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Phenotypic and molecular characterization of potential pathogens from raw fish, meat and milk samples sold and consumed in Calabar Metropolis, Cross River State, Nigeria

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Abstract
Objectives: This study isolated and characterized potential pathogens from raw fish, meat (goat and ram), and milk samples using cultural and molecular techniques, and their susceptibility to routine antibiotics.
Methods: This design was a cross-sectional study where raw fish, meat (goat and ram), and milk from various markets were characterised using cultural and molecular susceptibility to antibiotics used to manage infections they cause in clinical use. Furthermore, the isolates were subjected to pathogenicity tests using amylase and protease screening.
Results: Cultural technique identified a total of 42 isolates and these were: *Morganella* sp (n=6), *Providencia* sp (n=7), *Klebsiella* sp (n=10), *E. coli* (n=11) and *Salmonella* sp (n=8). All the isolates showed multi-drug resistance (MDR) to gentamicin, levofloxacin, clindamycin, ciprofloxacin, cefoxicin, piperacillin-azobactam, amikacin, and amoxicillin-clavulanic acid antibiotics. Following antimicrobial sensitivity, 9 isolates with multidrug resistance were selected for molecular characterisation and these were identified as *Escherichia coli, Klebsiella pneumoniae, Morganella morganii subsp. Morganii, Providencia stuartii, Klebsiella pneumoniae* and *Salmonella enterica subsp. Enterica.* All the isolates showed amylase and protease activity.
Conclusion: The MDR and pathogenicity potential of the isolates indicate their ability to elicit a potential foodborne infection in the study area and it is a cause for concern.

Keywords: Food; Pathogens; Resistance; Antibiotics; Calabar; Nigeria

Plain English Summary

The present study aimed to evaluate the presence of potential pathogens in raw fish, meat and milk samples. This was done using a combination of cultural and molecular techniques. The results revealed

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the presence of isolates that showed multiple resistance to various antibiotics. Furthermore, the isolates all showed the ability to be potential pathogens. Put together, the findings constitute a significant public health concern.

Introduction

Fish and meats are important sources of essential nutrients needed for proper growth, development, and maintenance of the human body (1). Meat can be obtained from various animals (domestic and wild), such as pork (pig), mutton (goat), goat (goat), poultry (chicken and other birds), beef (cow or cattle), sheep meat, and ram meat. Similarly, milk is also highly nutritious and a major and important source of proteins. It also contains important sources of nutrients that are needed for growth and development (2). The nutrient environment of both meat and milk provides a perfect milieu for the growth of many microorganisms. Milk, for example, has a near-neutral pH, a high amount of nutrients, and a high water activity that is ideal for microbial growth (2). Meat, especially unprocessed or raw meat, possesses the same physical and chemical properties that facilitate the colonisation and growth of many microorganisms (3).

The colonisation of milk is made possible in part because most nutrients in milk, especially the monomeric nutrients, are directly available to all microorganisms, while others are available following the metabolism of the major components into simpler components (3). Lactic acid bacteria (LAB) are the dominant bacterial species in raw milk samples of bovine, goat, sheep, and buffalo before pasteurization (2). The common LAB in milk includes *Lactococcus, Lactobacillus, Leuconostoc*, *Streptococcus,* and *Enterococcus*. Apart from the LAB, other microorganisms that can contaminate raw milk samples include *Pseudomonas* and *Acinetobacter sp* (4). In an earlier study, reported microbial contaminants included *Escherichia coli*, *Staphylococcus aureus*, *Shigella sp*., *Proteus sp.,* and *Salmonella sp.* in decreasing order of abundance (5). On the other hand, commonly associated microbial species with meat include those that belong to genera such as *Pseudomonas, Lactobacillus, Moraxella, Acinetobacter, Microbacteria, Brochotrix, Shigella,* and *Vibrio* (6). Other species include *Salmonella, Escherichia coli, Clostridium, Streptococcus faecalis, Flavobacterium, Bacillus, Leuconostoc, Proteus,* and *Micrococcus* (7).

