



Microbial Quality of Frozen Chicken Parts from three Import Countries into the Kumasi Metropolis of Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Author DDY conceptualized and drafted the manuscript. Author WKZ collected the data and conducted the laboratory analyses. Author WKJK (Meat Scientist) critically edited the manuscript. Author GIB reviewed and structured manuscript for publication. Author EKA (Laboratory Technician) supervised the laboratory analysis. Author GF reviewed the manuscript and accepted it for publication. All authors read and approved the final manuscript.

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ABSTRACT

There is an upsurge in the consumption of chicken meat leading to a high influx of imported frozen chicken parts into the Ghanaian markets with little information on their microbial qualities. This study examined the microbial quality of imported frozen chicken parts from three major import countries (USA, the Netherlands and Brazil) into the Kumasi Metropolis. A total of 45 chicken meat parts of 15 thighs, wings and backs from wholesale cold stores market in the Kumasi Metropolis were randomly sampled for laboratory examinations. A ten-fold serial dilution was performed on each homogenized chicken parts to determine microbiological quality using Plate Count Agar [1],

MacConkey Agar (MCA), Mannitol Salt Agar (MSA) and Desoxycholate Citrate Agar (DCA) for the total viable count (TVC), total coliform count (TCC), *Staphylococcus* and *Salmonella* spp counts respectively incubated at 37°C for 48 hours. Sabouraud Dextrose Agar (SDA) was used for fungal counts. We identified bacterial and fungal isolates using appropriate laboratory and biochemical tests. Descriptive data analysis was carried using SPSS-IBM version 16. Mean TVCs of 5.93, 5.98 and 6.14 log₁₀cfu/g were recorded for frozen chicken meats from the USA, the Netherlands and Brazil respectively. Means TCCs of 6.14, 5.93 and 5.98 log₁₀cfu/g were obtained for chicken meats from Brazil, USA and the Netherlands respectively. *Staphylococcus* spp. (G+) (35.4%), *E. coli* (G-) (26.2%), *Salmonella* spp. (G-) (24.6%), and *Klebsiella* spp. (G-) (13.8%) were isolated with *Aspergillus* spp (33.3%), *Rhizopus* spp (27.3%), *Penicillin* spp (24.2%), and *Cladosporium* spp (15.2%). Chicken thighs, backs and wings recorded 46.2%, 29.2% and 24.6% bacterial contaminants in this order. Bacterial isolates of 49.2%, 28.8% and 22.0% were recorded in frozen chicken meat products from Brazil, the Netherlands USA respectively. The results suggest that imported frozen chicken meats into the Ghanaian market have moderate quality with potential pathogens such as *E. coli* and *Salmonella* spp.

Keywords: Microbial quality; frozen chicken meat; pathogens; *E. coli*; *Salmonella*.

1. INTRODUCTION

Poultry production is dominated by chicken in many parts of the world due to the relative ease of its production [2] and serve as a major source of animal protein [3]. Meanwhile, chicken meat is known to cause many zoonotic foodborne infections in the world [4]. Global food production and consumption systems, therefore, required that food quality and safety be scrutinized and examined for public health safety [5,6]. Poultry and poultry meat are however prone to contaminations with potentially pathogenic microorganisms such as *Salmonella*, *Campylobacter*, *S. aureus*, *E. coli* and *Listeria* [7].

In Ghana, local broiler meat production is estimated at 35,000 tons, less than 25% of local demand [8]. Meanwhile, there is an upsurge in chicken meat consumption with a corresponding increase in demand for frozen chicken products [9,10]. An expected 14,000 to 158,000 tons is therefore required local demand [8] and this led to an influx of imported frozen chicken parts. Moreover, market prices of local broiler meat are 30-40% margin higher than imported frozen chicken meat making local poultry production uncompetitive [8,11,12]. Furthermore, consumers prefer imported frozen chicken products because these come pre-cut parts of legs-quarters, thighs, breasts and wings and are affordable [13,14].

Due to International Trade Liberation, the Ghanaian markets are inundated with frozen chicken products from major import nations such as the United States of America (USA), the

Netherlands and Brazil whose microbial qualities cannot be guaranteed [15]. The Kumasi Metropolitan Assembly and the Food and Drugs Authority of Ghana frequently ceased expired frozen chicken during routine surveillance at the Asafo market. However, there is a paucity of data on the microbial quality of pre-cut and packaged imported chicken meat products in the Kumasi Metropolis of Ghana [16].

