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Assessing the microbiological quality of food sold in various retail outlets in Libreville (Gabon)

Pierre Philippe MBEHANG NGUEMA[1], Gontran NSI AKOUE[2], Roméo Wenceslas LENDAMBA[3], Rachel ZOUGA ASSOUME ép. NZIENGUI[2], Christophe Roland NZINGA KOUMBA[1], and Ludovic MEWONO[2]

[1]Laboratoire de microbiologie, Institut de Recherche en Écologie Tropicale, Centre National de la Recherche Scientifique et Technologique (IRET, CENAREST), B.P. 13345, Libreville, Gabon

[2]Ecole Normale Supérieure (ENS), B.P. 17009, Libreville, Gabon

[3]Département des opérations cliniques, Centre de Recherches Médicales de Lambaréné (CERMEL), B.P. 242, Lambaréné, Gabon

ABSTRACT: Food safety is a critical aspect of public health, encompassing the hygienic quality and absence of harmful microorganisms or toxins in food products. In Libreville, Gabon's bustling economic hub, food markets range from traditional stalls to modern supermarkets. This study evaluates the microbiological quality of chicken, the most consumed food item among Libreville residents, by assessing contamination levels with *Escherichia coli, Salmonella spp.,* and *Staphylococcus spp.* Twenty-one chicken samples, including wings, thighs, and drumsticks, were randomly collected from various retail outlets. Microbiological analyses revealed contamination in all samples, with bacterial loads exceeding international safety thresholds. Wings exhibited the highest levels of contamination, potentially due to poor hygiene practices and frequent handling. Antibiotic susceptibility tests demonstrated resistance to multiple antibiotics, though no extended-spectrum beta-lactamase production was observed. Resistance was most pronounced in *E. coli* and *Staphylococcus spp.* for sulfamides and cefotaxime. The findings underscore the urgent need for improved food safety practices in Libreville's markets, highlighting the health risks associated with chicken consumption. This study calls for enhanced regulatory oversight, public awareness campaigns, and the adoption of stringent hygiene measures to safeguard consumers from foodborne illnesses.

KEYWORDS: Food safety and Chicken contamination, *Escherichia coli, Salmonella spp.,* Antibiotic resistance, Libreville.

1 INTRODUCTION

A food item is a substance that can be digested by a living organism to provide the nutrients necessary for development, functioning, and survival [1]. Foods may be of either plant or animal origin [2] and are generally consumed by humans as sustenance [1]. These foods are sold in various markets and shops. The sanitary quality of food refers to the set of characteristics and conditions ensuring that it is fit for human consumption and poses no health risks [3]. This includes the absence of contaminants, pathogenic microorganisms, or toxic substances [4]. Libreville, as Gabon's economic and commercial hub, offers a wide range of food items through its diverse marketplaces, spanning traditional markets to modern supermarkets [5]. One of the challenges faced by urban areas is ensuring the food security of their populations [6]. However, concerns often arise regarding the sanitary quality of food products due to inadequate handling, storage, or distribution practices. In this context, systematic evaluation is essential to assess compliance with sanitary standards and identify potential risk areas. Evaluating the sanitary quality of food is a significant concern globally in terms of food security and public health [7]. However, this issue is particularly important due to the intense commercial activity and diversity of food products available in local markets. Thus, this preliminary study aims to determine whether such food items, specifically chicken, are truly fit for human consumption.

Corresponding Author: Pierre Philippe MBEHANG NGUEMA

2 MATERIALS AND METHODS

PRE-STUDY SURVEY

Before commencing the study, a comprehensive local survey was conducted to identify the most commonly consumed food item among Gabonese residents, particularly those living in Libreville. This preliminary work revealed that chicken was the preferred food item (AGASA, DGCCRF), and thus it was chosen as the biological material for this study.

MATERIALS

BIOLOGICAL MATERIAL

Chicken parts, specifically thighs, wings, and drumsticks.

Sample Collection: Chicken samples (E1 to E21), each weighing approximately 1 kg, were purchased randomly from various food supply outlets in Libreville on 25 May 2024 (Table 1). The sampling was carried out across three types of retail outlets: supermarkets, mini-markets, and grocery stores, located in six neighbourhoods: Essassa, Bikélé, PK12, Nzeng-Ayong, Nkembo, and Mont-Bouet.

Samples were collected aseptically using sterile techniques, including gloves, freezer bags, and a clean cooler with ice to minimise contamination risks. During collection, a systematic visual analysis was also conducted to identify potential contamination factors such as product appearance, signs of partial thawing, texture degradation, or discolouration of chicken meat. These factors were recorded (Table 1).

