative	<i>Esherichia Coli</i>
D	Cream	Irregular	Flat	Entire	Coccus	Positive	<i>Staphylococcus sp</i>
E	Greenish	Circular	Flat	Smooth	Large rods	Negative	<i>salmonella sp</i>

Biochemical characteristics

ISOLATE	MOTILITY	COAGULASE	CATALASE	PROBABLE ORGANISM
A	-VE	-VE	+VE	<i>Bacillus sp</i>
B	-VE	-VE	-VE	<i>E. coli</i>
C	-VE	+VE	-VE	<i>Staphylococcus spp</i>
D	+VE	-VE	-VE	<i>Pseudomonas aeruginosa</i>
E	+VE	-VE	-VE	<i>Salmonella spp</i>

The results of the antibiotic resistance profiling of isolated bacteria revealed that most of the isolated bacteria demonstrated resistance to ceftazidime, cefuroxime, gentamicin, ciprofloxacin, amoxicillin clavulanate, ampicillin and nitrofurantoin (Table 4). Judging from their resistance to three or more classes

of antibiotics, multidrug resistance was observed among *E. coli*, isolated across all the meat and fish sampled,. The resistant phenotypes of these organisms reveal that the most prevalent multidrug phenotype is found among *E. coli* with a 37.1% occurrence in smoked fish and 21.7% in meat samples .

Table 4: Antibiotic susceptibility pattern of *E coli*

Sample	CEP	OFX	NA	PEF	CN	AU	CPX	STX	S	PN
<i>Esherichia Coli</i>	16	5	-	-	-	-	7	1	11	-

KEY

CEP = Ceporex

OFX = Tarivid

NA = Nalidixic acid

PEF = Reflacine

CN = Gentamycin

AU = Augmentin

CPX = Ciproflex

SXT = Septrin

S = Streptomycin

PN = Ampicillin

4. DISCUSSION

Meat and fish are increasingly becoming a more and more popular delicacy in Nigeria. Therefore, isolation of bacteria from meat and fish should raise public health concern. Values of total bacteria count (4.8×10^5 to 6.5×10^5 cfu/g) and total coliform count (4.0×10^5 to 7.6×10^5 cfu/g) obtained from the fish samples from this study have been identified to be higher than standard microbiological load ($\geq 10^4$) of ready-to-eat food.

The beef meat samples had the highest range of bacteria load in cfu/g. Its total viable count ranged from 3.4×10^5 to 7.7×10^5 cfu/g while coliform counts ranged from 2.1×10^5 to 6.2×10^5 cfu/g (Table 2). This agrees with the findings of Nester, *et al.*, 2017 who also reported a high range in coliform count (1.5×10^4 - 6.2×10^4 cfu/g) in a study conducted in Rivers State, Nigeria. The finding reports the presence of *S. aureus*, *E. coli*, *Pseudomonas* spp, *Enterobacter* spp, *Klebsiella* spp, *Shigella* spp, *Bacillus* spp, *Salmonella* spp and *Proteus* spp in the fish and smoked fishes sampled (Table 2). This is consistent with the report of Mensah, *et al.*, 2019), who isolated *Staphylococcus* spp, *E. coli*, *Pseudomonas* spp from ready-to-eat "suya" meat sold in Owo, Ondo State, Nigeria and that of Ingham *et al.*, 2017). who also stated that microbiological analysis of "suya" meat samples in Enugu State, Nigeria showed a contamination of meat samples with various bacterial species including *S. aureus* and some enteric bacteria. James, 2015 also stated that the presence of *Salmonella* spp as contaminant could be attributed to inadequate heating of meat product during its preparation.

The high bacteria count observed among the fish and smoked fish samples from our study may also be attributed to the poor hygienic condition under which they are produced (as observed during the time of sample collection) i.e. open space where they were sold and stored. It is also imperative to note that the

observed high microbial counts may be due to the original bio-load of slaughtered sick animals, the transportation by rickety vehicle and use of contaminated equipment (Chessbrough, 2021).

These reported values, therefore, place the meat and fish samples examined in this work in the "acceptable but not satisfactory" range (10^5 - 10^7 for meat and 10^6 - 10^7 for smoked fish) under the Public Health Laboratory Service guidelines for the bacteriological quality of ready-to-eat foods samples at the point of sale (De Boer, *et al.*, 2021).

Whereas the presence of some members of the family of *Enterobacteriaceae* may be due to contamination from long exposure of the "suya" meat to air, the organisms isolated in this study are the organisms usually suspected to be in connection with meat contamination and spoilage (Ochei, & Kolhatkar, 2021). When these findings were compared to that from smoked fish samples, an almost similar result was observed. *E. coli* was the highest isolated organism from meat and fish samples,

Most of the *E. coli*, isolated demonstrated multidrug resistance. The prevalence of multidrug resistant *E. coli* (37.1% prevalence in fish and 21.7% in meat samples). The prevalence reported from this study, agrees with a similar observation reported by OwegheUreghe and Afe (2021) In an assessment of frozen and dried fish, for multidrug resistant bacteria, conducted in Obollor-afor and Nsukka, Enugu State, Nigeria, reported that strains of *E. coli* demonstrated more than 36% resistance to 7 antibacterial agents tested.

The public health implication of this is of great concern. This is even more glaring in the light of the transferability of these resistance traits among both pathogenic and potentially pathogenic bacteria. In a study conducted by Amoah, (2022). on meat sold in Ado and Akure, Southwest Nigeria, bacteria, as well as molds, yeast and fungi have been reported.

5. Conclusion

Despite the wide spread popularity of meat and smoked fish delicacies in Nigeria, MDR bacteria with the ability to endanger human lives have been reported in high numbers from the samples studied. Hence, a great public health concern which calls for antibiotic resistant bacteria surveillance among clinicians and public health practitioners in this vicinity. While proper hygiene of the vendors, the processing environment and process-line of meat is highly recommended to be clean, the practice of preparation and distribution of "suya" and smoked fish in open places where there is no emphasis on hygiene standards should be discouraged. Proper sensitization of the local vendors on proper animal husbanding, hygienic slaughter and storage of meat, sanitation of utensils and equipment would help decrease the rate of infections from these foods. While a reduction in exposure to antibiotics is greatly advised, development of new and more effective antibiotics is recommended.

6. Recommendation

1. Another study should be implemented to measure resistance rate of pathogens to commonly used antibiotics; this study must include all positive confirmed cases for *Escherichia coli*.
2. A widespread screening program for *E coli* should be implemented to know the exact prevalence of *E coli*.
3. Policies must be put on antibiotic prescribing as the resistant rate in all antibiotics in this study is over 20%.
4. More health promotion programs are needed to be implemented at schools, to increase the awareness of students and their teachers and improve their healthy behaviors.

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