Phenotypic characterization and antibiogram of aerobic bacteria isolated from varieties of processed meat sold within Kafanchan Metropolis, Kaduna State

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Abstract

Meat and meat products are constantly threatened by pathogenic antibiotic-resistant bacteria, leading to foodborne diseases and huge economic losses. Therefore, this research aimed to isolate and identify aerobic bacteria and their antibiograms from varieties of meat products sold within Kafanchan metropolis. A total of nine ready-to-eat meat samples were aseptically collected, including "balangu, kilishi, and tsire," samples from each of Kafanchan ward A, Maigizo, and Takau wards, respectively. All the samples were processed according to the standard microbiological methods for bacterial isolation and identification. Five different bacteria were isolated and identified, including *Bacillus* spp., *Corynebacterium* spp., *Enterococcus* spp., *Staphylococci aureus*, and *Pseudomonas aeruginosa*. The Kilishi sample recorded the highest rate of contamination, with 3/5 (60%) of the total bacteria identified compared to the other meat samples. All the bacteria identified in this study were resistant to all the antibiotics tested. The bacteria isolated from the tsire sample in Takau ward recorded the highest antibiotic resistance with a 0.9% MDR index, while bacteria from Kilishi samples recorded the lowest MDR index value of 0.3%. Regulatory agencies such as the National Agency for Food and Drug Administration and Control, and Standard Organisation of Nigeria should ensure that food products are safe for consumption, that antibiotics are used appropriately at the recommended rate, and an accurate drug withdrawal period be observed during treatment of the animal before slaughter.

Keywords: Aerobic bacteria, Antibiogram, Kaduna state, Kafanchan, Meat, Phenotypic characterization.

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Introduction

Meat is a type of food that comes from animal flesh, including muscles and internal organs like the heart, liver, kidney, intestine, and bladder (Luyet, 2000). It provides the essential nutrients that our bodies need, such as protein, vitamins, and minerals, with zinc being a particularly important one. While some people rely heavily on meat and meat products for their diet, it's important to note that they can also be a breeding ground for harmful microbes that can cause spoilage and food poisoning (Pal, 2014). To prevent this, it's crucial to test for microbiological contamination at various stages of food production, as recommended by the (ICMSF, 2011).

Suya is a spicy barbecued, smoked or roasted meat product usually made from beef, rams, goats etc.; which is a popular food item in various parts of Nigeria (Eke *et al*., 2013). It is traditionally prepared by the Hausa people of Northern Nigeria, Cameroon, Niger and some parts of Sudan; where it is called *agashe* and is prepared from boneless Meat from animals that has been researched extensively (Ogbonna *et al*., 2012; Abdullahi *et al*., 2004). Suya, a type of grilled meat, comes in three varieties: tsire, balangu, and kilishi (Egbebi and Seidu, 2011). While tsire is commonly referred to as suya, balangu has become the most popular type. These convenient and tasty products (Kilishi, Balangu, and tsire) are popular street foods that can be enjoyed hot and are available for purchase at various locations such as street vendors, clubhouses, picnics, parties, restaurants, and institutions (Igene and Mohammed, 1983).

It is crucial to assess the microbial content of processed meat that humans consume for several reasons. Firstly, it ensures food safety by identifying and measuring harmful microorganisms, such as bacteria, viruses, and fungi that can cause foodborne illnesses (Madueke *et al*., 2014).

Secondly, it enhances the overall quality of the product by preventing spoilage and meeting the required standards for taste, texture, and freshness. Consumers have high expectations for the food they purchase, and microbial assessment plays a vital role in meeting these expectations (Madueke *et al*., 2014). Moreover, regulatory authorities have established specific microbial limits for different food products. Conducting microbial assessments allows producers to comply with these regulations (Soyiri *et al*., 2008). Lastly, antimicrobial resistance (AMR) is a severe threat to global public health. It increases morbidity and mortality and is associated with high economic costs due to the healthcare burden it imposes (WHO, 2020). Infections with multidrug-resistant (MDR) bacteria also have significant implications for clinical and economic outcomes. Therefore, it is essential to provide appropriate information, prescribing, and stewardship strategies to support treatment and prevent the use of antibiotic drugs and its consequences. There is limited literature on this study, especially in Kafanchan metropolis, and

there is a need for further research on related studies.

