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BACTERIAL LOAD, HAEMOLYTIC AND ENZYMATIC ACTIVITY PROFILE OF BACTERIAL STRAINS IN GOAT AND RABBIT MEAT SAMPLES OBTAINED FROM LOCAL MEAT VENDORS.

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

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

08068355322

**Ifylove247@yahoo.com*

ABSTRACT

Meat is a highly perishable food product, and its microbial quality is crucial for consumer safety. This study assessed the bacterial load and enzymatic activity profile of bacterial strains isolated from goat and rabbit meat samples obtained from local meat vendors in Nkwo Ogbe market Ihiala and Afor Nnobi market, both in Anambra state. Freshly slaughtered goat and rabbit meat samples were collected from five different vendors. Standard microbiological techniques, including enrichment technique, serial dilution, Spread plate and Streak plate methods, were used to determine the total bacterial count. Bacterial strains were isolated, purified, and identified using biochemical and molecular techniques. Haemolytic tests were carried out using blood agar, while enzymatic activity was assessed using qualitative and quantitative assays for Amylase, Caseinase, Gelatinase, Esculinase, Lipase, and Urease production. The total bacterial load in goat and rabbit meat samples were too numerous to count, *Escherichia, Salmonella, Staphylococcus, and Bacillus* count ranges from 6.15-6.36 log CFU/g, 5.83-6.10 log CFU/g, 5.26-5.32 log CFU/g, and 5.54-5.64 log CFU/g respectively in goat and rabbit meat. The predominant bacterial isolates include, *Escherichia coli*. *Salmonella sp, Staphyloccocus sp,* and *Bacillus* sp. The haemolytic analysis showed that all the 15 selected bacterial strains had capacity to produce haemolysin, while enzymatic activity profiling revealed that most isolates exhibited significant amylase ,lipase and urease activity, which are associated with meat spoilage and potential role in carbohydrate metabolism within the meat matrix. The study highlights the presence of diverse possible bacterial pathogens, with enzymatic activity that contribute to meat deterioration. Poor hygiene and improper storage practices among local vendors may enhance bacterial proliferation. Implementing proper meat handling, refrigeration, and hygiene measures can help minimize bacterial contamination and enzymatic degradation, ensuring better meat quality and safety for consumers.

Key; Antibiotic resistant, MDR

INTRODUCTION

The safety and quality of meat products are critical concerns in public health, particularly in regions where meat is sourced from local vendors with varying hygiene practices. Goat and rabbit meats are widely consumed in many communities due to their nutritional benefits and cultural significance. However, these meats can harbor bacterial contaminants that pose health risks to consumers. Understanding the bacterial load and enzymatic activity profiles of bacterial strains present in these meats is essential for developing effective strategies to ensure meat safety and quality. Studies have identified various bacterial pathogens associated with meat products. For instance, *Staphylococcus aureus* has been frequently isolated from animal-derived foods in Nigeria, raising concerns about its prevalence and antimicrobial resistance profiles.(Odetokun *et al*.,2023).Similarly, research on fresh meat marketed in Owerri, Imo State, Nigeria, revealed significant microbial loads, including coliforms and *Salmonella-Shigella* species, indicating potential health hazards(Mgbemena *et al* 2017).These findings underscore the need for continuous monitoring of bacterial contamination in meat products.

Beyond mere presence, the enzymatic activities of these bacterial strains play a pivotal role in meat spoilage and pathogenicity. Enzymes such as proteases and lipases, produced by bacteria, can degrade meat proteins and fats, leading to spoilage and off-flavors. A recent study highlighted that strains of *Candida zeylanoides* and *Candida sake* exhibiting high proteolytic and lipolytic activities effectively hydrolyzed beef fat and proteins, contributing to meat spoilage (Liu *et al*., 2025). Understanding these enzymatic profiles is crucial for developing targeted interventions to inhibit bacterial growth and enzymatic activity, thereby extending the shelf life of meat products.

The aim of this study is to assess the bacterial load and characterize the enzymatic activity profiles of bacterial strains isolated from goat and rabbit meat samples obtained from local meat vendors. By identifying the specific bacteria present and their enzymatic activities, this research seeks to provide insights into the potential health risks associated with the consumption of these meats and to inform strategies for improving meat safety and quality in local markets.

MATERIALS AND METHODS

Study Area/Sample 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.

Bacteria Enumeration

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.

Haemolytic test

Haemolysis test was carried out using blood agar. The isolated bacterial strains were introduced onto blood agar by streaking, incubated at 37 °C for 24 h and the reaction of the isolates on the plates were designated as: alpha (α) haemolysis which indicates a greenish cloudy zone around the colony; beta (β) haemolysis: also known as complete lysis, a clear zone with a clean edge around the colony and gamma (γ) haemolysis in which lysis does not occur indicating no change in the blood agar around the colony. Each bacterial strain was tested for its haemolytic activity on different blood agar plates using cow blood as described by Olusola – Makinde *et al.* (2021).

