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Product Quality and Safety

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, which can lead to foodborne diseases and significant economic losses. In order to address this issue, this research aimed to isolate and identify aerobic bacteria and their antibiograms from various 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. These samples were processed according to standard microbiological methods for bacterial isolation and identification. Five different bacteria were isolated and identified in the study, including Bacillus spp., Corynebacterium spp., Enterococcus spp., Staphylococci aureus, and Pseudomonas aeruginosa. The Kilishi sample had the highest rate of contamination, with 3 out of 5 (60%) of the total bacteria identified compared to the other meat samples. Furthermore, all the bacteria identified in this study were found to be resistant to all the antibiotics tested. The bacteria isolated from the tsire sample in Takau ward exhibited the highest antibiotic resistance, with a 0.9% Multiple Drug Resistance (MDR) index. On the other hand, bacteria from Kilishi samples had the lowest MDR index value of 0.3%. In light of these findings, it is imperative that regulatory agencies such as the National Agency for Food and Drug Administration and Control, and the Standard Organisation of Nigeria, take proactive measures to ensure the safety of food products for consumption. This includes monitoring and regulating the appropriate use of antibiotics at recommended rates, as well as enforcing accurate drug withdrawal periods during the treatment of animals before slaughter.*

Keywords: aerobic bacteria, antibiogram, Kaduna state, Kafanchan, meat, phenotypic characterization

Abbreviations: AMR-antimicrobial resistance, AST-antimicrobial susceptibility testing, CLSI-clinical and laboratory standard institute, LGA-local government area, MHA-Mueller Hinton agar, MDR-multidrug resistance, WHO-World Health Organization

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Introduction

Background of the Study

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 is 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 is 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.; it 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 (Abdullahi et al., 2014). 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, 1981).

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 (Bradeeba and Sivakumaar, 2013).

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.

Material 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 (Figure 1). Kafanchan is located at latitude 9º 59 North, longitude 8º 29 East and situated at an elevation of 733 m above sea level (world atlas, 2018).

Sample Collection Transportation and Storage: One hundred and fifty gram (150 g) 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.