Materials and methods

Study area

The study was carried out in Kafanchan metropolis of Jema'a local government area (LGA) of Kaduna State, Nigeria. Jema'a LGA is one of the 23 LGAs in Kaduna State, and belongs to the Kaduna South Agricultural Zones that encompasses eight LGAs. The local government share borders with Jaba, Kachia, Zangon-Kataf, Kaura and Sanga LGAs all of Kaduna State, and also share borders with Nasarawa and Plateau States, Nigeria (Fig. 1). Kafanchan is located at latitude 9^0 59 North, longitude 8^0 29 East and situated at an elevation of 733m above sea level (World Atlas, 2018).

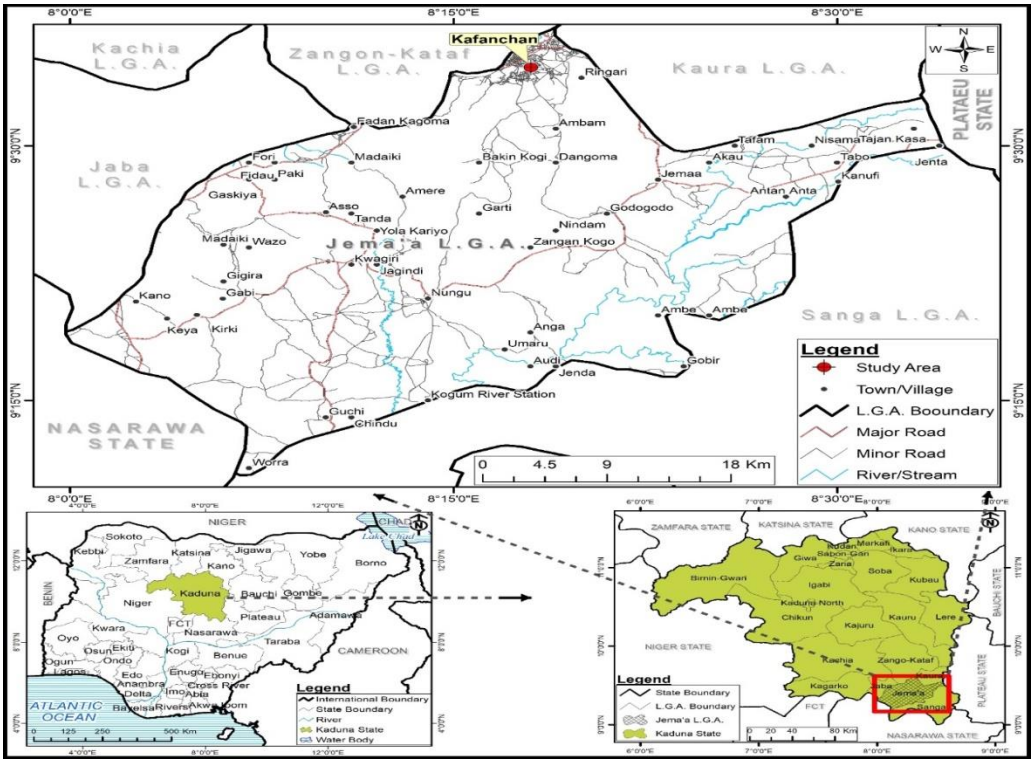

Figure 1: Map of Study Area (Kafanchan Metropolis) in Jema'a L.G.A. Source: Modified from https://www.openstreetmap.org/

Sample collection transportation and storage

One hundred and fifty gram (150g) each of freshly prepared meat products (Balangu, Kilishi and Tsire) were collected using convenient sampling method from three different wards (Kafanchan A, Maigizo and Takau, respectively) within Kafanchan metropolis from three different selling points i.e. points A, B, and C for Balangu, Kilishi and Tsire, the same trend follows for the other wards. After collection, the samples were wrapped on an aluminum foil paper, placed in an ice box and transported immediately to the Antimicrobial Resistance (AMR) Sentinel Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State for analysis.