Enzymatic/metabolite production/reaction

Caseinase production

To evaluate the production of caseinase, the strains were streaked on nutritive agar supplemented with 10 % soluble casein, Precipitation or zone of clearance surrounding the growth area after 24 h incubation indicates casein proteolysis as reported by Preda *et al*., (2021).

Esculinase production

For esculinase production, the strains were spotted on medium containing esculin and Fe^{3+} citrate. The positive result was indicated by the black colour after the enzymatic action of beta-galactosidase transforming esculin to esculethol and glucose. Esculethol reacts with the ferric citrate in the media to produce a dark precipitate as descried by Preda *et al*., (2021).

Lipase production

For lipase production, using the method described by Preda *et al.,* (2021), the strains were spotted on medium supplemented 1 % Tween 80 and incubated at 37 ° C for 24 h. After incubation, the plates were flooded with neutral red dye to increase the colour contrast precipitation and dark orange clearance zone indicate a positive reaction.

Amylase production

For amylase production, the strains were spotted on starch agar medium and incubated at 37 ° C for 24 h. After incubation, the plates were flooded with iodine solution and transparent clearance zone with a blue – black medium background indicate a positive reaction (Preda *et al*., 2021).

RESULTS/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: Gram reaction of the selected bacterial strains and their haemolytic activity

Isolate code	Gram reaction and shape	Haemolytic response
ECG 1	Gram negative rod shaped	Beta haemolysis
ECG 2	Gram negative rod shaped	Beta haemolysis
ECR 1	Gram negative rod shaped	Beta haemolysis
ECR 2	Gram negative rod shaped	Beta haemolysis
BCG 1	Gram positive rod shaped	Beta haemolysis
BCG 2	Gram positive rod shaped	Beta haemolysis
BCR 1	Gram positive rod shaped	Beta haemolysis
BCR 2	Gram positive rod shaped	Beta haemolysis
SAG 1	Gram positive coccus shaped	Beta haemolysis
SAG 2	Gram positive coccus shaped	Beta haemolysis
SAR 1	Gram positive coccus shaped	Beta haemolysis
SAR 2	Gram positive coccus shaped	Beta haemolysis
SG 1	Gram negative rod shaped	Beta haemolysis
SR 1	Gram negative rod shaped	Beta hemolysis
SR 2	Gram negative rod shaped	Beta hemolysis

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

Table 3: Enzymatic activity profile of the selected bacterial strains

Isolate code	Amylase	Caseinase	Gelatinase	Aesculinase	Lipase	Urease
ECG 1	+	+	-	-	-	+
ECG 2	+	+	-	-	-	+
ECR 1	+	+	-	-	+	+
ECR 2	+	+	-	+	+	+
BCG 1	+	-	-	-	+	+
BCG 2	+	+	-	+	+	+
BCR 1	+	+	-	-	+	+
BCR 2	+	-	-	-	-	+
SAG 1	+	-.	-	-	+	+
SAG 2	+	+	-	+	+	+
SAR 1	+	-	-	-	+	+
SAR 2	+	+	-	-	+	+
SR 1	+	+	-	+	+	+
SR 2	+	+	-	+	+	+
SG 1	+	+	-	-	+	+

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

Foodborne zoonotic agents such as *Shigella*, *Salmonella*, and *E. coli* are the focus of most food safety research (Edris *et al*., 2023). Bacteria contamination detected in the meat samples include *E.coli, Bacillus sp, Salmonella sp. Staphylococcus sp.* (Table 1). 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).

All the 15 selected bacterial strains had capacities to produce haemolysin (Table 2). The presence of these strains on the raw goat and rabbit meat samples could be strongly linked to human-related activities (Olusola - Makinde *et al*., 2021). Haemolysin is a pore-forming toxin that causes cell membrane injury and cell death. Since all the goat and rabbit bacterial strains presented haemolytic activities, production and exhibition of haemolytic activity by these strains is a factor of differential genetic expression. Haemolysin production and its virulence potential is strain specific and correlate with increased invasiveness and severity (Orole *et al*., 2022).

The Bacterial strains exhibited virulence factor expression, with the most common ones being the synthesis of amylase, urease, caseinase and lipase (Table 3) .Although the strains were isolated from raw goat and rabbit meat samples, it is crucial to identify these factors. The majority of the bacterial strains produced lipase, a factor that translates into the ability to convert local host tissues into nutrients vital for bacterial development and also augments the possibility to penetrate and affect host tissues (Preda *et al*., 2021).

CONCLUSION

In this work, goat and rabbit raw meat samples were found to contain diverse bacterial loads in which *E.coli, Salmonella* species, *Bacillus cereus* and Staphylococcus *aureus* species were implicated. From the results of this work the selected bacterial strains were found to possess variable degree of pathogenicity.

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