Tableau 1. Characteristics of Collected Samples

Sample No.	Type of Collection Outlet	Neighbourhood	Collected Food Items
E1	Grocery shop	Essassa	Thighs
E2	Convenience store	Essassa	Wings
E3	Supermarket	Bikélé (PK18)	Drumsticks
E4	Supermarket	Bikélé (PK18)	Wings
E5	Supermarket	PK12	Wings
E6	Supermarket	PK12	Drumstick
E7	Supermarket	Nzeng-Ayong	Drumstick
E8	Supermarket	Nzeng-Ayong	Thighs
E9	Convenience store	Nzeng-Ayong	Wings
E10	Convenience store	Nzeng-Ayong	Drumstick
E11	Grocery shop	Nzeng-Ayong	Wings
E12	Grocery shop	Nkembo	Thighs
E13	Convenience store	Nkembo	Wings
E14	Convenience store	Nkembo	Drumstick
E15	Supermarket	Nkembo	Wings
E16	Supermarket	Nkembo	Thighs
E17	Convenience store	Mt-Bouet	Wings
E18	Convenience store	Mt-Bouet	Thighs
E19	Convenience store	Mt-Bouet	Drumstick
E20	Supermarket	Mt-Bouet	Thighs
E21	Supermarket	Mt-Bouet	Wings

LABORATORY ANALYSIS

Upon arrival at the laboratory, samples were immediately stored at -18°C in a freezer until microbiological analysis was performed the following day. Microbiological tests were conducted at the Institute for Tropical Ecology Research (IRET) laboratory in the northern district of Libreville.

BACTERIAL ISOLATION AND IDENTIFICATION

Standardised bacterial culture techniques were employed to isolate and identify bacterial colonies within the samples [8].

1. Preparation of Stock Solutions: Chicken pieces were dissected both internally and externally using sterile forceps and scissors. Ten grams of each sample were weighed, mashed in a sterile mortar with 10 ml of sterile water for 5–7 minutes, and filtered through sterile gauze. The filtrate was transferred into pre-labelled, sterile 15 ml plastic tubes corresponding to the sample ID.
2. Dilutions: For each dilution, 1 ml of the stock solution was mixed with 9 ml of sterile water in a 15 ml tube using a vortex (Fisher Scientific, France). This formed the 10^{-1} dilution. Further dilutions (10^{-2} and beyond) were prepared by serially transferring 1 ml of the preceding dilution into 9 ml of sterile water.
3. Detection of Pathogens:

- ***E. coli***: Samples were inoculated on Eosin Methylene Blue (EMB) agar. Colonies displaying a green metallic sheen were presumptively identified as *E. coli* [8, 9].
- ***Salmonella spp***.: Samples were enriched in Rappaport-Vassiliadis broth before inoculation on *Salmonella Shigella* (SS) agar. Colonies with a colourless appearance and black centres were identified as *Salmonella* spp [10].
- ***Staphylococcus spp***.: Samples were cultured on Chapman agar. Gram staining was performed, and colonies appearing as Gram-positive cocci in grape-like clusters under 100x magnification were identified as *Staphylococcus spp* [11].

SURFACE COLONY COUNTING

Bacterial colonies were counted on each agar plate. Only plates with 15 to 300 colony-forming units (CFU) were considered valid for this analysis. In such cases, the enumeration was conducted according to the following formula:

Number of CFU = (Number of colonies counted x Dilution factor) /Volume of inoculum mL

This method allows for the accurate estimation of the number of viable microorganisms present in the sample. Each colony that develops on the agar surface is then assumed to originate from a single viable bacterium or a cluster of bacteria that have proliferated into a distinct colony.

For effective counting, it is essential to ensure that the colonies are well-distributed and fall within the acceptable range of 15 to 300 CFU per plate, as counting outside this range may lead to inaccurate estimations.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic susceptibility was determined using the Kirby-Bauer method on Mueller-Hinton agar (MH) [12]. The following antibiotics were used per the recommendations of the Clinical Laboratory Standards Institute (CLSI) [12, 13]: Amoxicillin + Clavulanic Acid (AMC, 20/10 µg), Aztreonam (ATM, 30 µg), Cefepime (FEP, 30 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Chloramphenicol (C, 30 µg), Ertapenem (ETP, 10 µg), Ofloxacin (OFX, 5 µg), Tetracycline (TEC, 30 µg), Tobramycin (TOB, 10 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75 µg), Vancomycin (VAN, 5 µg), and Oxacillin (OX, 30 µg).

