Tropical Journal of Applied Natural Science

Vol. 3 Issue 1 (2025)

ISSN: 2449-2043

https://tjansonline.org/view-paper.php?id=72;

Volume/Issue: Volume 3, Issue 1 **Published:** July 19, 2025

ANTIBIOTIC RESISTANT PROFILE OF THE BACTERIAL STRAINS ISOLATED FROM GOAT AND RABBIT MEAT OBTAINED FROM LOCAL MEAT VENDORS.

Okeke, M.I., Okpalla, J, and Uba, B.O

Department of Microbiology ,Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State.

**Ifylove247@yahoo.com, 08068355322*

ABSTRACT

Antibiotic resistance in bacteria from food sources is a growing public health concern, particularly in developing regions where meat hygiene standards vary. This study investigated the antibiotic resistance profile of bacterial strains isolated from goat and rabbit meat obtained from local meat vendors in Nkwo ogbe market Ihiala and Afor Nnobi market, both in Anambra State. Fresh samples of goat and rabbit meat were collected from selected vendors. Bacterial strains were isolated using standard microbiological techniques, including selective media, Spread plate, Streak plate technique, and biochemical identification tests. The antibiotic susceptibility of the isolates was determined using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included, Ciprofloxacin, Tarivid, Reflacin,Nalidixicacid,Ceporex,Ampicillin,Septrin,Augmentin,Gentamycin,Streptomycin,Ampiclox,Norfloxacin,Amoxil,,Chloramphenicol,Erythromycin,Rifampicin,Levofloxacin. A high bacterial load was observed in both goat and rabbit meat samples, with *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, and *Bacillus sp.* being the predominant isolates. Antibiotic susceptibility testing revealed that 100% of the tested Gram negative bacteria pathogens were resistant to Streptomycin and Augmentin, while 57.14% were Susceptible to Ciprofloxacin and Septrin. Also 87.50% of the tested Gram positive bacteria were resistant to Ampiclox, Erythromycin, and Chloramphenicol, while 100% and 87.50% were susceptible to Levofloxacin and Rifampicin respectively. Multidrug resistance (MDR) patterns were detected in 94% of the isolates, raising concerns about potential health risks associated with the consumption of contaminated meat. The findings highlight the presence of antibiotic-resistant bacteria in goat and rabbit meat from local vendors, posing potential health risks to consumers. The high prevalence of MDR strains suggests the misuse of antibiotics in animal husbandry and the need for stricter regulations on antibiotic use. Improved hygiene practices, routine surveillance, and public awareness campaigns are essential to mitigate the spread of resistant bacterial strains and ensure meat safety.

Key: Antibiotic resistance, MDR.

INTRODUCTION

The emergence of antibiotic-resistant bacteria in food products is a significant public health concern, particularly in developing countries where regulatory oversight may be limited. Goat and rabbit meats are staple proteins in many communities, including Nigeria, and their contamination with resistant bacterial strains poses serious health risks to consumers. Understanding the prevalence and resistance profiles of these bacteria is essential for developing effective control measures.

Several studies have highlighted the contamination of meat products with antibiotic-resistant bacteria. For instance, Uzeh *et al*. (2021) reported the presence of multidrug-resistant *Enterobacteriaceae* in retail meats in Lagos, Nigeria, emphasizing the potential health hazards associated with consuming such products (Uzeh *et al*.,2021).Similarly, Sun *et al*. (2024) identified antimicrobial resistance in *Escherichia coli* and *Enterococcus* species isolated from meat rabbits in Chengdu, China, underscoring the global nature of this issue. The misuse of antibiotics in animal husbandry is a significant factor contributing to the development of resistant bacterial strains. In Nigeria, the unregulated use of antimicrobials in livestock has been documented, leading to the proliferation of resistant pathogens in the food chain. (Oloso, *et.al*. 2018) This situation is exacerbated by inadequate hygiene practices during meat processing and handling, which facilitate the spread of these pathogens.

The aim of this study is to investigate the antibiotic resistance profiles of bacterial strains isolated from goat and rabbit meat obtained from local meat vendors. By identifying the specific bacteria present and their resistance patterns, this research seeks to inform public health strategies and policy decisions aimed at mitigating the spread of antibiotic-resistant bacteria through the food supply chain.

MATERIALS AND METHODS

Meat Sample Source and Collection: The fresh goat meat sample was obtained from local meat vendors at Nkwo Ogbe Market Ihiala situated in Ihiala Local Government Area while the rabbit meat samples was obtained from Afor Nnobi Market, Nnobi situated in Idemili South Local Government Area of Anambra State.

