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Assessment of the microbiological quality of bushmeat sold in southern Benin

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ABSTRACT

Bushmeat serves as a significant protein source in Benin; however, its distribution via informal channels poses microbial risks. This study aimed to assess the microbiological quality of the most consumed bushmeat species (francolin, grasscutter, hare, and squirrel) in the Tègon and Allada markets of southern Benin and determine their sources of contamination. A total of 118 samples were collected from two major markets (Tègon and Allada) in both raw and processed (grilled/smoked or fried) forms. Microbiological analyses were conducted to quantify total aerobic counts (TAC), fecal coliforms, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* (log_{10} cfu/g) according to relevant ISO standards. Pathogens, specifically *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*, were isolated and identified using ISO standards. The influence of location and species was assessed using ANOVA. Differences between preparation methods were analyzed using R, and p-values were reported. Results show that the preparation method significantly influenced microbial loads: fried samples exhibited the lowest contamination levels, followed by grilled and then raw meats (raw > grilled/smoked > fried). The prevalence rates were 100% for TAC, 75% for coliforms, 49% for *E. coli*, and 12% for *Listeria monocytogenes*. No samples tested positive for *Salmonella* spp. or *Staphylococcus aureus*. Location and species did not significantly affect microbial variability. The investigation found poor hygiene in meat handling before and after cooking. Consequently, inadequate handling and cooking affect bushmeat safety in Benin, not species or location. Standardizing thermal processing and improving hygiene are critical to reduce microbial risks for consumers.

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1. Introduction

Bushmeat refers to the meat of wild animals consumed by humans, especially in rural African settings (1). The meat's composition, characterized by a high protein and fat content, creates an optimal environment for bacterial proliferation. These proteins supply vital amino acids for microbial growth, while the lipids act as a carbon source (2). The nutritional composition of meat, combined with its high moisture content, promotes the proliferation of spoilage bacteria and pathogens, including *Pseudomonas*, *Yersinia*, *Listeria*,

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and *Salmonella,* leading to rapid decomposition and an increased risk of foodborne illness, even under refrigeration (3).

In contrast to meat sourced from butcher shops, which is derived from domesticated animals processed under regulated methods, bushmeat is obtained from animals captured using various methods, including traps and guns. These animals are then sold in their current state or after processing in markets and restaurants (4–6). Indeed, bushmeat value chains, informal handling, lack of hygiene norms, and transportation generally devoid of a proper cold chain facilitate infection and degradation of carcasses, consequently exposing customers to heightened health risks in comparison to conventional butchery chains (7,8).

A 2024 report by the World Health Organization (WHO) indicates that approximately 600 million individuals fall ill and 420,000 die annually due to the consumption of contaminated food, encompassing all causes (World Health Organization, 2024). A substantial portion of the global burden of foodborne diseases is acknowledged by health authorities as a significant and pervasive threat to public health, particularly due to their zoonotic origins. Consequently, foodborne zoonoses represent a substantial issue for global food safety (9,10).

Foodborne illnesses encompass a range of infections caused by various bacteria, including *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, pathogenic *E. coli*, and *Yersinia*. They also involve poisoning linked to bacterial toxins such as *Staphylococcus aureus*, *Clostridium perfringens*, *C. botulinum*, and *Bacillus cereus*, as well as parasitic infections from Trichinella, Toxoplasma, Cryptosporidium, and Giardia, and viral infections like norovirus/Caliciviridae, rotavirus, and hepatitis A and E, all transmitted through contaminated food (9). Investigations conducted in African markets indicate that bushmeat available for sale is contaminated with spoilage bacteria and pathogens, including *E. coli*, *Klebsiella*, *Staphylococcus*, and, occasionally, *Salmonella,* at elevated levels, thereby posing a health risk to consumers (11–13). A study conducted in Nigeria analyzed smoked rat meat sold in markets, revealing a prevalence of 1.3% for *E. coli* O157:H7 and *Salmonella* spp. at 0.5%, in addition to significantly elevated total loads (11). A study conducted in Ghana focusing on game (rodents, antelopes, civets) at the Kumasi market found mesophilic aerobic flora reaching up to 10.9 $\log_{10}$ cfu/g, along with *E. coli* and *Klebsiella* (13). The WHO Global Strategy for Food Safety 2022-2030 highlights that undertaking risk analysis is essential for regulatory bodies and the food industry to safeguard public health and ensure food safety (14). This approach integrates risk assessment and management with effective communication to identify hazards, select and apply control measures, and subsequently monitor and refine them.

In Benin, bushmeat consumption occurs for various reasons. In southern Benin, this meat is available in restaurants and along major roads, in both processed and unprocessed forms (15). These locations along major routes serve as specialized markets dedicated exclusively to the trade of bushmeat, particularly in Tègon and Allada in southern Benin. In most African countries, the predominant bushmeat consists of small animals, with grasscutter bushmeat being the most prevalent due to its availability, affordability, and

palatability (16–18). In Benin, Grasscutter and other small species are mostly consumed (8,19). This study assesses the microbiological quality of commonly consumed small bushmeat species available in various marketplaces, focusing on processing processes to identify risks associated with their sale and consumption, and to suggest corrective actions aimed at enhancing food safety in Benin.

2. Materials and Methods

2.1. Study area

The study was conducted in southern Benin, focusing on two major bushmeat markets, Tègon (Zogbodomey, Zou district) and Allada (Atlantique district). Both markets are supplied by the Lama Forest, a key forest reserve spanning 16,250 hectares across the Zou and Atlantique departments.