To highlight the presence of extended-spectrum beta-lactamase, the double disc diffusion method was utilised to test for synergy between third-generation cephalosporins and a beta-lactamase inhibitor (Patel and Nagel, 2015). The inoculum was prepared from a freshly cultured pure isolate. An inoculum suspension was created by mixing a pure colony, retrieved with an inoculating loop, with 700 µL of physiological saline (0.9% NaCl) in a sterile 2 ml tube, as previously performed by Mbehang Nguema et al [14]. Inoculation onto Mueller-Hinton agar was conducted using sterile swabs. Subsequently, the antibiotics were applied to the inoculated plates using sterile stainless steel forceps, and the plates were incubated at 37°C for 24 hours.

3 RESULTS

VISUAL ANALYSIS OF SAMPLES

Field observations highlighted factors likely to promote microbial growth, including poor hygiene practices, inadequate refrigeration, and evidence of partial thawing (Table 3).

MICROBIOLOGICAL ANALYSIS

IDENTIFICATION OF BACTERIA

A total of 21 poultry samples were collected, comprising: thighs (n=6), wings (n=9), and drumsticks (n=6). Following culture, isolation, and identification, the metallic green colonies on EMB agar and the black colonies on SS agar were pre-identified as *E. coli* [15] and *Salmonella spp*. [16], respectively. The identification of *E. coli* and *Salmonella spp*. was confirmed using the Api 20 E galleries.

For Staphylococci, enrichment was performed on Chapman agar, as previously described with dilutions E-1 and E-2. All colonies that grew on this medium were subjected to Gram staining. After microscopic observation at 100x magnification, only the purple colonies grouped in clusters resembling grapes were pre-identified as *Staphylococcus spp*.

ENUMERATION OF COLONIES OF SALMONELLA SPP., ESCHERICHIA COLI, AND STAPHYLOCOCCUS SPP.

Based on our results, all samples were contaminated by at least one species of the studied pathogens. Due to the absence of local standards, the microbial content of the samples was compared to Australian and New Zealand guidelines for the microbiological examination of ready-to-eat foods, which define acceptable limits for microbial load in a ready-to-eat food sample [17]. Consequently, the level of microbial contamination in the samples was classified as satisfactory, marginal, or unsatisfactory.

Our findings indicate that CFU/g (colony-forming units per gram) for each isolated bacterium ranged from very high to moderately high. This applies to all bacterial strains identified in the samples collected during our study (Table 2). These values also indicate varying levels of health risks associated with chicken consumption. It is noteworthy that we observed a higher quantity of isolated pathogens in the wings compared to other parts of the chicken (Table 2).

Furthermore, the presence of *Salmonella spp., Escherichia coli, and Staphylococcus spp*. was more frequent in samples obtained from retail outlets such as convenience stores and groceries than in those from supermarkets; moreover, this same food exhibited variations in terms of sanitary quality (Table 2).

Tableau 2. ***Microbiological quality of chicken sold in Libreville stores by category, according to Guidelines for the microbiological examination of foodstuffs***

	Microbiological guidelines		Microbiological quality of tested foods		
Microorganisms	**Classification**	**Limits (CFU/g)**	**Wings (n=9)**	**Thighs (n=6)**	**Drumsticks (n=6)**
E. coli	Satisfactory	<3	2	2	2
	Marginal	3-100	1	1	0
	Unsatisfactory	≥100	6	3	4
Staphylococcus spp	Satisfactory	102	0	2	2
	Marginal	10^2-10^3	3	3	1
	Unsatisfactory	10^3-10^4	6	1	3
Salmonella spp	Satisfactory	0 in 25g	0	0	0
	Marginal	-	0	0	0
	Unsatisfactory	0 in 25g	3	1	0
	Negative Culture		6	5	6

FOOD SAFETY PRACTICES AND MICROBIOLOGICAL QUALITY OF FOOD

The correlation between food safety practices and the microbiological quality of food indicates that poor food safety practices (abnormal food colours, improper thawing methods, unpleasant odours from food, foul smells in freezers, and unsanitary conditions in food preparation areas) (Table 3) are primarily associated with a very high health risk due to the presence of significant quantities of microorganisms in food. The emergence of specific microorganisms at unsatisfactory levels is linked to general hygiene practices, facilities, and surrounding environmental parameters. Unsatisfactory levels of food contamination were associated with inappropriate refrigeration methods (thawing food in freezers, improper refrigeration temperatures, etc.).