Pre- Enrichment and Enumeration of Enteric Bacteria: The raw meat samples (25 g) were mashed and then inoculated in buffered peptone water and Rapport Vassiliadis media for pre-enrichment cultures. The media were then stored at 37°C in a rotary shaker for 16 - 24 h, this enumeration process was done using the method described by Budiarso *et al.* (2021). Cell cultures were serially diluted to 10^{-7} with 0.1 % phosphate saline buffer solution (0.85%). Next, 0.1 mL cell cultures were inoculated from the 10^{-5}, 10^{-6}, and 10^{-7} dilutions using the spread plate technique on the surfaces of chromocult coliform agar (CCA), Salmonella Shigella Agar (SSA), Baird Parker Agar (BPA) supplemented with egg yolk tellurite emulsion, Mannitol Yolk Polymyxin Agar (MYP) and Plate Count Agar for *E. coli* (dark blue to violet colour), *Salmonella* (colourless with black centre), *S. aureus* (black colour), *Bacillus cereus* (pink – orange colour) and total aerobic mesophilic bacteria (TAMB), respectively. The plates were incubated at 37 ± 1 °C and 30 ± 1 °C for 48 h. The colonies that emerged were counted using a colony counter and

calculated coliform forming unit per gram (CFU/g) of raw meat.

Antimicrobial Resistance Testing: Preparation of microbial inoculums for antimicrobial sensitivity screening was carried out according to the method described by Umana *et al*. (2017). In this method, colonies of
pure bacteria isolates from their stock cultures was transferred into prepared nutrient broth (NB) using sterile inoculating wire-loop and incubated at 37 °C for 24 h.

The Clinical and Laboratory Standard Institute (CLSI) disc diffusion method of (2005) was used for the antibiotic sensitivity test. The turbidity of the inocula of various isolates was made to be equivalent to 0.5 of McFarland standard and each of the isolates was inoculated onto the surface of Muller Hinton agar using sterile swab sticks. The antimicrobial agents tested were: ciproflaxin 10 µg, norfloxacin 10 µg, gentamycin 10 µg, tarivid 10 µg, reflacine 10 µg, ceporex 10 µg, amoxicillin 20 µg, rifampicin 20 µg, ampiclox 20 µg, levofloxacin 20 µg, erythromycin 20 µg, streptomycin 30 µg, chloramphenicol 30 µg, augmentin 30 µg, nalidixic acid, septrin 30 µg, ampicillin 30 µg) (Opton Disc, Nigeria). These were aseptically placed on the surface of the inoculated agar plates. After 30 mins of applying the discs, the agar plates were inverted and incubated for 24 hrs at room temperature (Uba et al., 2018b). The clear zones that developed around each disc were measured as the zones of inhibition on the basis of CLSI guidelines. The recorded values were into standard resistant range: 1 – 10 mm; standard moderately susceptible range: 11 – 19 mm and standard susceptible range: 13 – 30 mm, respectively.

Multiple antibiotics resistance (MAR) index determination: The multiple antibiotic resistances (MAR) index was determined for each of the selected bacterial strains by dividing the number of antibiotics to which the strains were resistant by the total number of antibiotics tested as described by Umana *et al*., (2017).

Results and Discussion

Table 1: Bacterial loads (Log CFU/g) of rotten goat and rabbit meat sample

Bacterial flora/food sample	THBC	*Escherichia coli*	*Salmonella* sp.	*Staphylococcus aureus*	*Bacillus cereus*
Goat	TNTC	6.36	6.10	5.32	5.54
Rabbit	TNTC	6.15	5.83	5.26	5.64

Key: CFU/g = Coliform forming unit, THBC = Total heterotrophic bacterial count, sp = Species, TNTC = Too numerous to count.

Table 2: Antibacterial susceptibility pattern of the selected Gram negative bacterial pathogen (mm)

Test antibiotics	Test pathogen						
	ECG 1	ECG 2	ECR 1	ECR 2	SR 1	SR 2	SG 1
Ciprofloxcin	0.00± 0.00	24.00± 0.20	47.00± 0.20	2.00± 0.01	19.00± 0.20	0.00± 0.00	19.00± 0.10
Tarivid	0.00± 0.00	54.00± 0.20	2.00± 0.01	0.00± 0.00	16.00± 0.10	0.00± 0.00	0.00± 0.00
Reflacin	3.00± 0.02	18.00± 0.10	0.00± 0.00	25.00± 0.10	19.00± 0.10	0.00± 0.00	18.00± 0.10
Nalidixic acid	25.00± 0.10	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
Ceporex	24.00± 0.10	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
Ampicillin	53.00± 0.20	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
Septrin	22.00± 0.10	24.00± 0.03	21.00± 0.01m	38.00± 0.10	16.00± 0.01	15.00± 0.10	16.00± 0.2
Augmentin	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
Gentamicin	0.00± 0.00	2.00± 0.01	19.00± 0.10	0.00± 0.00	0.00± 0.00	0.00± 0.00	16.00± 0.10
Streptomycin	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00

Key; ECG=*Echerichia coli* from Goat meat, ECR= *Echerichia coli* from Rabbit meat, SR=*Salmonella* from Rabbit meat, SG=*Salmonella* from Goat meat.