Furthermore, several studies have shown the presence of multidrug-resistant (MDR) bacteria in meat and milk (8, 9, 10, 11). Bissong et al. (8) showed the presence of enterotoxigenic *S. aureus* in beef and milk samples in their study. In another study, their findings revealed the presence of potential spread of MDR *S. aureus* strains in dairy farms and abattoirs (9). They further inferred that these isolates could rapidly spread via food, posing serious health risks to consumers (9). Gizaw et al. (10) isolated MDR coagulase-negative *S. aureus* in milk, meat, equipment, and food handlers in their study. Ghabbour et al. (11) isolated *S. aureus* that were MDR and harboured various resistance genes. The presence of resistance amongst these pathogens poses a significant public health risk (12, 13). In addition to the risk posed by MDR microorganisms, they have been implicated in various instances of foodborne diseases across the globe (6, 14). Foodborne disease results from the consumption of food that is contaminated by pathogenic bacteria, viruses, and parasites (8). Foodborne disease severity can range from mild to severe, with the severe ones capable of causing death. The economic and public health implications of foodborne diseases are a significant public health issue that presents a significant burden to consumers and the health care systems (6, 14).

Despite these concerns, the microbial community in milk and meat sold and consumed in Calabar is still not fully understood, as earlier studies that examined the microbial diversity utilised only cultural techniques and none evaluated the antimicrobial sensitivity profiles of these isolates in their studies (15, 16, 17, 18, 19). In their studies to evaluate the microbiology of soymilk sold and Kunu drinks consumed in Calabar, cultural techniques were utilised to identify bacteria and fungi species in soymilk drinks (15, 16, 17). Similarly, cultural-based studies exist that have shown the presence of bacteria of public health importance in meat samples in the Calabar metropolis (18). These studies further revealed that the organisms exist in numbers that can cause foodborne diseases (6, 7, 14). Furthermore, using cultural techniques, *Listeria monocytogenes* was isolated from fresh and raw fish, chicken, and beef (19). None of these studies examined the antibiotic sensitivity profile of the various isolates. Furthermore, given the known limitations of cultural techniques (20), this study aimed to describe the microbial communities in raw fish, meat, and milk samples sold and consumed in the Calabar metropolis using cultural and molecular techniques in addition to microbial sensitivity and pathogenicity testing.

Materials and Methods

Study design

This study was based on a cross-sectional study design, a type of observational study (21). We purposively sampled several markets (Goldie, Marian, Watt, Akim, Edim Otop, and Bogobiri markets) in the Calabar Metropolis.

Study site

Calabar municipality, or metropolis is the capital of Cross River State. Calabar sits on coordinates 4°57′0″N 8°19′30″E, and occupies a total land mass of 20,156 km^2 and has a population density of 190 people per km^2 (22, 23, 24). The population of the capital city has increased from 10,000 during the colonial era to 371,022, and 2.6 million for the entire state according to the last census exercise (22, 23, 24).

Sample collection

From the selected markets, 70 samples were collected as shown in Table 1 over three months (June to August 2021). All the samples that were collected were displayed in the open. The samples were randomly collected and held in sterile Ziploc plastic bags, kept in ice, and transported within 1 hour to the laboratory for analysis.

Table 1: Distribution of samples according to locations and types

Sampling location	Meat/Chicken/Fish	Milk
Bogobiri	16	6
Watt	16	0
Marian	16	0
Akim	16	0
Total	64	6

Sample preparation and enumeration of THBC and TCC

Samples were made to assume room temperature before they were processed further. From the various samples (fish, meat, and milk), total heterotrophic bacteria counts and total coliform counts were enumerated as previously reported (25, 26, 27).

Cultural and biochemical identification of the isolates

Following both counts, discrete colonies of the putative isolates were then subcultured twice onto freshly prepared nutrient agar plates to purify them. A total of 42 purified isolates were obtained, and these were stored in sterile Bijou bottles containing nutrient agar slants as previously reported (28, 29). Using a battery of cultural, morphological, and biochemical tests, the isolates were identified (30, 31, 32, 33, 34, 35).

