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Antibiogram of *Escherichia coli* Isolated from Fish (Salmon Fish)/Meat (Beef)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Although meat and fish are prized delicacy in Nigeria, they can also spread germs that are multidrug resistant (MDR). Moreover, research on these MDR bacteria from Awkametropolies is scarce. Thus, the bacteriological characteristics and antibiogram profiles of E coli isolated from meat and fish were studied in this investigation. Twenty different meat and fish samples from the research sites were gathered and microbiologically examined. Standard microbiological procedures were used to conduct a total viable count, a coliform count, a bacterial characterisation, and an identification of the bacteria. The results showed that "meat" samples had both the highest coliform

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count (2.1105 to 6.2105 cfu/g) and total viable bacteria count (3.4105 to 7.7105 cfu/g). In all, 78 and 85 microorganisms were found in samples of smoked fish and beef, respectively. The disc diffusion technique was used to determine the antibiotic sensitivity pattern. Gentamicin, Amoxycillin/clavulanate, Streptomycin, Cloxacillin, Erythromycin, Chloramphenicol, Cotrimoxazole, Tetracycline, Penicillin, Ciprofloxacin, Ofloxacin, Levofloxacin, Ceftriaxone, Amoxycillin, and vancomycin were the antibiotics that E. coli was most sensitive to, with sensitivity rates of 92. The findings of the current study demonstrate that fluoroquinolones are efficient in treating E. coli infections, including those caused by methicillin-resistant strains, in this setting.

Keywords: Antibiotics; bacteria; fish; meat.

1. INTRODUCTION

Meat is the animal meat consumed as food, and it may be found on sheep, cattle, goats, and pigs [1]. Fish is one of the most significant sources of animal protein and other essential elements that are consumed by people of all races and socioeconomic backgrounds (Collignon et al., 2015). Fish flesh is preferred to other white or red meats because it has lower lipid levels and more water than beef or chicken (Nestel, 2020). For the majority of people in many areas of the globe, meat and fish are substantial sources of protein and a significant source of vitamins; as a result, they are crucial for the development, maintenance, and repair of body cells, which are required for our daily activities [2].

Because meat and fish provide all the necessary amino acids the body needs, the protein profile of these foods has been praised as being great [3-7]. Plant alternatives cannot provide the protein and vitamins found in meat, particularly vitamin A and vitamin B12 [8]. Although the meat and fish markets contribute significantly to people's health, they are not without health risks [9].

Typhoid fever, cholera, and other food-related illnesses are quite common and are often reported in hospitals and clinics across the world [10-16]. Some have previously raised concern about the connection between meat and animal products and food poisoning, although data show that more than 74% of food poisoning incidents globally are caused by meat dishes [8]. Meat has a significant risk of microbial contamination since it contains a lot of nutrients that are ideal for bacterial development [17-21].

The microbial proliferation can cause meat to decay and food-borne diseases in people, which would cost money [22].

Widespread bacterial food poisoning is influenced by an unclean environment and unsanitary food handling [23]. *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, Salmonella, *Escherichia coli*, and *Staphylococcus aureus* are some of the major bacterial pathogens that may be discovered in meat [24,25]. Contamination may result through unsanitary handling, processing, and slaughter procedures or from naturally occurring microorganisms in normal animal tissues, the surroundings, and the air [26].

A significant difficulty in clinical therapy has been the widespread occurrence of antibiotic resistance among pathogenic bacteria (Li et al., 2008). Some bacteria showed resistance by the change or alteration of the target site and the metabolic pathway [27].

According to Frost et al. (2005), mobile genetic elements such plasmids, transposons, interferon, bacteriophages, and insertion elements might make it easier for genes encoding resistance to be spread across different bacterial strains. Yet, the coexistence of antibiotic-resistant microbes in restaurant waste that is released into the environment would undoubtedly cause the antibiotic resistance gene to spread quickly among other creatures in the ecosystem [28].

In order to detect and propose a solution to the establishment of antimicrobial resistance mechanisms, effective surveillance is needed to address the apparent increase in the occurrence of antibiotic resistance among bacteria from many sources, including clinical, foods, and water.