Sample processing for bacteria isolation and identification

All the samples collected were processed according to the standard microbiological methods for bacterial culture and isolation as described by Markey *et al.* (2013). Briefly, samples were chopped and homogenous suspensions made using sterile distilled water, and sterile swab sticks were used to make direct inoculations onto blood agar and MacConkey agar plates and incubated aerobically at 37˚C for 24 hours. Colonies were sub-cultured to obtain pure colonies for identification using colony morphology, Gram staining and other biochemical tests such as catalase, coagulase, oxidation-fermentation, triple sugar iron, indole, motility, Voges-Proskauer, methyl red and citrate tests. Two to three each of the identified colonies were stored on nutrient agar slants until needed for antibacterial susceptibility testing.

Antimicrobial susceptibility testing (AST)

The Kirby-Bauer disc diffusion method, also known as the disc diffusion susceptibility test, was carried out according to the Clinical and Laboratory Standard Institute (CLSI, 2021) guidelines. Six different antibiotics were used, including ceftriaxone, ECF (30 µg) and Oxoid™ amoxicillin clavulanic acid, AMC (30 µg), cefixime, CFM (30 µg), cefoxitin, FOX (30 µg), azithromycin, AZM (15 µg), Gentamycin, CN (10 µg), and chloramphenicol, C (30 µg). A standard inoculum was prepared of overnight cultures of all the identified bacteria in a 3 mL of sterile distilled water. The suspension was adjusted to 0.5 McFarland, and using a sterile swab stick, a standardized inoculum of each bacteria were then cultured on a freshly prepared Mueller Hinton Agar (MHA) plate, this was then allowed to absorb the excess moist (dry) and antibiotics were then placed on the lawned plates. The plates were then incubated at 37˚C for 24 hours, the diameter of zone of inhibition was then measured using transparent ruler and result interpreted as resistance, intermediate or sensitive in accordance with the CLSI, 2021 guidelines. A multidrug resistant bacterium was defined as the one resistant to at least three different antibiotics (Mohd Asri *et al.*, 2021).

Results and discussion

In this study, five different bacteria were isolated and identified which include; *bacillus* spp. *corynebacterium* species, *Enterococcus* species. *Staphylococci aureus*, and *Pseudomonas aeruginosa* (Fig. 2). Kilishi from Takau and Kafanchan wards A record the highest contaminants with three bacteria identified of the five isolated which include, *Staphylococci aureus*, *Bacillus* species and *Enterococcus* species while those from Maigizor ward record the least level of contaminants in the meat samples with only two bacteria identified. Tsire samples from Takau and Kafanchan ward A. recorded the least contaminants with only one bacteria identified each which is, *Staphylococci aureus* (Fig. 3).