Antibiotic sensitivity test

The antimicrobial assay was carried out using the disc diffusion method as outlined by the Clinical Laboratory Standard Institute (CLSI) (36, 37). The isolate's turbidity was adjusted to 0.5 McFarland standard by comparing it with the turbidity of freshly prepared 1% barium chloride, which corresponds to ~10^8 cfu/ml. The utilised antibiotics were six (6) selected beta-lactam antibiotics (Imipenem (10μg), Ceforuxime (30μg), Augmentin (30μg), Ranicef (5μg), Cefoxitin (30μg), and Graxone (30μg); three (3) fluoroquinolones (Levofloxacin (5μg), Ciprofloxacin (5μg), and Ofloxacin (5μg); and one aminoglycoside, Gentamicin (10μg). Using freshly prepared Mueller Hinton Agar (MHA), the 42 isolates were subjected to susceptibility testing (34, 35). The observed zones were then recorded and interpreted as (S), intermediate (I), and resistant (R) for all the isolates. Zones greater than or equal to 18 mm were considered sensitive, 13-17 mm = I, and those less than or equal to 12 mm = R (22).

Molecular identification of the MDR isolates

Following antibiotic sensitivity, a total of nine (9) MDR isolates were further identified using molecular technique. The isolates were first revived by subculturing them onto freshly prepared nutrient agar and incubated overnight at 37°C. DNA was then extracted using a ZR fungal/bacterial DNA (Zymo, USA) mini-prep extraction kit supplied by Inqaba South Africa, and the extraction was done following the instructions of the manufacturer. The ultra-pure DNA was then stored at -20°C for other downstream reactions. This was then identified using the Sanger BigDye Terminator kit on a 3510 ABI sequencer at Inqaba Biotechnological, Pretoria, South Africa. Resulting sequences one after the other were then loaded onto the Basic Local Alignment Search Tool (BLAST) hosted at the National Centre for Biotechnology Information (NCBI) to identify them (38, 39). The sequences have been submitted to GenBank (SUB14731409). Raw sequences and BLAST results are attached as supplementary data.

Screening of the isolates for amylase and protease enzyme activities

The isolates were screened for the ability to elaborate protease and amylase enzymes as previously reported (40). The stored isolates were revived using freshly prepared nutrient agar, and the resulting pure colonies were then streaked centrally on freshly prepared starch and milk agars at the rate of one isolate per plate and prepared as reported (40). The inoculated milk and starch agar plates were then incubated in an inverted position for 24 to 48 hours and thereafter observed for zones of clearance around the streak lines for each isolate. The zones were then described qualitatively and quantitatively (40).

Statistical analysis

Simple descriptive statistics such as percentage and mean were used in the analysis of the collected data. In addition, the replicate data for the THBC from the samples were subjected to analysis of variance (ANOVA), with significance set at 0.05 (95%). All the analyses were done using the Statistical Package for Social Sciences (SPSS) version 20.

Results

Table 2 shows the result of the total heterotrophic bacteria counts (THBC) for the various locations for fish, chicken, goat (meat and milk) and ram (meat) samples in this study. From Bogobiri, the counts were 4.60±1.50 x 10^6 cfu/ml, 5.30±2.60 x 10^5 cfu/ml, and 6.47±2.60 x 10^6 cfu/ml respectively for goat nose (GN1), goat ear (GE1) and raw milk. For Watt, the counts were 6.40±2.30 x 10^6, and 6.10±1.65 x 10^5 cfu/ml respectively for goat nose (GN2) and goat ear (GE2). For Marian, the counts were 6.30±1.90 x 10^6, and 5.40±1.80 x 10^5 cfu/ml respectively for goat nose (GN3) and goat ear (GE3) and finally for Akim, the THBC were 6.90±2.10 x 10^6, and 6.80±1.40 x 10^5 cfu/ml respectively for goat nose (GN4) and goat ear (GE4). Furthermore, the result further revealed that the location with the highest microbial load for goat ear and goat nose was Akim with counts 6.90±2.10 x 10^6, and 6.80±1.40 x 10^5 cfu/ml. For ram, the counts were 5.60±1.20 x 10^6, 6.40±1.80 x 10^6, 7.30±1.90 x 10^6, 8.15±3.60 x 10^6, 4.30±0.80 x 10^6, 7.25±3.89 x 10^6, 8.14±2.65 x 10^6, and 7.49±2.98 x 10^6 cfu/ml, for RN1, RN2, RN3, RN4, RE1, RE2, RE3, and RE4, respectively.