2.2. Sampling and data collection

A total of 116 samples were randomly collected from bushmeat vendors across the two markets, including grasscutter, squirrel, francolin, and hare carcasses. Raw and grilled/smoked bushmeat samples were collected in the Tègon, while raw and fried samples were collected from Allada markets. Samples were collected weekly from each market, with variations in the sampling days. The samples were collected according to the species present, with four selected species. Sixty-one samples were collected in Tègon Market, including six samples of grasscutter raw and nine grilled, seven raw and ten grilled squirrel, three raw and ten grilled francolin, six raw and ten grilled hare, and fifty-five samples were collected in Allada Market including five raw, nine fried grasscutter, three raw, ten fried squirrel, five raw, ten fried francolin, four raw nine hare, samples. The samples were obtained under aseptic conditions, sealed in sterile zip bags, preserved in a cooler with ice packs, and transported to the laboratory for analysis within 2 to 4 h after collection.

2.3. Determination and enumeration of bacteria present in bushmeat

2.3.1. Sample preparation and serial dilution

Twenty-five grams of each prepared sample were aseptically collected into sterile stomacher bags, subsequently followed by the addition of 225 mL of buffered peptone water (BPW). The mixture was homogenized using a stomacher, yielding a 10^{-1} serial diluted solution. Further serial dilutions were performed by transferring 1 mL of the prior dilution into 9 mL of sterile peptone water, repeating this procedure until the target dilution was attained. These dilutions were used to enumerate and identify the bacterial species present in the sample.

2.3.2. Determination of total aerobic count (TAC)

The total aerobic count (TAC) was evaluated in accordance with ISO 4833-2:2013 criteria. For each homogenized sample, suitable dilutions were prepared, and 1 mL aliquots were inoculated onto Plate Count Agar (PCA). Plates were incubated at 30°C for 72 h, after which colony counts were conducted to assess the total aerobic bacterial load (cfu/g). The average TAC values for each sample were subsequently determined using the formula:

$$\text{No. of bacteria in } \left(\frac{\text{cfu}}{\text{g}}\right) = \frac{\text{Number of colonies x reciprocal of dilution factor}}{\text{Inocilum size (mL)}}$$

2.3.3. Isolation and identification of *Salmonella* spp. from bushmeat samples

The identification of *Salmonella* spp. was carried out in four distinct phases in accordance with ISO 6579:2002 criteria. A pre-enrichment phase was conducted using a non-liquid selective medium by immersing 25 g of bushmeat in buffered peptone water at ambient temperature. Subsequently, incubation occurred at 37 ± 1°C for 18 ± 2 h. Subsequently, the enrichment procedure involved inoculating 1 mL and 0.1 mL of the pre-enriched solution into 10 mL of MKTTn broth and 10 mL of RVS broth, respectively. The prepared solutions were then incubated at 37 ± 1°C for 24 ± 3 h. The isolation procedure involved inoculating XLD agar and incubating at 37°C for 24 h to promote the growth of potential colonies. *Salmonella* confirmation was achieved by subculturing isolated colonies, followed by biochemical and serological tests to ensure precise identification.

2.3.4. Isolation and identification of *Staphylococcus aureus* from bushmeat samples

The isolation and identification of *Staphylococcus aureus* were performed according to the protocols specified in ISO 6888-2:2021. A 25 g sample of bushmeat was obtained and subsequently diluted to prepare the suspension required for inoculation. Sample suspensions were prepared and inoculated onto Baird-Parker agar supplemented with Rabbit Plasma Fibrinogen (RPFA) using a double-layer technique. The inoculated dishes were subsequently incubated at 37°C for 24 h under aerobic conditions. Following incubation, colonies exhibiting a gray-black hue with a surrounding halo were enumerated to evaluate the quantity of *Staphylococcus aureus* in the sample.

2.3.5. Enumeration of *Escherichia coli* from bushmeat samples

The enumeration of *Escherichia coli* was conducted in accordance with ISO standard 16649-2:2001. An initial stock suspension was prepared from each sample of bushmeat, from which a series of decimal dilutions was made. For each sample, 1 mL of the stock suspension and each dilution was plated in duplicate by pouring into plates containing Tryptone Bile X-Glucuronide (TBX) medium. Subsequently, all plates were incubated under aerobic conditions at 44°C for 24 h. Presumptive *E. coli* colonies, identified by their characteristic blue coloration, were enumerated.

2.3.6. Quantification of *Listeria monocytogenes* in bushmeat samples

The quantification of *Listeria monocytogenes* was performed in five steps, according to ISO 11290:2017. Briefly, a stock suspension of bushmeat samples was initially prepared in accordance with the guidelines outlined in the standard for microbiological examination. Subsequently, 0.1 mL of the appropriate dilution was spread-plated onto the surface of Agar Listeria according to the Ottaviani & Agosti (ALOA) method. The seeded plates were incubated at 37°C for 48 ± 2 h. Presumptive *L. monocytogenes* colonies, characterized by a blue-green halo resulting from phosphatidylinositol hydrolysis and a white precipitate due to esculin hydrolysis, were counted to provide a precise assessment of their occurrence in the sample.

2.4. Statistical analysis

All statistical analyses were performed using R software (R version 4.5.1 (2025-06-13 ucrt) R foundation for statistical computing). Data manipulation and visualization were conducted using the dplyr and ggplot2 packages, respectively. Microbial counts, expressed as colony-forming units per gram (cfu/g),

were transformed to log_{10} to provide a normal distribution suitable for parametric analysis before comparison.

The prevalence of contamination (presence/absence) across categorical factors (Bacteria, Mode, Species, Locality) was assessed using Pearson's chi-square (χ^2) tests of independence performed on contingency tables. Results are presented as counts (n) and percentages (%).