This study, therefore, assessed and compared the microbial quality of imported frozen chicken parts from three major import countries (United States of America, the Netherlands and Brazil) into the Kumasi Metropolis of Ghana.

2. METHODOLOGY

2.1 Study Area

The study site was the Asafo Market in the Kumasi Metropolis. The market is a major hub for wholesale cold stores and is a repertoire for about 95% of all imported frozen meats and fishes from the Tema harbour of Ghana. Poultry production in the metropolis accounted for 28.07% of the total poultry production in Ghana [17]. Ashanti Region is located in the middle belt and the third largest of the 16 administrative regions in Ghana. Kumasi Metropolis has an estimated population of 1,730,249; 36.2% of the regional population [17]. The region has 10.2% of the total land area of Ghana with a population of 4,780,380. The region lies between longitudes 0.15W and 2.25W, and latitudes 5.50N and 7.46N and has 27 districts with Kumasi the regional capital.

Conceptual Framework

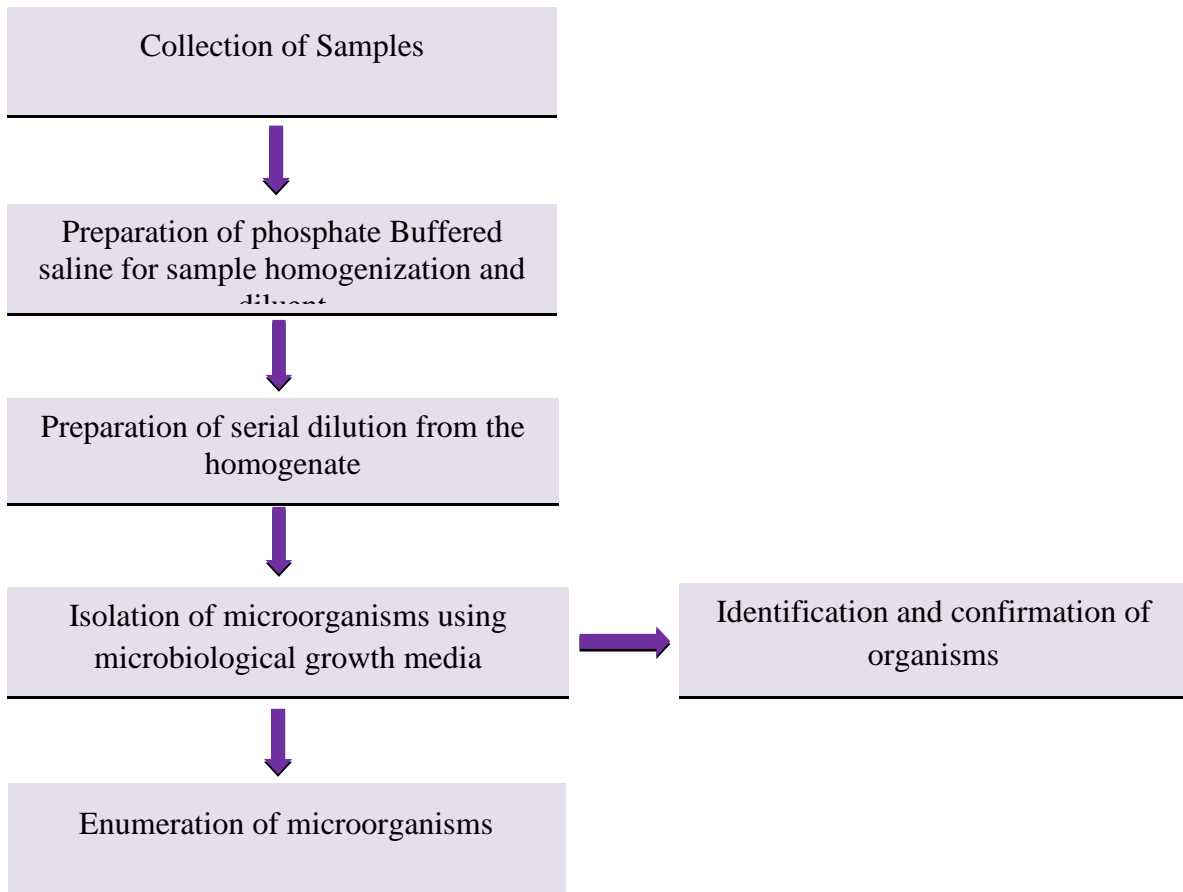


Fig. 1. Schematic flow chart of the research design used in this study

2.2 Study Design

This was a descriptive cross-sectional study designed to assess the microbiological quality of imported chicken parts sold in wholesale cold stores at the Asafo Market in the Kumasi Metropolis of Ashanti Region, Ghana. Fig. 1 shows the schematic flow chart of the research design used in this study (collection of samples to enumeration of microorganisms).