Table 2: Total heterotrophic bacteria count (THBC) from raw goat and ram meat from the locations

Sample code	Locations	THBC (cfu/ml)
Goat		
	Bogobiri	
GN1		^{a}4.60±1.50 x 10^6
GE1		^{a}5.30±2.60 x 10^5
RM		^{a}6.47±2.60 x 10^6
	Watt	
GN2		^{a}6.40±2.30 x 10^6
GE2		^{a}6.10±1.65 x 10^5
	Marian	
GN3		^{a}6.30±1.90 x 10^6
GE3		^{a}5.40±1.80 x 10^5
	Akim	
GN4		^{a}6.90±2.10 x 10^6
GE4		^{a}6.80±1.40 x 10^5
Ram		
	Bogobiri	
RN1		^{a}5.60±1.20 x 10^6
RN2		^{a}6.40±1.80 x 10^6
RN3		^{a}7.30±1.90 x 10^6
RN4		^{a}8.15±3.60 x 10^6
RE1		^{a}4.30±0.80 x 10^6
RE2		^{a}7.25±3.89 x 10^6
RE3		^{a}8.14±2.65 x 10^6
RE4		^{a}7.49±2.98 x 10^6

RN = Ram nose sample; RE = Ram ear; GN = Goat Nose, GE = Goat Ear, RM = Raw milk, Similar superscript represent ANOVA values that were significant ($p < 0.05$)

Table 3 shows the THBC for raw chicken samples that were obtained from various locations (Bogobiri, Watt, Marian and Akim markets). From Bogobiri, the counts were 8.47±2.56 x 10^7, and 4.92±2.47 x 10^6 cfu/ml for chicken intestine (CI 1) and chicken gizzard (CG1), respectively. From Watt, the counts were 6.76±1.54 x 10^7, and 5.36±1.35 x 10^5 cfu/ml for chicken intestine (CI 2) and chicken gizzard (CG2), respectively. For Marian, the counts were 6.58±2.89 x10^7, and 4.25±1.89 x 10^6cfu/ml for chicken intestine (CI 3) and chicken gizzard (CG3), respectively. For Akim, the counts were 8.10±2.79 x 10^7, and 3.98±1.30 x 10^5cfu/ml for chicken intestine (CI 3) and chicken gizzard (CG3), respectively. The result further revealed that the highest count for chicken (CI) was observed at Bogobiri with a count of 8.47±2.56 x 10^7 cfu/ml while for chicken gizzard, the highest count was observed at Bogobiri with a count of 4.92±2.47 x 10^6 cfu/ml.

Furthermore, the result of the microbial load showed the THBC for the raw Catfish samples obtained from various locations. The result indicated that the THBC for the various catfish samples were 8.10±3.59 x 10^6, and 6.70±2.51 x 10^5cfu/ml respectively for fish gill (FG1) and fish flesh (FF1) for Bogobiri. For Watt, the THBC were 7.80±3.94 x 10^6, and 5.46±2.78 x 10^5cfu/ml respectively for fish gill (FG2) and fish flesh (FF2). For Marian, the THBC count was 7.18±3.10 x 10^6, and 3.74±1.41x 10^5cfu/ml respectively for fish gill (FG3) and fish flesh (FF3). For Akim, the THBC were 8.47±2.42 x 10^6, and 4.60±0.74x 10^5 10^5 cfu/ml respectively for fish gill (FG4) and fish flesh (FF4). The highest count was observed for fish gill at Akim with a count of 8.47±2.42 x 10^6 cfu/ml, while the highest load for fish flesh was observed at Akim market with a value of 8.47±2.42 x 10^6cfu/ml.