1.1 Statement of the Problem

As other bacteria with therapeutic relevance for humans can acquire the antibiotic resistance determinants, food contamination by antibiotic-resistant bacteria poses a serious risk to public

health. Recent decades have seen a rise in the incidence of antimicrobial resistance among food-borne infections, probably as a result of selection pressure brought on by the use of antibiotics in animals raised for food [29].

Especially in underdeveloped nations like Nigeria, outbreaks of diseases that were often brought on by inadequate hygiene and eating tainted food have been on the rise. Moreover, food-borne infections like e coli have higher rates of antibiotic resistance than other pathogens. The presence of such bacteria in food items may contribute to the spread of antimicrobial resistance among food-borne pathogens, even though it is difficult to demonstrate a direct connection between drug-resistant bacteria contaminating food items and the rise in clinical cases of resistant infections [30].

Hence, sufficient data should be obtained to create an efficient plan to lower the burden of resistance in the population and the emergence of food-borne diseases.

1.2 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 Awkametropolies.

1.3 Specific Objectives

1. To identify and isolate the bacterial species that cause contamination of meat and seafood.
2. To estimate the amount of E coli in the isolated bacterium.
3. To ascertain the isolated E coli's pattern of antibiotic susceptibility

1.4 Scope of Study

The goal is to gather fish and meat samples from Awkametropolies and identify the pattern of isolated bacteria' antibiotic susceptibility.

1.5 Significance of Study

This work is important because it will contribute to a decrease in contamination and disease brought on by food pathogen contamination.

The goal was to provide the foundation for advancing traditional knowledge and practises using contemporary drug development methods.

2. MATERIALS AND METHODS

2.1 Materials

Nutrient agar, MacConkey agar, Peptone water, Petri dish, autoclave, inoculating wire loop, forceps, Bunsen burner, conical flask, antibiotic discs, weighing scale, test tube rack, plastic pipette, wire loop, microscope, incubator, beakers, glass slide, sterile cotton wool, test tube rack, and universal container are among the media used in this work.

2.2 Sample Collection

The ten (10) eateries in Awka metropolies that provided the fish and meat samples. Within six hours of collection, the samples were transported to the laboratory for analysis.

2.3 Analysis of Sample

Using sterile distilled water as diluents, each sample was serially diluted [2]. Using different sterile pipettes, 9ml of distilled water was measured out into test tubes, and 1g of the sample was metered out into the first test tube after being thoroughly mixed. 1 millilitre from the first test tube was pipetted into the second test tube, which already had 9 millilitres of distilled water in it, using a separate sterile pipette. This process was repeated until the last dilution (ie the last test tube). Using the pour plate method, 1ml of each sample unit from the test tubes was pipetted into the sterile Petri dishes with their duplicates using separate sterile pipettes for each sample. Then, while the plate was flat on the bench, the prepared MacConkey agar was aseptically poured into each Petri dish and stirred. Furthermore, nutrient agar medium was employed for this. The plates were incubated for 24 hours at 37°C.

To obtain pure cultures of the isolates, the representative colonies on the plates were subcultured on new nutrients agar after incubation. After that, the pure cultures were put onto nutrient agar slants for biochemical analysis.

2.4 Microbial Load Assay

The pour plate method, which Adegoke described, was used to analyse total aerobic counts (TAC), total fungal counts (TFC), total staphylococcus count (TSC), total feacal coliform

count (TFC), total feacal streptococci count (TSC), and total coliform counts (TCC) (2004). For each sample of a serial dilution, the pour plate technique was performed in triplicates, and the average microbial growth was discovered and recorded as;

$$n = a+b+c/3$$

Also, the colony forming unit is given as:

$$C = n/Vd$$

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 aliqoute (sample solution) = 0.1 ml.

2.5 Identification of Isolates

The bacteria were identified on the basis of their morphological, biochemical and fermentation tests as mentioned by Chees Brough [31].