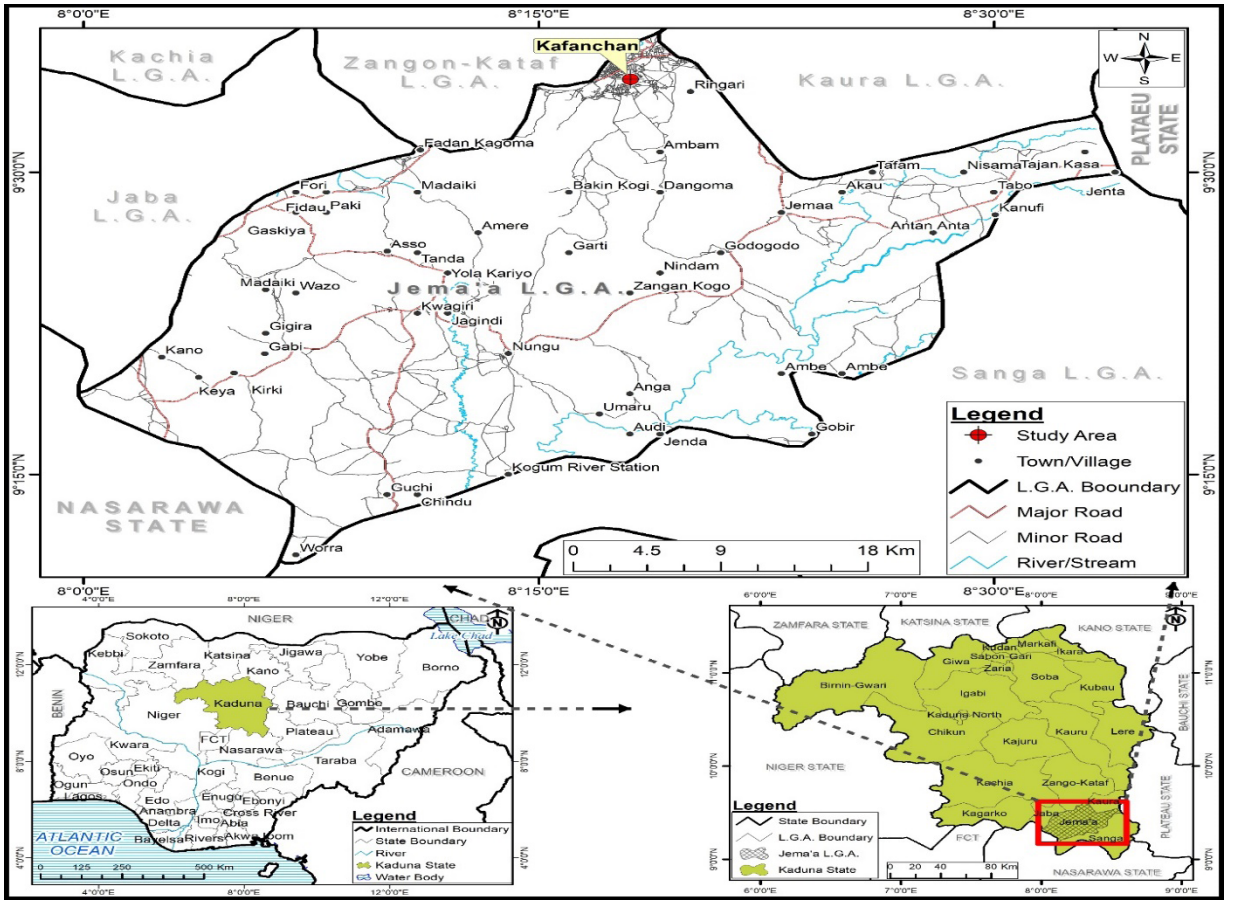

Figure 1. Map of Study Area (Kafanchan Metropolis) in Jema'a L.G.A. Source: Modified from https://www.openstreetmap.org/

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 the 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

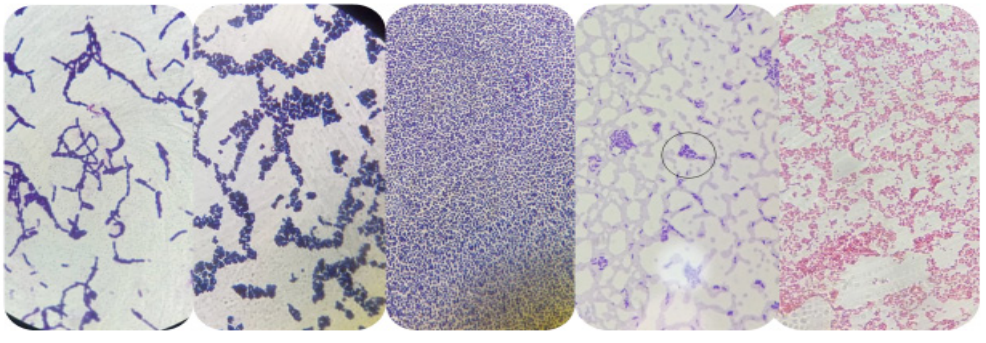

Figure 2. Photomicrograph of bacteria isolated from varieties of processed meat samples collected within the study area. A = Gram-positive rods, B= Gram-positive cocci in clusters, C= Gram-positive cocci in pairs, D= Gram-positive rods parallel to each other, and E= Gram-negative rods.

In this study, five different bacteria were isolated and identified which include; *Bacillus* spp. *Corynebacterium* species, *Enterococcus* species. *Staphylococci aureus*, and *Pseudomonas aeruginosa* (Figure 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 bacterium identified each which is, *Staphylococci aureus* (Figure 3).

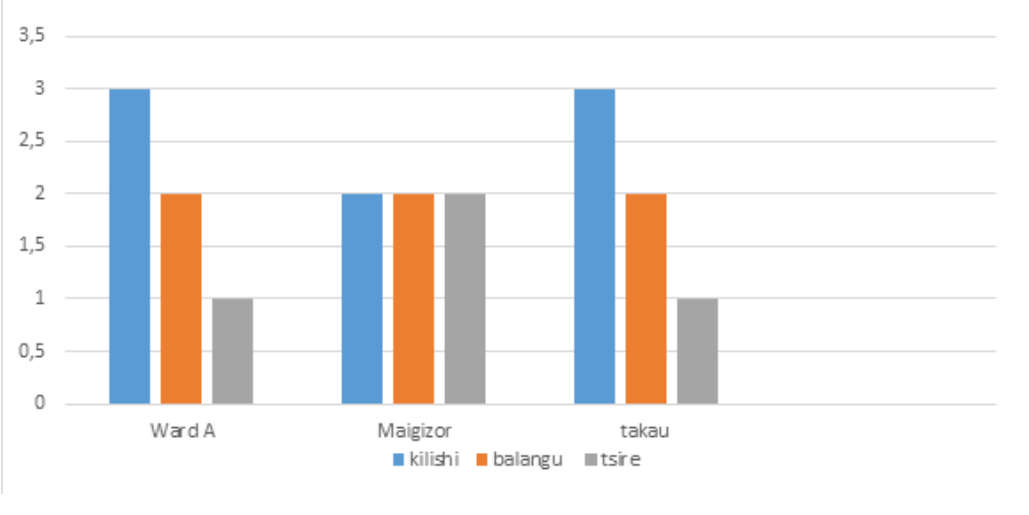

Figure 3. Frequency of Bacteria Isolated from Processed Meat Products Sold within Kafanchan Metropolis.

Meat and meat products play an important role in the maintenance of human health by providing all essential nutrients 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 (Swanson et al., 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.