Table 3: Total heterotrophic bacteria count (THBC) from the various raw chicken (Broiler) and catfish samples from various locations

Sample code	Locations	THBC (cfu/ml)
Chicken		
	Bogobiri	
CI 1		^{a}8.47±2.56 x 10^7
CG 1		^{a}4.92±2.47 x 10^6
	Watt	
CI 2		^{a}6.76±1.54 x 10^7
CG 2		^{a}5.36±1.35 x 10^5
	Marian	
CI 3		^{a}6.58±2.89 x10^7
CG 3		^{a}4.25±1.89 x 10^6
	Akim	
CI 4		^{a}8.10±2.79 x 10^7
CG 4		^{a}3.98±1.30 x 10^5
Fish	Locations	THBC (cfu/g)
	Bogobiri	
FG1		^{a}8.10±3.59x 10^6
FF1		^{a}6.70±2.51 x 10^5
	Watt	
FG2		^{a}7.80±3.94 x 10^6
FF2		^{a}5.46±2.78 x 10^5
	Marian	
FG3		^{a}7.18±3.10 x 10^6
FF3		^{a}3.74±1.41x 10^5
	Akim	
FG4		^{a}8.47±2.42x 10^6
FF4		^{a}4.60±0.74x 10^5

CI = Chicken intestine; CG = Chicken gizzard; FG = Fish gill; FF = Fish flesh. Similar superscripts represent ANOVA values that were significant ($p < 0.05$)

Table 4 shows the total coliform counts (TCC) of the various samples from the various locations. From the result, the total coliform count for ram (ear) samples were 141, 90, 94, and 56 cfu, respectively while for nose sample, the counts were 81, 78, 47, and 43 cfu for locations Bogobiri,

Watt, Marian, and Akim. For goat nose samples, the total coliform counts were 79, 56, 71, and 40 cfu while for goat nose, the coliform counts were 34, 44, 49, and 19 cfu respectively, for locations Bogobiri, Watt, Marian, and Akim. For the chicken samples, the the TCC in the intestine were 56, 56, 71, and 40 cfu while for the gizzard, the counts were 34, 70, 50 and 44 cfu respectively, for locations Bogobiri, Watt, Marian and Akim respectively. For the fish samples, the gill had total coliform counts were 78, 101, 81 and 56 cfu while for the fish flesh, the counts were 93, 46, 79 and 42 cfu, respectively for locations Bogobiri, Watt, Marian and Akim. For the milk sample, the total coliform count was 50 CFU. Table 5 shows the results of the biochemical, cultural, and morphological characterisation of the isolates.

Table 4: Total coliform counts (cfu) from various locations per 100ml of sample

Sample type	Parts	Total coliform count			
		Bogobiri	**Watt**	**Marian**	**Akim**
Ram					
	Ear	141	90	94	56
	Nose	81	78	47	43
Goat					
	Ear	79	56	71	40
	Nose	34	44	49	19
Chicken					
	Intestine	56	56	71	40
	Gizzard	34	70	50	44
Fish					
	Gills	78	101	81	56
	Flesh	93	46	79	42
Milk		50			

Table 5: Morphological, cultural and biochemical characterisation of the isolates

Probable isolates	Growth on NA	Gram staining/ Morphology	Catalase	Oxidase	Citrate	MR	VP	Indole/ Motility	Growth on EMB
Morganella sp.	Circular colonies	opaque-/Rods	+	-	-	+	-	+/+	Colourless
Providencia sp	Large colonies	dull-/Rods	+	-	+	+	-	+/+	Colourless
Klebsiella sp	Circular colonies	yellow-/Rods	+	-	+	-	+	-/-	Dark purple
Escherichia coli	Large colonies	circular-/Rods	+	-	-	+	-	+/-	Green metallic sheen
Salmonella sp	Colourless colonies	-/Rods	+	-	-	+	-	-/+	Colourless

MR = Methl red; VP = Voges Proskauer

Figure 1 shows the distribution (percentage) of the isolates obtained in this study which were *Morganella* (n = 6), *Providencia* (n = 7), *Klebsiella* (n = 10), *E. coli* (n = 11) and *Salmonella* (n = 8). The percentage distribution was 14.3, 16.7, 19.0, 23.8 and 26.2%, respectively for *Morganella*, *Providencia*, *Salmonella, Klebsiella*, and *E. coli.*