2.6 Bacteria Identification

2.6.1 Gram staining

The approach employed was that which Thomas and Carpenter (1977) outlined (1973). On clean, grease-free slides, smears of the isolates were created and heated fixed. The smears were dyed with crystal violet for a minute. This was removed with softly flowing tap water. The diluted Gram's iodine solution was poured all over the slides. The blue hue was removed by washing it off with water and then decolorizing the streaks with 95% alcohol (about 30 seconds). The smears were then counter stained for about 10 seconds with saffranin solution. The slides were then air dried, cleaned with tap water, and examined with oil immersion objectives.

2.6.2 Motility test

Which isolates were motile was determined using this assay. The motility test is often used to distinguish between motile and non-motile organisms. The hanging drop technique was used for this test, and it was carried out in accordance with Kirk et alinstructions. .'s (1975). The chamber of a hanging drop slide was given a little coating of Vaseline jelly. On a cover slip, a drop of peptone water containing only the pure culture was inserted. The drop of peptone water was then covered by the hanging drops slide, which was positioned so that the depression's centre was on top of the drop. Using an oil immersion objective, the slide was swiftly inverted and examined under the microscope.

2.7 Biochemical Tests

2.7.1 Urease test

The isolates' capacity to generate the enzyme urease, which breaks urea into ammonia, was demonstrated using this assay. The test is often used to distinguish between urease-positive species like proteus and non-urease positive organisms (Baker and Breach 1974). The approach was that mentioned by speck (1971). A tube of urea-agar was inoculated using a loop filled with the isolates. The presence of urease was determined when the tubes were incubated at 37°C and their colour changed from yellow to red.

2.7.2 Analyze catalase

Typically, this test is used to help distinguish Staphylococci from Streptococci and other catalase-positive organisms from catalase-negative organisms (Barker 1976). The technique used in this case was that which Speck explained (1976).

The pure colony was transferred in loops into a flat, spotless glass slide. A drop of hydrogen peroxide with a concentration of 3% was then added to the sample. The response was noticed right away. The existence of catalase was confirmed by gas generation, as shown by the creation of gas bubbles.

2.7.3 Methyl red test

With the use of this test, it was determined which of the isolates was capable of fermenting glucose in a stable acid product. Typically, the test is utilised to help identify and distinguish between distinct Enterobacteriaceae (Baker 1976). According to Kirk et al instructions, .'s this test was conducted (1975). Lightly inoculating the isolates were tubes of buffered glucose-peptone broth. For a minimum of 48 hours, the tubes were incubated at 37°C. The culture was diluted into 5 ml with about 5 drops of the methyl red reagent. Methyl red test indication is made up of 0.lg of methyl red and 300ml of 95% ethyl

alcohol. When the reagent was added, a brilliant red colour was produced right away, indicating a successful test.

2.7.4 Voges -proskeur test (V.P. test)

This test was used to determine which isolates were capable of producing either butylene glycerol, a reductive byproduct of the fermentation of glucose, or acetyl methyl carbinol (acetoin), a neutral end point. Typically, the test is used to distinguish between Gram-negative organisms, particularly Enterobacteriaceae members (Baker, 1976). The test was conducted in accordance with Kirk et al (1975). A young culture of the isolates was softly injected into tubes of buffered glucose peptone broths. The tubes were incubated for at least 48 hours at 37°C. The test was conducted using Burrits reagent. 0.2ml of solution B and 0.6% v/v of solution A were alternately applied to 1ml of the culture. After each addition, the mixtures were well shaken. A pink colour that develops at the top of the tube either right away or after five minutes is a sign of a positive reaction.

2.7.5 Indole test

This test was performed to identify which isolates were able to separate indole from the tryptophan in buffered peptone water. It is typically used to help differentiate between different types of Gram-negative bacteria, particularly those belonging to the Enterobacteriaceae family (Baker 1976). The test was conducted in accordance with Kirk et al (1975). Young cultures of the isolates were injected into tubes containing peptone water. 48 hours were spent incubating the tubes at 37 °C. In each of the 1ml culture tubes, 4 drops of Kovac reagent were introduced. A red colour that appears right away at the top of the test tube indicates a positive result.