Tableau 3. ***Food safety practices and microbiological quality of food***

Sample	Food sampled	Observations (hygiene: Colour, texture ...)	Health risk		
			E. coli	***Salmonella***	***Staphylococcus***
E1	Thighs	PHP	Very high	Very high	None
E2	Wings	PHP	Very high	None	Very high
E3	Drumsticks	GHP	None	None	Low
E4	Wings	GHP	None	None	Very high
E5	Wings	PHP	Very high	Medium	Moyen
E6	Drumsticks	PHP	Medium	None	Low
E7	Drumsticks	GHP	Medium	None	Low
E8	Thighs	GHP	Low	None	Low
E9	Wings	PHP	None	None	Medium
E10	Drumsticks	PHP	Low	None	Very high
E11	Wings	PHP	Very high	None	Low
E12	Thighs	PHP	None	None	Low
E13	Wings	PHP	Very high	Very high	Very high
E14	Drumsticks	PHP	Low	None	None
E15	Wings	GHP	Low	None	Low
E16	Thighs	GHP	Low	None	Medium
E17	Wings	PHP	None	None	Medium
E18	Thighs	PHP	Medium	None	Very high
E19	Drumsticks	PHP	Very high	None	High
E20	Thighs	MPH	None	None	Low
E21	Wings	MPH	Low	High	Very high

PHP*: Poor Hygiene Practice (storage issues, abnormal colour of chicken flesh, cutting tools);* ***GHP****: Good Hygiene Practice.*

ANTIBIOTIC SUSCEPTIBILITY TESTING

After manually placing antibiotic diffusion discs on each Muller Hinton agar plate previously inoculated with identified colonies and incubating for 24 hours, plates exhibiting colonies surrounded by inhibition diameters were interpreted to obtain various resistance profiles.

PREVALENCE OF ANTIBIOTIC RESISTANCE IN FOOD

Among the 11 antibiotics tested, resistance was observed in only 7, representing 63.6%. However, this resistance remains low. It is noteworthy that the production of extended-spectrum beta-lactamase was not detected in either *E. coli* or *Salmonella*. Nevertheless, in *E. coli,* the resistance was higher for Sulphonamides, followed by Cefotaxime, Nitrofurantoin, Ofloxacin, and Ertapenem. In *Staphylococcus* spp., resistance was found solely for Sulphonamides (Table 4).

Tableau 4. ***Prevalence of Antibiotic Resistance of E. coli, Salmonella et Staphylococcus***

Bacteria	AUG	CTX	ETP	SUL	TOB	NIT	OFX
E. coli	0	22,2%	5,5%	27,8%	16,7%	22,2%	5,5%
Salmonella	50%	0	0	0	0	0	0
Staphylococcus	ND	ND	ND	4,76%	0	ND	0

AUG: Amoxcicillin/Clavulanic Acid; CTX: Cefotaxime; ETP: Ertapenem; SUL: Sulphamethoxazole; TOB: Tobramycin; NIT: Nitrofurantoin; OFX: Ofloxacin; ND: Not determined

4 DISCUSSION

MICROBIOLOGICAL ANALYSES

E. coli is a bacterium frequently used as an indicator of faecal contamination and general hygiene in food products [18]. However, certain strains, such as *E. coli O157,* can cause severe illnesses [19]. According to the standards of the Codex Alimentarius and the International Organisation for Standardisation (ISO), acceptable contamination levels are ≤100 CFU/g for *E. coli* and *Staphylococcus spp.,* and 0 CFU/25 g for *Salmonella spp* [17].

The findings of our study revealed that the majority of the pathogens isolated exceeded these thresholds, indicating insufficient hygiene practices in the storage and handling of food in the studied commercial settings [20]. As detailed in Table 3, there is a significant correlation between the presence of specific micro-organisms and poor hygiene practices ($p = 0.0128$). Inadequate practices were observed in approximately 71.4% of cases, compared to only 2.8% of cases where good practices were identified, which explains the high levels of contamination [21].

The substantial presence of pathogenic bacteria in food, particularly in chicken wings, raises concerns about the health risks associated with their consumption. These results are consistent with studies conducted in Algeria [22] and Tanzania [23]. Contamination may result from inappropriate handling by customers or poor storage conditions, such as repeated thawing or deterioration in texture and colour. The popularity of chicken wings in the dietary habits of Libreville's population may also explain this finding. Furthermore, wings are often identified as areas where antibiotic-resistant pathogens tend to concentrate [24]. These findings underscore the need for concerted efforts from producers, vendors, consumers, and authorities to improve food safety standards and safeguard public health.

PREVALENCE OF ANTIBIOTIC RESISTANCE

Antibiotic resistance was observed, albeit moderately, particularly in *Staphylococcus spp.* for sulphonamides and *Salmonella spp.* for AUG. However, it was more pronounced in *E. coli,* especially for sulphonamides. These observations align with studies conducted in Algeria [22] and Tanzania [23].

5 CONCLUSION

This study assessed the sanitary quality of food sold in commercial outlets in Libreville, the economic and administrative capital of Gabon. It highlights deficiencies in hygiene and food safety practices, with high microbial loads observed in certain food types.

The results demonstrate that the sanitary quality of food is largely unsatisfactory, posing public health risks to consumers. These findings should prompt corrective actions involving all stakeholders in the food supply chain to ensure consumer safety.

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