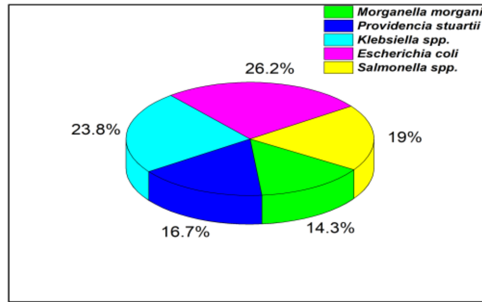

Figure 1: Percentage distribution of the isolates obtained

Antibiotic sensitivity test of the isolates

Figure 2 shows the result of the antibiotic sensitivity for the various isolates. The results were interpreted as follows zones greater than or equal to 18 mm were considered sensitive, 13 - 17 mm and those less than or equal to 12 mm were considered resistant. The result indicated that all the isolates were multidrug resistant to the test antibiotics used in this study.

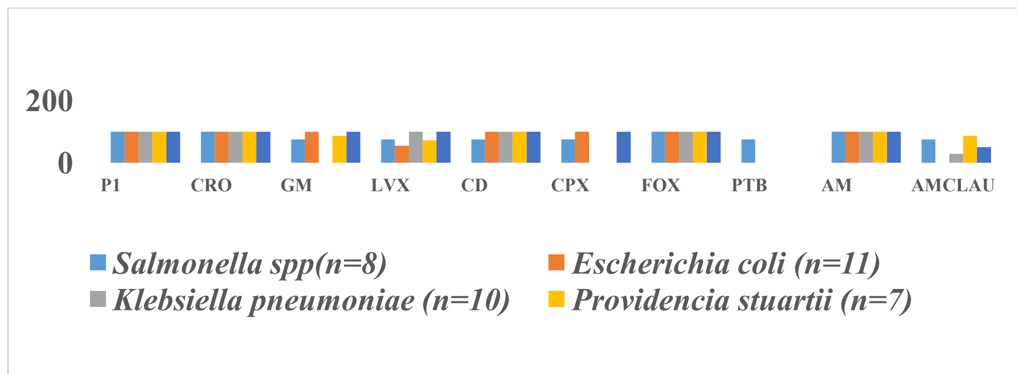

Figure 2: Resistance profile of the isolates

PI = PENICILLIN G; CRO = CEFRIAXON; GM= GENTAMICIN; LVX=LEVOFLOXACIN; CD=CLINDAMCIN; CPX= CIPROFLOXACIN; FOX=CEFOXICIN; PTB=PIPERACILLIN-AZOBACTAM; AM=AMIKACIN; AMCLAU= AMOXICILLIN-CLAVULANIC ACID

For the molecular characterisation, a total of nine (9), were selected based on their antimicrobial sensitivity (multidrug resistance). A total of six isolates gave positive bands (600 bp) and these were *Escherichia coli, Klebsiella pneumoniae, Morganella morganii subsp. Morganii, Providencia stuartii, and Salmonella enterica subsp. Enterica* (Figure 3 and **Supplementary Table 1**).

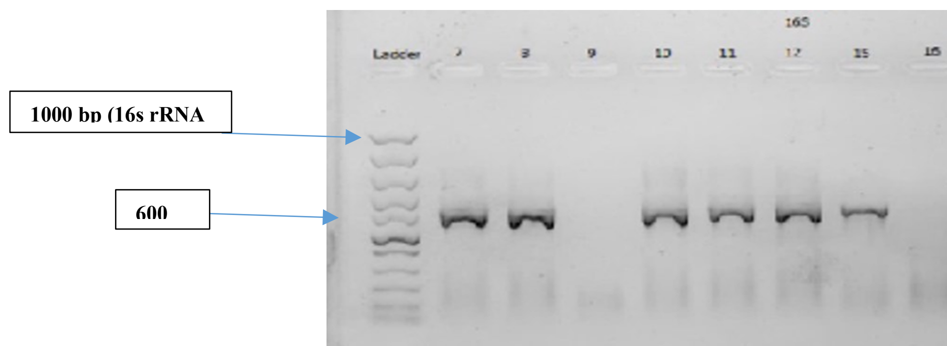

Figure 3: Gel electrophoresis for the identified isolates.