2.7.6 Citrate utilization test

Which isolates may use citrate as their exclusive source of carbon for metabolism was determined by this assay. For differentiating organisms in the Enterobacteriaceae and the majority of other genera, the test is typically utilised as a tool. (Baker1976). The Simon's citrate agar was the testing media. Young cultures of the isolates were added to slant tubes of Simon's citrate agar for inoculation. By employing sterile straight inoculating wire that contained the culture, the inoculation was carried out by stabbing the medium onto the test tubes. The tubes were subsequently incubated for around 24 hours at 37°C. Positive results were shown by the colour changing from green to blue after around 24 hours of incubation.

2.7.7 Antimicrobial screening tests

By utilising paper discs impregnated with antibiotics, the disc diffusion technique (Bauer et al., 1966) was used to test the isolates' sensitivity to various antibiotic agents (s). The Gram-negative antibiotics Ciprofloxacin (CIP), Tetracycline (TET), Norfloxacin (NOR), Amoxycillin (AMX), Ofloxacin (OFL), Chloramphenicol (CHL), Cefuroxime (CEF), Ampicillin (AMP), and Gentamycin (GEN) were employed in the study. The antibiotic disc(s) for gram-positive bacteria are Ciprofloxacin (CPX) (10g), Norfloxacin (NOR) (10g), Gentamicin (GEN) (10g), and Amoxil (AMX) 2015; 1(3): 34–40 36 (20 g), Streptomycin (30 g), Rifampicin (20 g), Erythromycin (30 g), Chloramphenicol (30 g), Ampiclox (20 g), and Levofloxacin (20 g).

2.7.8 Standardization of the inoculums

Fresh test culture colonies will be injected into sterile distilled water to create the inoculum. The turbidity will be compared to a 0.5 McFarland standard made using Cheesbrough's technique (2004).

2.7.9 Screening test

Mueller-Hinton agar will be produced, placed into plates, and allowed to set up. Five holes will be drilled into the agar with a 5mm cork borer, each hole being properly spaced apart from the others. Following that, a 0.2 ml aliquot of the antibiotics was placed into a Mueller-Hinton plate's 5-mm well created using a sterile cork-borer. The plates were then incubated aerobically at 370°C for 24 hours. After that, the plates will be checked for any zones of inhibition. Using a ruler and the bottom of the covered plates, the diameter of the growth inhibition zones will be measured. We will measure the diameter.