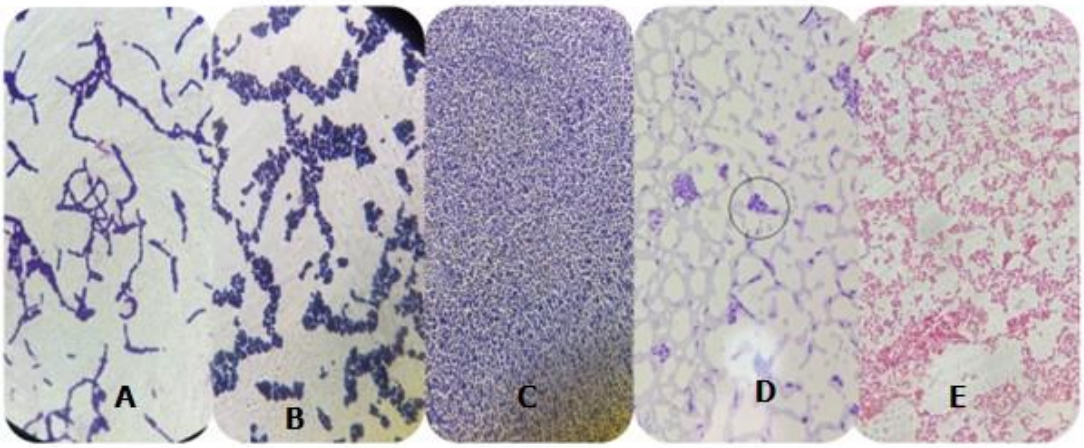

Figure 2: Photomicrograph of bacteria isolated from varieties of processed meat samples collected within the study area. A = Gram-positive rods, B= Gram-positive cocci in clusters, C= Gram-positive cocci in pairs, D= Gram-positive rods parallel to each other, and E= Gram-negative rods.

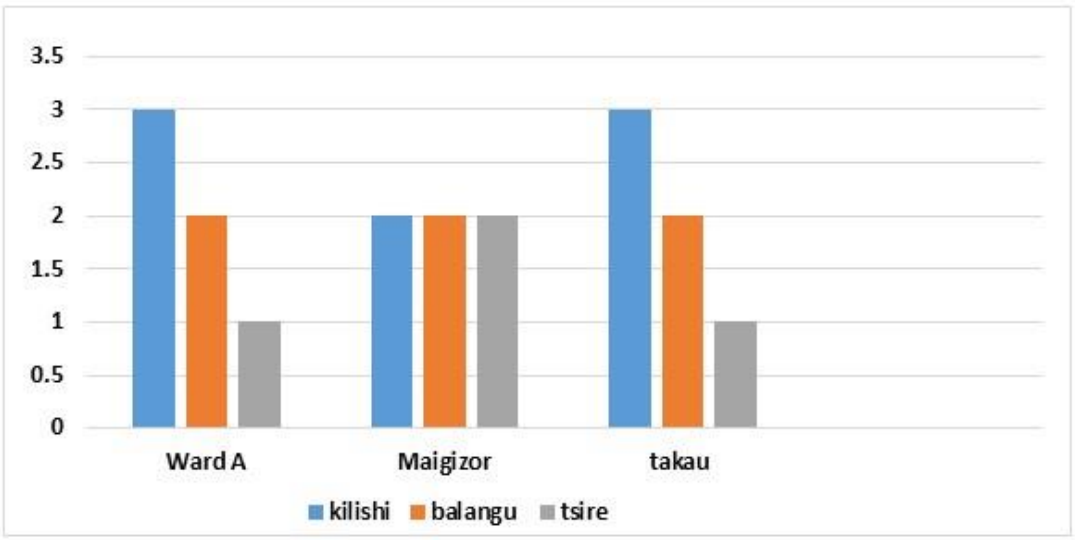

Figure 3. Frequency of bacteria isolated from processed meat products sold within Kafanchan Metropolis.

Meat and meat products play an important role in the maintenance of human health by providing all essential nutrient such as protein, vitamins and minerals. On the other hand they act as an important medium for many microbes which can either cause food spoilage or food poison (ICMSF, 2011). In this study, it was clear that all the meat samples from the three selling points were contaminated with at least one of the microorganisms tested. The high level of contamination for meat samples in this study especially the kilishi sample may be due to the processing methods used, mixture of spices and the micro-climate within these areas. This is in line with the findings of Madueke *et al*., (2014) who reported that even with the increase in the shelf life of the kilishi product, it is still subjected to microbial spoilage as a result of contamination

during processing. Again, most of the meat products vendors are found in certain location and lack formal education thus, still uses traditional method of processing which aid the growth of these microorganisms naturally in the processing environment or sometimes in the live animals themselves (Sofos, 2014). In another study by Marias *et al*. (2007) stated that the occurrence of microorganisms on meat may be due to some human factors such as poor farm animal management, the practice involve during slaughter, processing method, condition of storage and lack of safety knowledge on meat and meat handlers. This can only be obtainable through better management practices, maintenance of good hygiene and creating awareness on food security to all the meat processors involve in the production system (Haileselaessei *et al*., 2013; Sofos, 2014).