7 = *Escherichia coli*; 8 = *Klebsiella quasivariicola*; 10 = *Morganella morganii*
11: *Providencia stuartii*; 12= *Klebsiella pneumoniae*; 13 = *Salmonella enterica subsp.enterica*

The isolates obtained via cultural identification were further subjected to primary amylase and protease screening, and the results are presented in Table 6. The result indicates that all the isolates were positive for amylase, and quantitatively, the range of the zones of inhibition ranged from 9 to 14 mm while for protease, they ranged from 7 to 15 mm, respectively.

Table 6: Primary screening of the isolates for amylase and protease

Isolates	Amylase		Protease	
	Qualitative	Quantitative (mm)	Qualitative (mm)	Quantitative
Morganella sp	++	14	++	15
Providencia sp	+	12	+	11
Klebsiella sp	++	13	+	10
E. coli	+	11	++	14
Salmonella sp	+	9	+	7

+ = Positive; mm = millimeter

Discussion

The microbial load observed in our study varied from sample to sample and locations or markets sampled, and this is in line with previous reports (36, 37). In their study (37), analysed the THBC of raw goat meat sold in Uyo, Akwa Ibom State reported lower counts (9.1 x 10^2 cfu/g to 1.07 x 10^4 cfu/ml) than our observed counts for all the locations examined in this study. On the other hand, our reported counts for goat meat were only slightly lower than those reported by another study for various markets, with the highest count of 2.90 x 10^5 cfu/g. In our study, ram was only sampled from the Bogobiri location alone because it is a Muslim cluster and they are the main consumers of ram meat. In an earlier study, they reported THBC counts for beef, mutton, and smoked pork meat samples. Their THBC showed that ram had the third highest microbial load (38) reported counts that were within my reported range of counts with a value of 6.0 x 10^6 cfu/g. The microbial load for the catfish in our study was within range for that reported earlier for the wet and dry seasons which ranged from 1.95 to 3.46 x10^5cfu cfu/ml (39). The microbial load for chicken also varied for the various locations for the various chicken parts examined. The presence of microbial species in chicken was in line with previous reports with similar counts to our findings (41, 42). In addition to the THBC, TCC was also examined, and it ranged from 34 to 141 cfu per 100 ml of rinsed water for the meat samples and per 100 ml of the raw milk samples for Bogobiri. For the other locations, the total coliform counts were 44 to 101, 47 to 94, and 19 to 56 cfu for Watt, Marian and Akim markets, respectively. This was lower than the total coliform counts reported previously (2, 43). Overall, the high microbial counts recorded for the various meat and milk samples are capable of eliciting a foodborne illness in consumers, especially if the food is not properly processed or consumed raw.

Raw fish, raw meat, and milk as mentioned earlier, create an important milieu that supports the growth of various microorganisms. From the various samples, a total of 42 bacterial isolates belonged to the following genera *Morganella* (n = 6), *Providencia* (n = 7), *Klebsiella* (n = 10), *E. coli* (n = 11) and *Salmonella* (n = 8), were isolated from the samples except for the milk sample, which had no *Morganella* species. In part, the presence of these microorganisms has been attributed to the unhygienic steps that are employed in the processing of meat and milk (41). *E. coli* was isolated from all the samples and locations in this study, and this was in line with the reports of (37) who isolated *E. coli* from their studied sample. Globally, cattle and other meat products are known as the main reservoirs of this strain of *E. coli*. Infections in humans result mainly from the consumption of raw or undercooked contaminated meat (44, 45). The *E. coli* isolates in our study showed multi-drug resistance and have been associated with infections in humans (44).

Klebsiella pneumoniae and *Salmonella enterica subsp. enterica* was characterised *from* various samples and all the locations in this study. An earlier study isolated *Salmonella* from a poultry processing plant (42). *Salmonella* is an important foodborne pathogen that contains over 2587 serotypes (46). Although poultry is regarded as one of the main vehicles for the transmission of Salmonellosis, a zoonotic infection of great public health implications (47). Estimates indicate that the outbreak of salmonellosis often associated with food brings about substantial economic losses in both developing and developed nations of the world when it happens. The main route of infection remains the consumption of animal products that are poorly cooked or cross-contaminated by