Log_{10}-transformed microbial loads were compared across the levels of individual factors (Locality, Species, and Bacteria) using one-way analysis of variance (ANOVA). When ANOVA assumptions were violated, the non-parametric Kruskal-Wallis test was employed as a robust alternative. Model results are reported with their respective test statistic (F or H), degrees of freedom, and p-value.

All analyses employed a significance threshold of $\alpha = 0.05$. Data are summarized using descriptive statistics (mean ± standard deviation) and presented graphically as boxplots with the mean annotated.

3. Results

The microbiological analyses conducted on bushmeat, specifically francolin, grasscutter, hare, and squirrel, enabled the evaluation of the presence of various pathogens, fecal coliforms, and total aerobic counts (TAC). The samples were sourced from the Tègon market, encompassing both raw and grilled/smoked varieties, as well as from the Allada market, which included raw and fried options. The findings indicate that the preparation method has a significant impact on microbial loads for all examined bacteria, except for variations attributable to location or animal species.

3.1. Bacterial loads in raw, grilled, and fried bushmeat samples (Log_{10} cfu/g)

The assessment of mean bacterial loads (Log_{10} cfu/g ± standard deviation) by preparation method is presented in Table 1. Statistically significant differences were identified among all investigated microorganisms, as shown in Table 1. The highest TAC values were observed in raw meat (6.39 ± 0.51), followed by grilled meat (5.96 ± 1.25) and fried meat (5.88 ± 0.92), with significant differences among the groups ($p = 0.0498$). A highly significant difference was observed ($p = 0.0016$) in fecal coliform levels depending on the method of preparation. Raw meat had 3.47 ± 1.18 log_{10} cfu/g, compared to 2.47 ± 1.89 for grilled meat and 1.63 ± 1.61 for fried meat. Regarding *E. coli*, counts also differed significantly by preparation method ($p < 0.001$). Raw meat had 3.23 ± 1.14 log_{10} cfu/g, compared to 1.18 ± 1.52 for grilled meat and 0.13 ± 0.47 for fried meat. The loads exhibited variability based on the preparation method ($p = 0.0076$). The analysis revealed that for *Listeria monocytogenes,* raw meat had a count of 0.90 ± 1.72 log_{10} cfu/g, while grilled meat had a count of 0.76 ± 1.56 log_{10} cfu/g. Notably, no bacterial presence was detected in the fried bushmeat. However, the microbial loads exhibited no significant variation based on location; the loads were comparable in Allada and Tègon ($F(1, 590) = 2.33$, $p = 0.128$), and this was also the case for the bushmeat species analyzed ($F(3, 588) = 0.60$, $p = 0.617$). Fig. 2 illustrates the nonparametric comparison of bacterial loads between the two markets, showing a modest but statistically insignificant increase in Tègon.