Table 3: Antibacterial susceptibility patterns of the isolated Gram positive bacterial pathogen (mm)

Test antibiotics	Test pathogen							
	BCG 1	BCG 2	BCR 1	BCR 2	SAG 1	SAG 2	SAR 1	SAR 2
Norfloxacin	0.00± 0.00	0.00 ± 0.00	0.00± 0.00	0.00± 0.00	18.00± 0.10	0.00± 0.00	0.00± 0.00	23.00± 0.10
Ampiclox	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	15.00± 0.20	0.00± 0.00	0.00± 0.00	0.00± 0.00
Amoxil	52.00± 0.20	0.00± 0.00	0.00± 0.00	0.00± 0.00	22.00± 0.10	00.00± 0.10	27.00± 0.10	24.00± 0.10
Gentamicin	0.00± 0.00	0.00± 0.00	37.00± 0.20	15.00± 0.10	20.00± 0.10	25.00± 0.10	26.00± 0.10	24.00± 0.20
Levofloxacin	53.00± 0.40	53.00± 0.30	53.00± 0.10	21.00± 0.00	24.00± 0.20	25.00± 0.20	25.00± 0.10	23.00± 0.10
Erythromycin	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	25.00± 0.10	0.00± 0.00	0.00± 0.10	0.00± 0.00
Ciprofloxacin	36.00± 0.00	0.00± 0.00	36.00± 0.20	0.00± 0.00	25.00± 0.20	26.00± 0.20	24.00± 0.10	20.00± 0.10
Chloramphenicol	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	28.00± 0.10	0.00± 0.00	0.00± 0.00	0.00± 0.00
Streptomycin	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	20.00± 0.10	00.00± 0.20	26.00± 0.20	25.00± 0.10
Rifampicin	42.00± 0.20	0.00± 0.00	45.00± 0.10	42.00± 0.00	21.00± 0.10	27.00± 0.10	25.00± 0.20	25.00± 0.20

Key: BCG = *Bacillus sp* from goat meat sample; BCR = *Bacillus sp* from Rabbit meat sample, SAG=*Staphylococcu sps* from Goat meat, SAR=*Staphylococcus sp* from Rabbit meat sample

Table 4: Multiple antibiotics resistance (MAR) index of the selected bacterial strain exposed to gram negative and positive antibiotics

Isolate code	Total number of antibiotics tested	Number of resistance	MAR index
ECG 1	10.0	5.0	0.5
ECG 2	10.0	5.0	0.5
ECR 1	10.0	6.0	0.6
ECR 2	10.0	7.0	0.7
BCG 1	10.0	6.0	0.6
BCG 2	10.0	9.0	0.9
BCR 1	10.0	6.0	0.6
BCR 2	10.0	7.0	0.7
SAG 1	10.0	0.0	0.0
SAG 2	10.0	6.0	0.6
SAR 1	10.0	4.0	0.4
SAR 2	10.0	3.0	0.3
SR 1	10.0	6.0	0.6
SR 2	10.0	9.0	0.9
SG 1	10.0	6.0	0.6

Key; ECG=*Escherichia coli* from Goat meat, ECR= *Escherichia coli* from Rabbit meat, SR=*Salmonella* from Rabbit meat, SG=*Salmonella* from Goat meat, BCG = *Bacillus sp* from goat meat sample; BCR = *Bacillus sp* from Rabbit meat sample, SAG=*Staphylococcus sp* from Goat meat, SAR=*Staphylococcus sp* from Rabbit meat sample

DISCUSSION

The current study sampled goat and rabbit raw meat from local meat vendors at Nkwo Ogbe Market Ihiala and Afor Nnobi Market, both in Anambra state. The Enteropathogenic contamination was detected using enrichment and spread plate technique The prevalence and distribution of *E.coli*, *Salmonella* sp. *Bacillus sp* and *Staphylococcus aureus* pathogens in goat meat sample was more than the rabbit meat sample except in the *Bacillus cereus* pathogens (Table 1). The high count of these isolated pathogens in especially the members of enterobacteriaceae from the tested animals has been implicated in a clinical and public health crisis. The *Enterobacteriaceae* family is a normal and healthy component of animal gut microbiota, which may explain its widespread distribution in tested meat. Furthermore, the origin of these bacteria, as well as multiple transmission routes during the production and handling of animal derived foods, prompted food safety authorities to adopt *Enterobacteriaceae* and/or their members as a valuable microbiological indicator of food safety, quality, and hygiene (Edris *et al*., 2023).

The majority of isolated bacteria strains from the raw goat and rabbit meat samples especially the suspected *Bacillus cereus* and *Salmonella enterica* possessed high level of resistance and MAR index values up to 0.9(Tables 2,.3, and 4.) to several gram negative or positive antibiotics used in this study. The bacterial isolates may have been resistant to some of these antibiotics because of the production of enzymes which inactivate or modify antibiotics, cause changes in bacteria cell membrane, modification of target site and development of metabolic pathways by bacteria (Uba *et al*. 2018a). Similar findings were obtained by Ekpo *et al*. (2017) in their study on food borne pathogens. Moreso, uncontrolled use of antibiotics for empirical treatment of infectious diseases in the test animals has been implicated as a cause of high prevalence of these antibiotics resistance (Ekpo *et al*., 2017; Umana *et al*., 2017).

CONCLUSION

In this study, goat and rabbit raw meat samples were found to contain variable bacterial loads in which *E.coli, Salmonella* species, *Bacillus cereus* and *Staphylococcus aureus* species were implicated. These selected bacterial strains were found to possess, multiple antibiotics resistance.

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