In this study, results had shown that all the meat samples are contaminated with one or more of the bacteria identified, with kilishi samples having the highest rate of contaminants compared with the other meat samples. Four gram positive bacteria were identified which include; *Corynebacterieum specie, Staphylococci aureus*, *Enterococcus* species and *Bacillus* species (Table 1).

Table 1: Phenotypic identification of gram-positive bacteria isolated from varieties of meat samples sold within Kafanchan Metropolis.

Isolate	Morphology	Grams reaction	Biochemical tests			Identification
			Catalase	Coagulase	MSA	
1.	Large, gray, flat ß-haemolytic colonies on blood agar plate	Gram-positive rods	+	NA	NA	*Bacillus* species
2.	Small, white, round, smooth, raised, ß-haemolytic colonies on blood agar plate	Gram-positive cocci in clusters	+	+	Golden yellow	*Staphylococcus aureus*
3.	Medium, yellow, round, smooth γ-haemolytic colonies on blood agar plate	Gram positive cocci in pairs	+	+	-	*Enterococcus* species
4.	Large, white, flat α-haemolytic colonies on blood agar plate	Gram-positive rods parallel to each other	+	–	NA	*Corynebacterium* species

Key: + = Positive, - = Negative, NA = Not applicable, MSA = Growth on mannitol salt agar

Staphylococci aureus and *staphylococci* species were prevalent in meat samples from all the three wards. *Corynebacterium* species was present in meat samples from both Takau and Maigizor wards and *Enterococcus* species were more prevalent in Takau and Kafanchan ward A, while *Bacillus*

species was found only in Kafanchan ward A.

Similarly**,** in addition to other Gram-positive bacteria identified, only one Gram-negative bacterium (*Pseudomonas aeruginosa*) was isolated and identified from Kafanchan ward A, in one of the varieties of meat sampled (Table 2).

Table 2: Phenotypic identification of gram-negative bacterium isolated from variety of meat sample within Kafanchan Metropolis.

S/n	Biological tests	Remark	Presumed bacteria
1	Triple sugar iron (TSI)	K/NG	
2	Indole	-	
3	Methyl red (MR)	+	*Pseudomonas aeruginosa*
4	Voges-Proskauer (VP)	-	
5	Motility	+	
6	Urease	+	
7	Citrate	+	

Key: += positive, -= negative, K/NG= Alkaline/no colour change.

This study indicate that meat samples from the different vendors for (kilishi, balangu and tsire) within the selling points in Kafanchan metropolis of Kaduna State were heavily contaminated with at least one of the microbes tested. This high level of contamination in kilishi samples in this study indicates a potential breakdown of hygiene at various stages of the meat processing. Also, most of the suya vendors in Kafanchan use inked paper, old and abandoned newspapers for packaging which may be considered dirty, dusty and contaminated.

This is similar to study by Odey *et al*. (2013), Okono *et al*. (2013), and Inusa and Said, (2017), whose studies were found to have the same samples to be contaminated with almost the same organisms that pose threat to public health.

In another study by Yusuf *et al*. (2012), he stated that *Bacillus* spp may be present in processed meat samples as a result of contamination from the aerial spores carried in air. Another study also report that, acidity, pH, temperature, water activity, atmospheric gas, available nutrients and competition with other microbes are factors that can influence microbial multiplication in ready to eat meat products (Madueke *et al*., 2014). Climate condition in the tropics also favors the persistence and proliferation of most pathogenic microorganisms (Ekere *et al*., 2014). After vending, leftover soya products are often kept to be sold the next day thus, providing opportunity for rancidity and spoilage to occur in the products (Onuorah *et al*., 2015). Such products if not properly reheated for consumption it may lead to food borne infections. Material used for packaging processed meat and meat products pose a significant means of contamination (Eke *et al*., 2013).