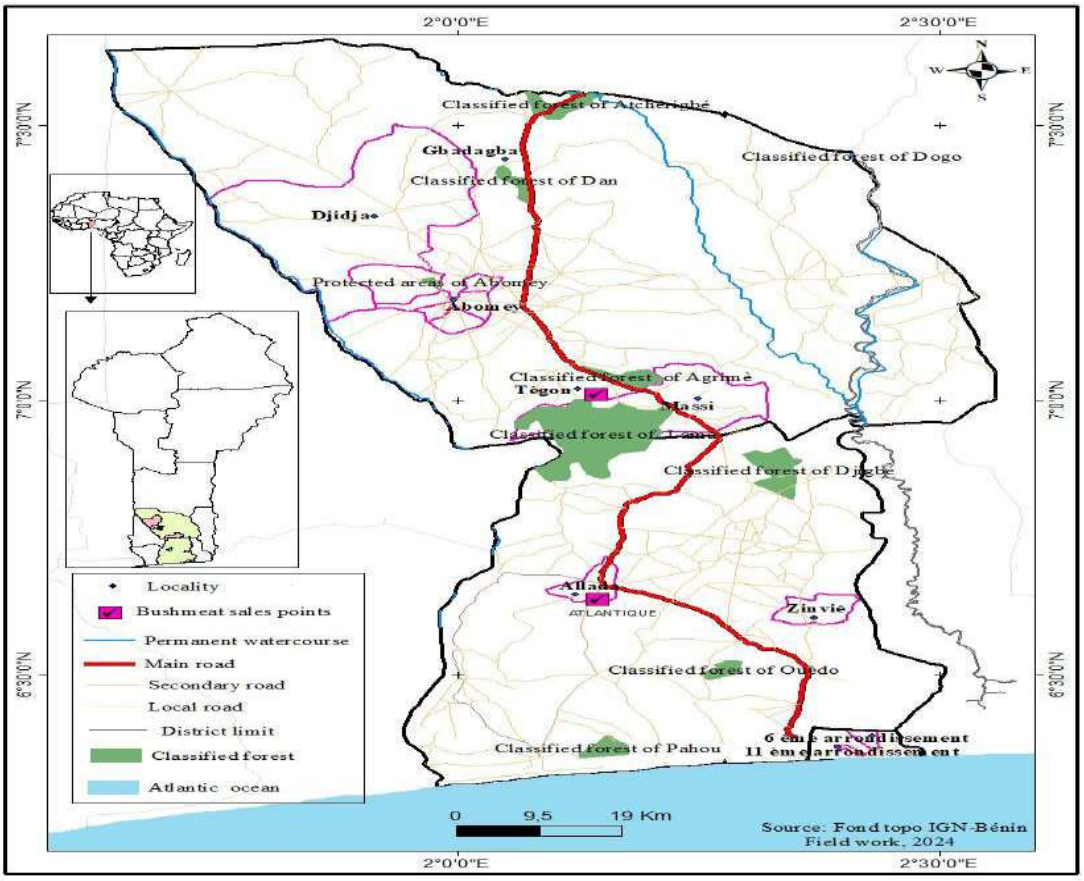

Figure 1. Map of study area

Table 1. Mean microbial loads (log_{10} cfu/g) according to preparation method

Bacteria	Preparation method	Mean bacterial load (Log_{10} cfu±SD)	p-value
TAC	Raw	6.39±0.51	0.0498
	Grilled	5.96±1.25	
	Fried	5.88±0.92	
Fecal coliforms	Raw	3.47±1.18	0.0016
	Grilled	2.47±1.89	
	Fried	1.63±1.61	
E. coli	Raw	3.23±1.14	0
	Grilled	1.18±1.52	
	Fried	0.13±0.47	
Listeria monocytogenes	Raw	0.90±1.72	0.0076
	Grilled	0.76±1.56	
	Fried	0	

SD: Standard deviation

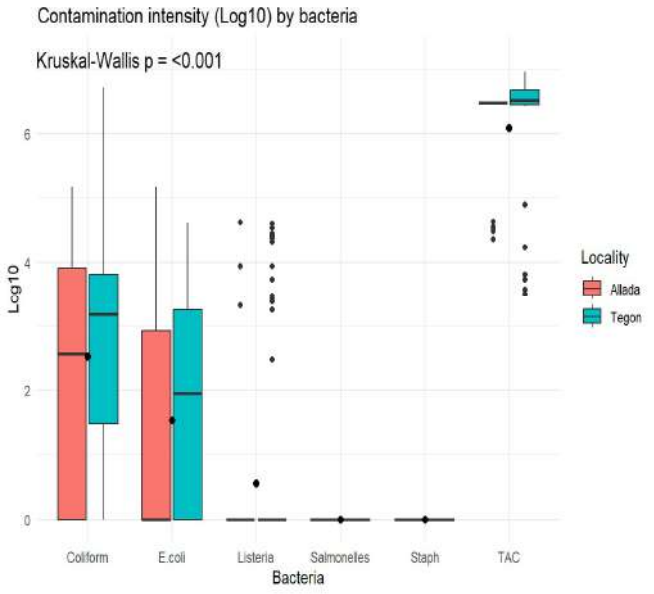

Figure 2. Non-parametric comparison of bacterial loads between the two markets

3.2. Mean total aerobic count (TAC) in bushmeat (Log_{10} cfu/g) from Tegon and Allada markets

Table 2 presents the mean of TAC (log_{10} cfu/g) load in bushmeat samples at Allada and Tègon market. The microbial loads of TAC showed minimal variation across locations and species; however, the highest levels (>6 log_{10} cfu/g) were observed in the majority of raw and grilled meats, especially in francolin, hare, and grasscutter. The lowest TAC values were observed in grilled squirrel (4.26 log_{10} cfu/g) and fried squirrel meat (4.89 log_{10} cfu/g), in Tègon and Allada markets, respectively. The findings indicate that cooking methods, especially frying, generally reduce the microbial load, although they do not eliminate the total aerobic flora.

3.3. Mean fecal coliform and *E. coli* load in bushmeat (Log_{10} cfu/g) from Tegon and Allada markets

The mean load of fecal coliform and *E. coli* in bushmeat (log_{10} cfu/g) at the Tègon and Allada markets is illustrated in Table 3. The highest fecal coliform counts were observed in raw meat, especially francolin and grasscutter species, which had the highest concentrations (>4 log_{10} cfu/g). Grilled and fried samples showed lower fecal coliform loads, with the lowest in fried hare (0.53 log_{10} cfu/g) and grilled squirrel (1.01 log_{10} cfu/g). About *E. coli*, the counts of fried squirrel meat were free of *E. coli*, while raw hare in Allada had the highest load (3.99 log_{10} cfu/g).

3.4. Mean *Listeria monocytogenes* level (log_{10} cfu/g) in bushmeat samples from Tègon and Allada markets

The mean level of *Listeria monocytogenes* (log_{10} cfu/g) in bushmeat samples from the Tègon and Allada markets is shown in Table 4. No presence of *Listeria monocytogenes* was found in any of the fried bushmeat samples. The higher levels of *Listeria monocytogenes* were recorded at 1.77 log_{10} cfu/g in raw francolin in Tègon, followed by 1.18 log_{10} cfu/g for grilled hare and 1.16 log_{10} cfu/g for raw hare in Tègon. The notable variability suggests that *Listeria* presence is inconsistent, but it can still occur after partial cooking.

3.5. Prevalence of microbial contamination in bushmeat samples

The Fig. 3 presents the prevalence of bacterial contamination in the bushmeat sample. The microbiological investigation indicated a significant prevalence of contamination in the bushmeat samples. The TAC was identified in 100% of the samples examined across all markets within the study area. Indicators of fecal contamination were prevalent, with fecal coliforms detected in 75% of samples and *E. coli* in 49%. *Listeria monocytogenes* was identified in 12% of the samples. Conversely, no samples tested positive for *Salmonella* spp. or *Staphylococcus aureus.*