3. RESULTS

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

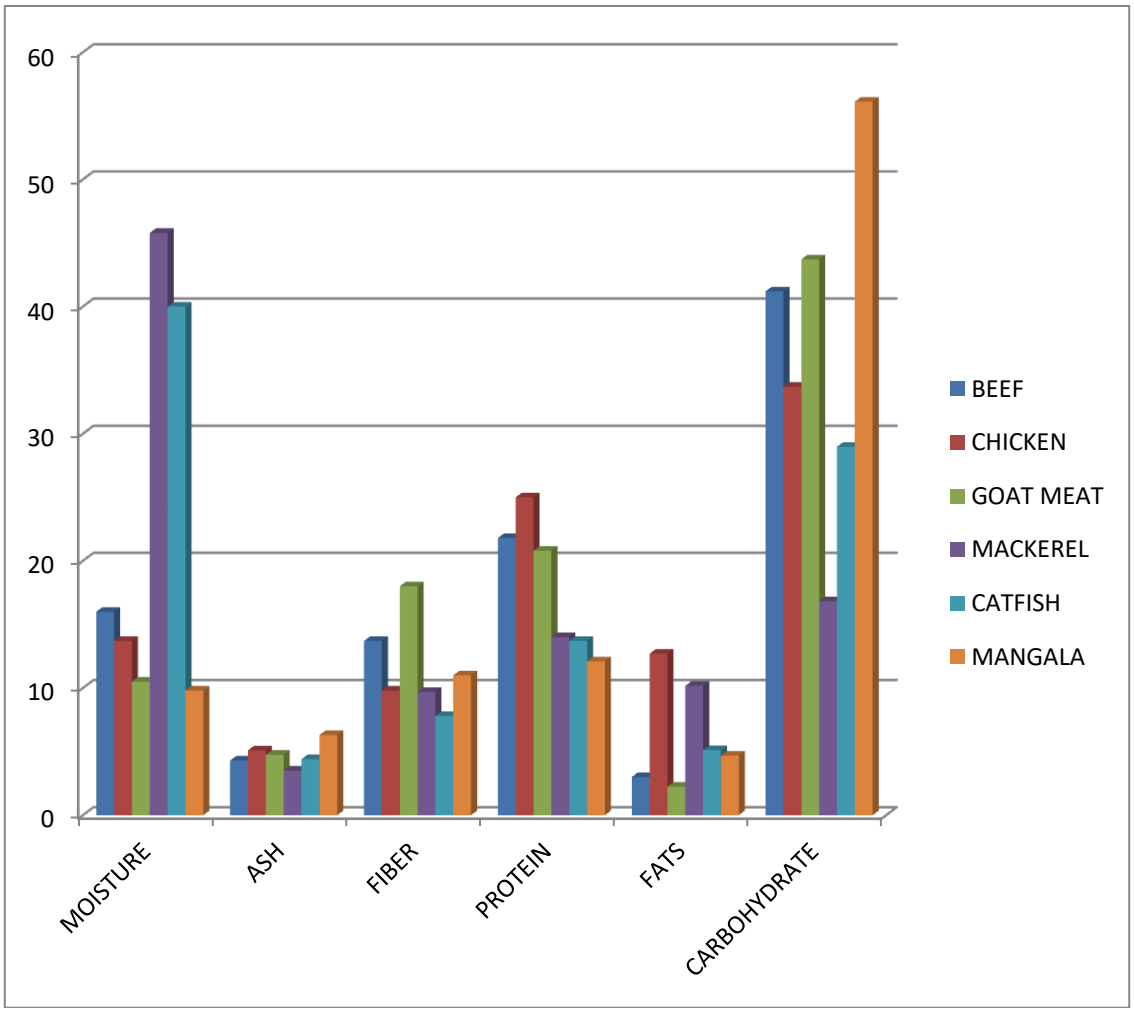

Fig. 1. Proximate composition of different meat and fish samples

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.

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 2. Microbial Analysis of fish and meat samples

Sample	Total viable count (x 10^4cfu/g)	Total coliform count (x 10^4cfu/g)	Total fungi count (x 10^4cfu/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	$1.8x10^2$	$1.0x10^2$	$8.8x10^2$

Table 3. Morphological characteristics

Isolate	Colony characterization			Cell characterization			
Colour	**Form**	**Elevation**	**Margin**	**Cell arrangement**		**Gram reaction**	**Organism**
A	Grennish	Irregular	Flat	Undulate	Small rods	Positive	*Bacillus sp*,
B	Cream	Circular	Flat	Smooth	Short rods	Negative	*Pseudomonas spp*
C	pink	Irregular	flat	Smooth	Short rods	Negative	*Esherichia Coli*
D	Cream	Irregular	Flat	Entire	Coccus	Positive	*Staphyloccus sp*
E	Greenish	Circular	Flat	Smooth	Large rods	Negative	*Salmonella sp*

Biochemical characteristics

Isolate	Motility	Coagulase	Catalase	Probable organism
A	-VE	-VE	+VE	*Bacillus sp*
B	-VE	-VE	-VE	*E. coli*
C	-VE	+VE	-VE	*Staphylocuccusspp*
D	+VE	-VE	-VE	*Pseudomonas aeruginosa*
E	+VE	-VE	-VE	*Salmonella spp*

Table 4. Antibiotic susceptibility pattern of *E coli*

Sample	CEP	OFX	NA	PEF	CN	AU	CPX	STX	S	PN
Esherichia Coli	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