Table 3 Showed multidrug resistant pattern of the bacteria isolated from different meat samples processed within Kafanchan metropolis (Fig. 4).

Table 3: Multidrug resistant pattern in bacteria isolated from different meat sample processed within Kafanchan Metropolis.

Sample	Ward	Microorganism	Resistant Pattern	Class of Antibiotics	MDR Index
Kilishi	Takau ward	*Corynebacterium* sp.	AMC, CFM	2	0.3
		Staphylococcus aureus	FOX, AMC, ECF, CFM	4	0.6
		Staphylococcus sp.	FOX, AMC, CFM	3	0.4
	Kaf. Ward A	*Bacillus* sp.	FOX, AMC, CFM	3	0.4
		Staphylococcus sp.	FOX, CFM	2	0.3
		Enterococcus sp.	FOX, AMC, ECF, CFM	4	0.6
	Maigizor ward	*Corynebacterium* sp.	FOX, AMC, CFM	3	0.4
		Staphylococcus aureus	FOX, AMC, CFM	3	0.4
		Staphylococcus sp.	FOX, AMC, ECF, CFM	4	0.6
Tsire	Takau ward	*Staphylococcus aureus*	C, FOX, AMC, AZM, ECF, CFM	6	0.9
		Staphylococcus sp.	FOX, AMC, ECF, CFM	4	0.6
	Kaf. ward A	*Staphylococcus aureus*	FOX, AMC, AZM, CN, CFM	5	0.7
		Staphylococcus sp.	C, FOX,CFM	3	0.4
	Maigizor ward	*Bacillus* sp.	FOX, AMC, ECF, CFM	4	0.6
		Staphylococcus aureus	FOX, AMC, AZM, ECF, CFM	5	0.7
		Staphylococcus sp.	FOX, AMC,CFM	3	0.4
Balangu	Takau ward	*Bacillus* sp.	FOX, AMC, ECF, CFM	4	0.6
		Staphylococcus aureus	FOX, AMC, CFM	3	0.4
		Staphylococcus sp.	FOX, AMC, ECF, CFM	4	0.6
	Kaf. Ward A	*Staphylococcus* sp.	FOX, AMC, ECF, CFM	3	0.4
		Pseudomonas sp.	C, FOX, AMC, CFM	4	0.6
	Maigizor ward	*Bacillus* sp.	FOX, AMC,CFM	3	0.4
		Staphylococcus aureus	C, FOX, AMC, CFM	4	0.6
		Staphylococcus sp.	FOX, AMC, AZM, CFM	4	0.6

Key: MDR = Multidrug resistant, AMC = Amoxycillin Clavulanic acid, CFM = Cefixime, FOX = Cefoxitin, AZM = Azithromycin, ECF = Ceftriazone, CN = Gentamycin, and C = Chloramphenicol

From the result obtained, it showed that all the samples collected from the three selling points were resistant to at least one or more of the Antibiotic tested which include: Amoxicillin clavulanic acid, cefixime, cefexotin, azithromycin, ceftriaxone and gentamycin. Tsire sample from Takau ward record the highest resistance level of the antibiotic tested with 0.9% MDR Index, followed by Kafanchan ward A with 0.7% MDR Index. Balangu sample from two wards that is, Takau and Maigizor wards record a lower MDR Index values of 0.6 compared to those from Tsire samples.