Table 2. Mean TAC (log_{10} cfu/g) load in bushmeat at Tegon and Allada market

Bacteria	Species	Preparation method	Market	Mean TAC (log_{10} cfu/g)
TAC	Francolin	Fried	Allada	6.48
TAC	Francolin	Grilled	Tègon	6.66
TAC	Francolin	Raw	Allada	6.48
TAC	Francolin	Raw	Tègon	6.52
TAC	Grasscutter	Fried	Allada	6.11
TAC	Grasscutter	Grilled	Tègon	6.37
TAC	Grasscutter	Raw	Allada	6.48
TAC	Grasscutter	Raw	Tègon	6.64
TAC	Hare	Fried	Allada	6.08
TAC	Hare	Grilled	Tègon	6.59
TAC	Hare	Raw	Allada	6.48
TAC	Hare	Raw	Tègon	5.73
TAC	Squirrel	Fried	Allada	4.89
TAC	Squirrel	Grilled	Tègon	4.26
TAC	Squirrel	Raw	Allada	6.48
TAC	Squirrel	Raw	Tègon	6.48

Table 3. Mean fecal coliforms and *E. coli* load in bushmeat samples (log_{10} cfu/g)

Bacteria	Species	Preparation method	Market	Mean TAC (log_{10} cfu/g)
Fecal coliform	Francolin	Fried	Allada	2.14
Fecal coliform	Francolin	Grilled	Tègon	2.47
Fecal coliform	Francolin	Raw	Allada	4.43
Fecal coliform	Francolin	Raw	Tègon	3.89
Fecal coliform	Grasscutter	Fried	Allada	2.51
Fecal coliform	Grasscutter	Grilled	Tègon	3.57
Fecal coliform	Grasscutter	Raw	Allada	4.41
Fecal coliform	Grasscutter	Raw	Tègon	4.09
Fecal coliform	Hare	Fried	Allada	0.53
Fecal coliform	Hare	Grilled	Tègon	2.88
Fecal coliform	Hare	Raw	Allada	3.92
Fecal coliform	Hare	Raw	Tègon	1.94
Fecal coliform	Squirrel	Fried	Allada	1.36
Fecal coliform	Squirrel	Grilled	Tègon	1.01
Fecal coliform	Squirrel	Raw	Allada	2.3
Fecal coliform	Squirrel	Raw	Tègon	2.84
E.coli	Francolin	Grilled	Tègon	0.7
E.coli	Francolin	Raw	Allada	3.82
E.coli	Francolin	Raw	Tègon	3.57
E.coli	Grasscutter	Fried	Allada	0.15
E.coli	Grasscutter	Grilled	Tègon	0.87
E.coli	Grasscutter	Raw	Allada	3.59
E.coli	Grasscutter	Raw	Tègon	2.68
E.coli	Hare	Fried	Allada	0.24
E.coli	Hare	Grilled	Tègon	2.12
E.coli	Hare	Raw	Allada	3.99
E.coli	Hare	Raw	Tègon	2.46
E.coli	Squirrel	Fried	Allada	0
E.coli	Squirrel	Grilled	Tègon	1.02
E.coli	Squirrel	Raw	Allada	3.8
E.coli	Squirrel	Raw	Tègon	2.85

Table 4. Amount of *Listeria monocytogenes* (log_{10} cfu/g) in bushmeat from Tegon and Allada markets

Bacteria	Species	Preparation method	Market	Mean TAC (log_{10} cfu/g)
Listeria monocytogenes	Francolin	Grilled	Tègon	0.45
Listeria monocytogenes	Francolin	Fried	Allada	0
Listeria monocytogenes	Francolin	Raw	Allada	0.66
Listeria monocytogenes	Francolin	Raw	Tègon	1.77
Listeria monocytogenes	Grasscutter	Fried	Allada	0
Listeria monocytogenes	Grasscutter	Grilled	Tègon	0.85
Listeria monocytogenes	Grasscutter	Raw	Allada	0.98
Listeria monocytogenes	Grasscutter	Raw	Tègon	0.56
Listeria monocytogenes	Hare	Fried	Allada	0
Listeria monocytogenes	Hare	Grilled	Tègon	1.18
Listeria monocytogenes	Hare	Raw	Allada	1.16
Listeria monocytogenes	Hare	Raw	Tègon	0
Listeria monocytogenes	Squirrel	Fried	Allada	0
Listeria monocytogenes	Squirrel	Grilled	Tègon	0.6
Listeria monocytogenes	Squirrel	Raw	Allada	1.31
Listeria monocytogenes	Squirrel	Raw	Tègon	1.18