Table 1. Phenotypic Identification of Gram-positive Bacteria Isolated from Variety 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

Four Gram-positive bacteria were identified, which include; *Corynebacterieum specie*, *Staphylococci aureus*, *Enterococcus species* and *Bacillus species* (Table 1). *Staphylococci aureus* and *staphylococci* species were prevalent in meat samples from all the three wards. C*orynebacterium* 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 Bacteria 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 indicated 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 indicated a potential breakdown of hygiene at various stages of the meat processing. Also, most of the suya vendors in Kafanchan used inked paper, old and abandoned newspapers for packaging which may be considered dirty, dusty and contaminated.

Table 3 showed multidrug resistant pattern of the bacteria isolated from different meat samples processed within Kafanchan metropolis. From the results obtained, it is evident that all the samples collected from the three selling points in Kafanchan were resistant to at least one or more of the antibiotics tested, including Amoxicillin clavulanic acid, cefixime, cefexotin, azithromycin, ceftriaxone, and gentamycin. The Tsire sample from Takau ward exhibited the highest resistance level to the antibiotics tested, with a Multi-Drug Resistance (MDR) Index of 0.9%. This was followed by Kafanchan ward A, which had a MDR Index of 0.7%. The Balangu sample from Takau and Maigizor wards had lower MDR Index values of 0.6% compared to the Tsire samples. The Kilish samples had the lowest MDR Index values of 0.3% compared to the other meat samples.

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

In this study, *Pseudomonas aeruginosa* was the only Gram-negative bacterium identified among others that were Gram-positive. The presence of Pseudomonas in the samples may be attributed to poor or improper heating of meat by the vendors in Kafanchan metropolis. This resistance to antibiotics could be a result of the bacteria developing resistance during production processes. It also highlights the presence of antibiotic-resistant bacteria in meat samples from Kafanchan, emphasizing the importance of proper food handling and preparation practices to prevent the spread of antibiotic resistance. Further research is needed to explore the mechanisms of antibiotic resistance in these bacteria and develop strategies to mitigate its impact on public health.

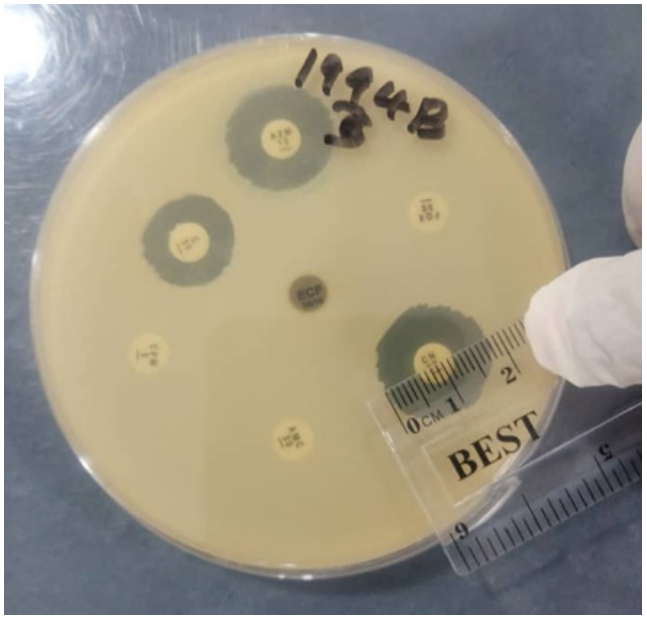

Figure 4. Multidrug-resistant pattern observed in Enterococcus sp. isolated from Kilishi meat sample in Kafanchan ward A of Jema'a LGA, Kaduna State.

Conclusion

In conclusion, the presence of pathogenic bacteria in meat products such as balangu, kilishi, and tsire poses a significant public health risk to consumers. The identification of *Staphylococcus aureus* (45%), *Corynebacterium species* (10%), *Enterococcus species* (10%), *Bacillus species* (20%), and *Pseudomonas aeruginosa* (5%) in these products highlights the need for improved sanitation practices and proper handling of meat during processing and preparation.

The potential for foodborne infections and outbreaks of food poisoning due to the presence of these bacteria underscores the importance of implementing strict food safety measures. The National Agency for Food and Drugs Administration and Control should play a key role in educating both producers and consumers on the importance of sanitation and personal hygiene in food production.

Consumers can also take steps to protect themselves by ensuring that meat products, such as suya, are properly reheated before consumption to eliminate any harmful bacteria. Additionally, the use of clean packaging materials, such as foil paper or polythene bags, can help prevent contamination during storage and transportation.

Furthermore, the responsible use of antibiotics in animal husbandry is crucial in preventing the development of antibiotic-resistant bacteria in meat products. By following recommended dosages and withdrawal periods before slaughter, producers can help reduce the risk of antibiotic residues in meat that could pose a threat to human health.

Overall, addressing the presence of pathogenic bacteria in meat products requires a collaborative effort between regulatory agencies, producers, and consumers. By implementing proper food safety practices and promoting awareness of the risks associated with contaminated meat products, we can work towards ensuring the safety and well-being of consumers.

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