handlers. An estimate by (48), indicated that 1.8 million people die due to foodborne infections every year around the world. Even more worrisome was the fact that the *Salmonella* isolates in the study showed muti-drug resistance to fifty per cent of the antibiotics (n=10). This same pattern of resistance is in line with an earlier report that evaluated the prevalence and antibiogram of *Salmonella* and *S. aureus* from poultry meat (48). One unique isolate that was identified via molecular characterisation was *Morganella morganii*. *M. morganii* is a member of the family, *Enterobacteriaceae*. Although they have low pathogenicity, it is well known that immune-compromised patients can develop diarrhoea, urinary tract infections, bacteremia, and sepsis if they are exposed to this pathogen (49). Its presence is linked to histamine poisoning, as *M. morganii* is a well-known histamine decarboxylase elaborator and is responsible for the accumulation of histamines in foods (49). Another unique isolate obtained in this study was the *Providencia* species. *Providencia* species are common uropathogens in people with long-term indwelling urinary catheters who were hospitalised or resided in a nursing care facility (50). *Providencia species* belongs to the family *Enterobacteriaceae* and has been implicated as a causative agent of diarrhoea (51). *Providencia* is not a common foodborne pathogen. Their presence in food is largely driven by the poor personal hygiene status of the handler (51). *Providencia* species such as *P. alcalifaciens* most frequently affect children, including travellers from developing countries. Furthermore, two large outbreaks of foodborne infection caused by *P. alcalifaciens* have been reported in Japan and the Czech Republic, thus providing evidence of causing gastroenteritis (51).
Furthermore, the isolates were further evaluated for their ability to produce amylase and protease enzymes. Evaluation of both enzymes is a crude way of checking isolates for pathogenicity (12, 13, 40). Thus, the presence of amylase and protease activities in all the MDR isolates further confirms their ability to become virulent on the one hand and, on the other hand, their ability to spoil the meat and milk samples.

Conclusion
Microbial isolates were characterised from raw chicken, milk, fish, goat, and ram from various markets (Akim, Bogobiri, Marian, and Watt) located in the Calabar metropolis. Isolation was identified using cultural and molecular techniques. From a total of 70 samples collected, 42 bacterial species were isolated. These were *Morganella sp* (n=6), *Providencia* (n = 7), *Klebsiella sp* (n = 10), *E. coli* (n = 11) and *Salmonella sp* (n = 8). The microbial loads (THBC) obtained in this study varied according to sample type and location. All the isolates in the study showed MDR to the test antibiotics used in the study. In addition, the isolates were all positive for amylase and protease enzymes, a further indication of their pathogenicity. Furthermore, all the isolates obtained in our study have been implicated in various episodes of foodborne diseases and poisoning around the world.

Limitations of the study
The study has some limitations. First, the study utilised only four of the most popular markets used by the public in the study area. Second, the identification of the isolates was done using cultural and molecular methods. However, molecular characterisation was only performed for nine (9) isolates.

List of Abbreviations
ANOVA: Analysis of Variance
BLAST: Basic Local Alignment Search Tool
Bp: base pair
cfu: colony forming unit
CLSI: Clinical Laboratory Standard Institute
DNA: Deoxyribonucleic acid
MDR: Multidrug resistance
MR: Methyl red
rRNA: Ribosomal Ribonucleic Acid
SPSS: Statistical Package for Social Sciences
THBC: Total heterotrophic bacteria count
TCC: Total coliform count
USA: United States of America
VP: Voges Proskauer

Declarations
Ethical approval and consent to participate
All the necessary permissions for the study were obtained.

Consent for publication
All authors gave consent for publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials
All essential data supporting the findings of this case are available within the article. Additional data are available upon request from the corresponding author.

Competing interests
The authors declare no conflict of interest.

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Authors' contributions
All the authors were involved in various aspects of the study and this is given as a breakdown below. The study was conceived by UJC, UOE, GPB and NFO. Data acquisition was carried out by UJC, UOE, GPB, NFO, AMO, UAC, and UAA. Data analysis was conducted by UJC, GBP, and UOE. Manuscript writing was done by UJC and UOE. All the authors reviewed the articles while a UJC, JE and IEO were involved in the supervision and funding of the project. All the authors approved the publication of the manuscript.

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