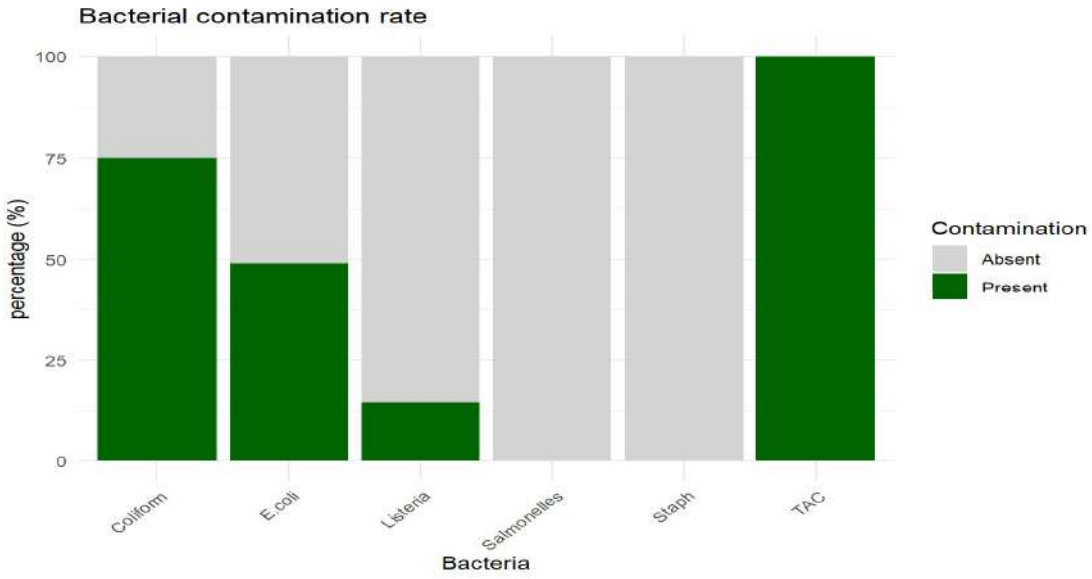

Figure 3. Prevalence of bacterial contamination in bushmeat

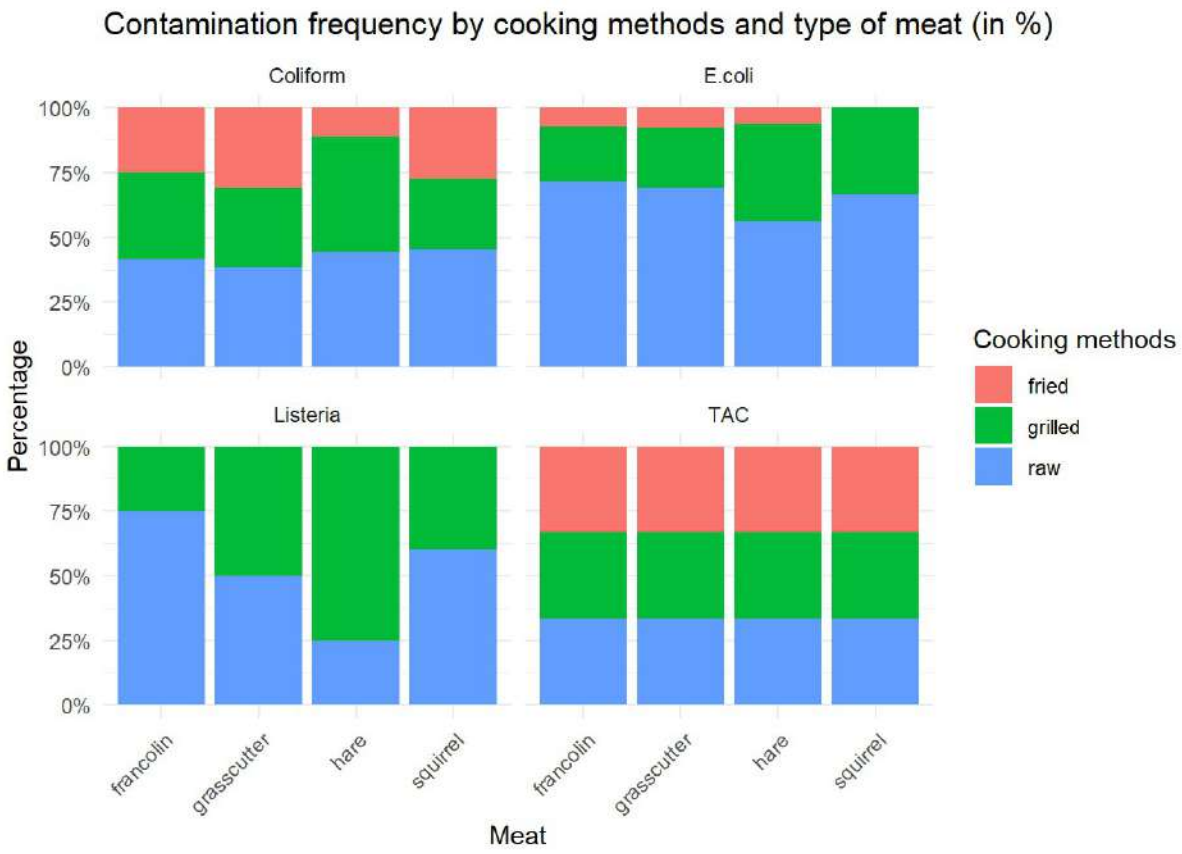

Figure 4. Prevalence of bacterial contamination by cooking methods in the different species of bushmeat

The frequency of contamination for the various pathogens across species and preparation methods is depicted in Fig. 4. The consistent presence of TAC (33%) across all species and preparation methods indicates a high initial microbial load and suggests potential recontamination after cooking. A distinct gradient in fecal indicators was observed by processing method: fecal coliform prevalence was significantly lower in grilled and fried bushmeats than in raw bushmeats, although fried bushmeat exhibited the lowest incidences, with Hare at 15%, Francolin at 25%, and Squirrel at 26%, respectively. Grasscutter exhibited a consistent fecal coliform contamination rate of 33% across various preparation methods. The prevalence of *E. coli* followed a comparable pattern: markedly elevated in raw bushmeat and dramatically diminished in grilled and fried bushmeat. Furthermore, *E. coli* was not present in any fried squirrel samples. In comparison, *Listeria monocytogenes* was detected exclusively in raw and grilled meat and was not detected in any fried samples. The prevalence was typically higher in raw bushmeats; however, in the hare species, the incidence was higher in grilled samples (75%) than in raw samples (25%).

4. Discussion

4.1. Hygiene indicators and overall microbial quality

Meat is highly perishable due to its nutrient-dense composition, which promotes microbial growth (20).