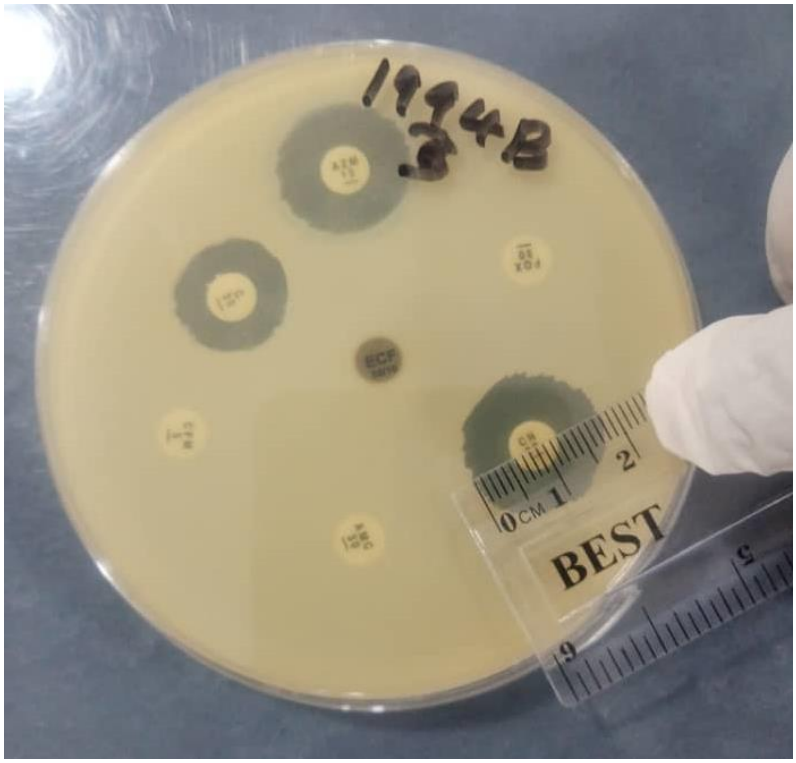

Figure 4: Multidrug-resistant pattern observed in *Enterococcus* sp. isolated from Kilishi meat sample in Kafanchan ward A of Jema'a LGA, Kaduna State.

Kilish samples record the least MDR Index values of 0.3% compared to the other two meat samples.

Pseudomonas aeruginosa is an aerobic Gram-negative bacterium which is commonly found in soil and can grow well in range of temperature levels from 2 to 35°C (Madueke *et al*., 2014), and can easily be found in chilled food products, as well as food prepared at room temperature. They have the ability to grow at a low temperature and have a tendency of secreting enzymes that can affect the overall quality of the food including cold store food (Madueke *et al*., 2014).

In this study, it is clear that *pseudomonas aeruginosa* was the only Gram negative bacteria identified among others which are gram positive. Therefore the Gram negative pseudomonas identified in this study may be as a result of poor or improper heating of meat by the vendors in Kafanchan metropolis. This could be due to the fact that they develop resistance to certain antibiotics during production. This is in line with the study of (Odey *et al*., 2013) who isolate *staphylococcus aureus*, *Escherichia coli*, *streptococcus spp Salmonella spp*, *Bacillus spp*, *Pseudomonas spp*, and *prosteus spp* from selected suya samples on sale at Calabar, Cross River State, Nigeria. Also, in a similar study by Inusa and Said (2017), suya samples from Kano metropolis of Kano State, Nigeria. In conclusion A total of five bacteria were identified which include; *staphylococcus aureus* (0.45%), *Corynebacterium* species (0.1%), *Enterococcus* species (0.1%), *Bacillus* species (0.2%), and

Pseudomonas aeruginosa (0.05%). Most of the bacteria isolated and identified in the meat product (balangu, kilishi and tsire) are of public health importance thus, their presence in such meat product continues to be a concern especially among consumers. Similarly, the presence of pathogenic bacteria and antibiotic resistance in meat and meat products gives a warning signal for the possible occurrence of foodborne infections and capable of producing outbreak of food poisoning and economic loss. We therefore recommend that the National Agency for Food and Drugs Administration and Control should provide adequate knowledge on sanitation, personal hygiene and its relevance to food product. Consumers should insist on proper or effective reheating of the suya before purchase to ensure proper destruction of micro bacteria cells. Consumer also should insist on using foil paper or polythene bags for packaging the roasted meat product when purchase instead of using unclean or ink newspaper. We also recommend responsible use of antibiotics at recommended dose during treatments of animals as well as ensuring an accurate withdrawal period before slaughter.

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