In Nigeria, fish and meat are becoming increasingly popular delicacies. Thus, the discovery of germs in meat and fish should cause worry for the general public's health. The results of this study's fish samples showed total bacterial counts that ranged from 4.8 x 105 to 6.5 x 105 cfu/g and total coliform counts that ranged from 4.0 x 105 to 7.6 x 105 cfu/g that were greater than the typical microbiological load (104) of ready-to-eat food. The range of bacteria load in cfu/g was largest in the samples of beef meat. While the coliform counts ranged from 2.1 105 to 6.2 105 cfu/g, the total viable count ranged from 3.4 105 to 7.7 105 cfu/g (Table 2). This is consistent with the results of Nester et al. [32], who similarly noted a wide range of coliform counts (1.5 104 - 6.2 104 cfu/g) in a research carried out in Rivers State, Nigeria. The finding indicates that the analysed fish and smoked fish included S. aureus, E. coli, Pseudomonas spp., Enterobacter spp., Klebsiella spp., Shigella spp., Bacillus spp., Salmonella spp., and Proteus spp (Table 2). This is in line with the findings of Mensah et al. [33] and Ingham et al. [34], who recovered Staphylococcus spp., E. coli, and Pseudomonas spp. from ready-to-eat "suya" meat marketed in Owo, Ondo State, Nigeria. He added that a microbiological investigation of "suya" meat samples in Enugu State, Nigeria revealed bacterial species, including S. aureus, contamination of the meat samples and some enteric bacteria. James, 2015 further claimed that improper heating of the beef product during preparation may have contributed to the presence of Salmonella spp. as a contaminant [35-40].

The high bacteria count found in the samples of fish and smoked fish from our investigation may possibly be related to the unsanitary conditions in which they were created, such as the open area where they were sold and stored [41-46]. It is crucial to remember that the elevated microbial counts may have originated from the bio-load of diseased animals that were slaughtered, the use of contaminated tools, and transportation in shaky vehicles [31].

As a result, the meat and fish samples analysed in this paper fall into the category of "acceptable but not adequate." according to the Public Health Laboratory Service's recommendations for the bacteriological quality of ready-to-eat food samples at the point of sale, range (105-107 for meat and 106-107 for smoked fish) [47].

The organisms discovered in this study are the ones that are often linked to meat contamination and spoiling, although the presence of certain members of the Enterobacteriaceae family may be related to contamination from protracted exposure of the "suya" meat to air [48]. These results were virtually identical to those from samples of smoked salmon when they were compared. The majority of the E. coli that were identified showed multidrug resistance. E. coli was the most frequently isolated bacterium from meat and fish samples. The frequency of multidrug-resistant E. coli (21.7% in meat samples and 37.1% in fish). The frequency shown in this study is consistent with an observation made by OweghеUreghe and Afe in the year [49]. At Obollor-afor and Nsukka, Enugu State, Nigeria, researchers evaluated frozen and dried fish for the presence of multidrug-resistant bacteria. They found that certain *E. coli* strains had more than 36% resistance to the seven antibacterial drugs they tested.

This has serious implications for public health. Given the transferability of these resistance characteristics among pathogenic and potentially harmful microorganisms, this is even more obvious. Amoah's examination of meat marketed in Ado and Akure, Southwest Nigeria, in [50] found bacteria as well as moulds, yeast, and fungi have been reported.

5. CONCLUSION

MDR bacteria with the potential to damage human life have been discovered in significant amounts from the samples examined, despite the widespread appeal of meat and smoked fish delicacies in Nigeria. So, there is a serious public health issue that requires close monitoring of antibiotic-resistant microorganisms among local doctors and public health professionals. The practise of preparing and distributing "suya" and smoked fish in open areas without a focus on hygiene standards should be avoided, even if it is strongly recommended that the vendors, the processing environment, and the meat-processing line all be clean. The risk of illnesses from these items might be reduced with thorough sensitization of the local vendors on correct animal husbandry, hygienic slaughter and storage of meat, and cleaning of utensils and equipment. While it is highly advocated to reduce antibiotic exposure, it is also urged that new and more potent antibiotics be developed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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