Bushmeat is derived from hunted wildlife and is not subject to food regulations. Therefore, ensuring its quality, particularly its microbiological quality, is crucial. Our findings indicate that four common species, grasscutter, francolins, hares, and squirrels, exhibited consistently higher TAC (log_{10} cfu/g) across various cooking methods: raw, grilled, or fried. TAC serves as a general indicator of hygiene in slaughter processes and initial spoilage, as it quantifies mesophilic bacteria that proliferate within the temperature range of 20 to 45°C (21). The level of TAC reflects the quality of the meat, manufacturing hygiene, and the degree of degradation. The mean TAC across various meat species remained consistent regardless of the processing method. Fried and grilled squirrel meat were excluded, with average counts of 4 log_{10} cfu/g. Although the mean TAC obtained in this study was above 5 log cfu/g, which is the level required by the European Commission's guidelines for carcasses of cattle, sheep, goats, and horses (22). The TAC averages are consistently high and align with the findings of (23) concerning bushmeat sold at the Kumasi market, which displayed TAC loads between 5.6 ± 1.2 log_{10} cfu/g and 10.9 ± 1.9 log_{10} cfu/g. Additionally, these values correspond with those documented by Ikeh et al. (2021) for ready-to-eat bushmeat from grasscutters (7.62 ± 0.9 log_{10} cfu/g) and antelope (8.09 ± 0.15 log_{10} cfu/g). In Benin, while explicit rules specifying appropriate TAC criteria for bushmeat are absent, this measure is widely acknowledged as a critical determinant of the meat's microbiological purity and freshness. TAC reflects the cleanliness and sanitation standards for slaughtering, processing, and preservation methods, with higher levels typically associated with increased spoilage risk and reduced shelf life (24). The observed consistency in mean TAC values across species and processing methods suggests a widespread deficiency in cleanliness and insufficient meat handling, even after processing. The frying and grilling methods result in marginally lower mean TAC levels; these differences are statistically significant (p=0.0498), albeit slight. The reduced TAC levels of fried and grilled bushmeat were observed in squirrel, with values of 4.89 and 4.26 log_{10} cfu/g, respectively. This observation regarding squirrel meat may be associated with the species, as its small size limits the body's ability to segment after evisceration, thereby hindering its manipulation. The texture of this meat is significantly firm. It probably has a lower moisture content than other meat varieties, thereby limiting the presence of substantial amounts of TAC, particularly after frying and smoking. The high levels observed across various species, which show slight variation due to the treatments applied to the bushmeat, are thought to be linked to handling and exposure in unsanitary conditions. Additional contamination may arise from handling meat with unclean hands, as well as from basins and bags that are likely to harbor contaminants. Additionally, smoked meats are exposed to ambient air, making them vulnerable to dust, and consumers may handle them before selection. Grilled or smoked meats are sometimes coated with blood to improve preservation, as observed by Ahouanse et al. (25), despite blood being a medium rich in microbes.

After grilling, the meats are coated with vegetable oil before being presented for sale in Tègon. Vegetable oil is typically applied using feathers from slaughtered birds, which are not sanitized and may introduce

additional microbial contamination to the smoked carcasses. This observation accounts for the notably elevated and unusual TAC load recorded in grilled francolins (6.66 log_{10} cfu/g). Fried meat in Allada is presented in transparent, well-protected bags or within transparent glass cabinets. Nevertheless, the women engaged in the sale of this meat often do not maintain hygienic practices, as they hurriedly attempt to draw in customers, sometimes resorting to aggressive tactics such as forcibly opening car doors or seizing customers' hands. These hands are frequently employed to serve customers without prior washing. Customers occasionally select meat by handling it directly, neglecting to wash their hands beforehand, despite the availability of a fork for this task. The various situations are considered the foundation for the elevated TAC charges on smoked and fried bushmeat, aligning with the findings of Emelue et al (26) regarding smoked bushmeat in Nigeria.

4.2. Indicators of fecal contamination

Fecal or thermotolerant coliforms are bacteria that digest lactose at 44 °C. These bacteria may include *E. coli, Klebsiella*, *Enterobacter*, and *Citrobacter* (27). The detection of fecal coliforms and *E. coli* signifies fecal contamination, typically resulting from inadequate evisceration, substandard hygiene, or non-compliant washing water (28,29). The concentration of fecal coliforms was significantly influenced by the preparation procedure ($p = 0.0016$). Mean values decreased progressively across the preparation methods, with the highest load detected in raw meat (3.47 ± 1.18 log_{10} cfu/g). Grilling reduced the load to 2.47 ± 1.89 log_{10} cfu/g, while frying resulted in the most substantial reduction to 1.63 ± 1.61 log_{10} cfu/g, establishing a distinct descending gradient of contamination relative to thermal treatment. The mean reduction was roughly 1.0 log_{10} cfu/g from raw to grilled, and 1.84 log_{10} cfu/g from raw to fried, indicating a genuine yet heterogeneous thermal effect (notable standard deviations in grilled meat), aligned with diverse cooking techniques and potential recontamination during sale and other services. Fecal coliform levels exceeding 4 log_{10} cfu/g were predominantly identified in specific raw meats, notably francolin and grasscutter, suggesting potential enteric contamination and a critical need for improved handling practices. Similarly, *E. coli* loads were significantly influenced by the preparation method ($p<0.001$). The highest mean count was recorded in raw meat (3.23 ± 1.14 log_{10} cfu/g), which was significantly reduced by grilling (1.18 ± 1.52 log_{10} cfu/g) and further diminished by frying (0.13 ± 0.47 log_{10} cfu/g). This represents mean reductions of approximately 2.05 log_{10} cfu/g and 3.10 log_{10} cfu/g from raw to grilled and raw to fried, respectively. Notably, *E. coli* was absent in fried squirrel samples, while the maximum concentration was observed in a raw hare sample from Allada (3.99 log_{10} cfu/g). This disparity (high in raw meat, residual or absent post-frying) is characteristic of initial fecal contamination that is significantly diminished by heat, however, not invariably eradicated by grilling when heat penetration is insufficient and/or recontamination transpires downstream. The potential origins of these loads (and their variability) are consistent for both measures. Before cooking, skinning, and particularly evisceration are pivotal processes in the transmission of enteric flora to the carcass (including contact with skin, intestinal contents, and knives) (30). The capture

method significantly influences outcomes, as certain studies indicate that when animals are shot with rifles, and the bullet penetrates the abdomen, the internal contents contaminate the entire carcass, leading to elevated levels of enteric bacteria, particularly if the carcasses are inadequately managed thereafter (13,31). Several variables account for the recontamination observed at the retail level: unwashed hands, reused utensils and containers, variable microbiological purity of the washing water, exposure to air, and multiple contacts between the seller and buyer (32). This pattern aligns with our findings: Fried meats exhibit the most tremendous thermal impact (reductions of over 3 $\log_{10}$ for *E. coli* and around 1.8 $\log_{10}$ for coliforms) and the lowest residual levels, whereas grilled meat shows considerable variability, indicative of inconsistent cooking and inadequate post-cooking management. ANOVA results indicate that neither location ($F(1,590) = 2.33$; $p = 0.128$) nor species ($F(3,588) = 0.60$; $p = 0.617$) accounts for the observed variation, underscoring that method and practices predominantly influence contamination dynamics.

4.3. Pathogenic microorganisms contamination

All tested samples exhibited no evidence of *Staphylococcus aureus* or *Salmonella* spp. The absence of *Salmonella* spp. in the present study is consistent with findings by Oduro et al. (23) and Amponsah et al. (33) in Ghana using comparable sample matrices. The findings of the current study contrast markedly with those of aMpalang et al. (34) in Lubumbashi, DRC, where *Salmonella* spp. was detected in 26 of 186 bushmeat samples. Results in the present study also contrast with data from Nigeria, where Ikeh et al. (35) reported *Staphylococcus aureus* detection in 25% of grasscutter samples and 28% of ready-to-eat bushmeat products. The variability among research may indicate disparities in supply chains, processing methodologies (temperature/time efficacy), analytical techniques (detection methods, thresholds), or sampling frameworks (species, anatomy, season). Unlike *Salmonella* spp. and *S. aureus*, *Listeria monocytogenes* was detected in 12% of samples in this study, exclusively in raw and smoked products. This result aligns with the findings of Adeyeye et al. (36), who identified *L. monocytogenes* in smoked grasscutter meat in Nigeria. *Listeria monocytogenes* prevalence may be due to multiple variables. Smoking may initially fail to achieve the time-temperature combinations required for microbial inactivation in the product core. Secondly, *Listeria* spp. survives on processing surfaces and equipment because of their environmental resistance and mild temperature tolerance. Thirdly, the poor hygiene measures, including surface sanitization, handwashing, and utensil cleaning, can lead to post-processing recontamination, especially for heavily handled and exposed items. The persistent absence of *L. monocytogenes* in fried meats can be attributed to the elevated, uniform frying temperatures achieved on the surface and internally, along with reduced post-cooking exposure due to improved protective packaging, display cases, or containers. The distinction between smoked and fried foods underscores the importance of the thermal barrier, which must be augmented by post-cooking hygiene measures (hands, surfaces, water, utensils) to avert recontamination that may reintroduce *L. monocytogenes* into ready-to-eat items.

5. Conclusion

This study shows that the microbiological quality of bushmeat in southern Benin is primarily influenced by post-harvest handling and preparation techniques (grilling/smoking/frying) rather than by geographical origin or animal species. The absence of *Salmonella* spp. and *S. aureus* is a positive finding; however, the high levels of TAC (log_{10} cfu/g), which are hygiene indicators and fecal contamination markers (fecal coliforms and *E. coli*), along with the detection of *L. monocytogenes,* even at low prevalence in raw and grilled/smoked products, indicate a significant public health concern. The data indicates a distinct contamination gradient: raw > grilled/smoked > fried, highlighting the vital role of thermal processing in reducing risk. The persistence of microbial loads post-grilling and the detection of *L. monocytogenes* indicate inconsistent cooking practices and considerable post-processing recontamination resulting from unhygienic handling at the retail level. The safety of bushmeat is primarily compromised by vulnerabilities within its informal value chain, encompassing practices from slaughter to point of sale, rather than its wild origin. The results indicate a primarily process-related risk, influenced by conditions during animal slaughter, transport, and handling, as well as inconsistent cooking and recontamination at the point of sale, including hands, surfaces, and water, and exposure.

Multiple approaches are needed to assure bushmeat safety for consumers. This involves standardizing cooking methods by specifying time and temperature (time/°C) requirements, particularly for smoked and grilled items, to eliminate microorganisms. Hygiene should be emphasized throughout the supply chain by separating raw and cooked meats, washing hands, using potable water, sanitizing utensils, and using protective packaging at the point of sale to reduce handling and recontamination. A systematic monitoring program that includes regular inspections and microbiological testing for TAC, coliforms, and *E. coli* is crucial for objectively assessing risks, assessing the effectiveness of implemented measures, and informing corrective actions. Sustainable microbiological risk mitigation and bushmeat safety can be achieved by integrating controlled cooking, hygienic procedures pre- and post-cooking, and protective measures at the point of sale.

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Authorship contribution

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Declaration of competing interest

The authors declare no competing conflict of interest.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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