2.3 Study Population and Size

A total of 30 licensed importers with large wholesale cold stores for frozen chicken at the Asafo market were involved. Three chicken parts (thighs, wings, and backs) were each collected from the three import countries (United State of America, Brazil and the Netherlands). A sample size of 45 comprising of 5 each of pre-cuts chicken thighs, wings, and backs from each import country was collected.

2.4 Sampling Technique

Multistage sampling; purposive, stratified and simple random techniques were employed. Firstly, wholesale import cold stores with frozen chicken meats from the three import countries (USA, Brazil and the Netherland) were purposively selected. Stores were further stratified into three import countries from which at least four cold stores were randomly sampled from each group. Five newly arrived cartons of frozen chicken parts (chicken thighs, wings, backs) were then purchased from each import country for the study (Table 1). These were transported under the ice to the laboratory for analysis.

2.5 Sampling Procedures

Aseptic sampling techniques were used to sample one kilogram of frozen chicken parts (thighs, wings and back) from each carton into

sterile plastic envelope bags, sealed, and stored under the ice for the microbiological analysis.

2.6 Laboratory Methods and Analysis

2.6.1 Media and reagent for bacteriological study

Media used for microbial analysis were Plate Count Agar [1], MacConkey Agar (MCA), Mannitol Salt Agar (MSA), Desoxycholate Citrate Agar (DCA) and Sabouraud Dextrose Agar (SDA). All commercial media were prepared as previously described [18,19].

2.7 Sample Preparation

A 25 g portion of each chicken parts were excised aseptically into a sterile stomacher bag containing 225ml of sterile buffered peptone water and homogenized. Each homogenate was then used for serial dilution.

2.8 Serial Dilution and Culturing

A ten-fold serial dilution was performed on each homogenate for cultivation and identification of microbial contaminants as previously described [18,19]. Meanwhile, fungal isolates were enumerated and identified by morphological characteristics on Sabouraud Dextrose Agar (SDA) incubated at room temperature [20].

2.9 Microbial Analysis

Total Viable Counts (TVC), Total Coliform Counts (TCC), *Salmonella* species counts, *Staphylococcus* species counts were determined using 1ml dilution of each sample on suitable media and incubated at 37°C for 24 hours as previously described [18,19]. Bacterial isolates on the different media were identified using standard microbiological methods [21]. Using representative colonies, isolates were sub-cultured and identified using morphological

characteristics and standard biochemical tests previously described [18,19].

2.10 Identification of Fungi

The fungal isolates were identified by their morphological characteristics on Sabouraud Dextrose Agar (SDA) and microscopic examination using lactophenol cotton blue staining technique as previously described [20,22].

2.11 Statistical Analysis

Data obtained from the microbial analysis were entered into Microsoft excel 2007 spreadsheet. The means microbial counts were computed (cfu/g) and transformed into log₁₀cfu/g. The data was subsequently imported into IBM-SPSS software version 16.0 and analyzed. Descriptive statistics were used to summarize the distribution of various variables into tables and figures. The mean counts of the three chicken parts and the country of import were compared using one-way analysis of variance (ANOVA) at a 95% confidence interval (P<0.05).

3. RESULTS

3.1 Mean Bacterial Counts of Frozen Chicken Parts Per Import Country

Table 2 shows the mean log cfu/g counts of TCC, TVC, and *Salmonella* and *Staphylococcus aureus* for chicken meats from the three import nations. Chicken meat parts from Brazil had means TCC of 6.14 mean log₁₀cfu/g whereas the Netherlands was 5.98 mean log₁₀cfu/g. Chicken meats from Brazil recorded TVC of 6.14 mean log₁₀cfu/g whereas the least was from the USA. The highest counts for *Salmonella species* and *Staphylococcus aureus* were from the USA. There were no significant differences (P > 0.05) in mean counts for chicken parts in the USA, the Netherlands and Brazil.

Table 1. Sample size from major cold stores

Country of import	Chicken parts			
	Wings	Backs	Thighs	Total
USA	5	5	5	15
Brazil	5	5	5	15
the Netherlands	5	5	5	15
Total	15	15	15	45

Table 2. Mean bacterial load log count of chicken meat per import country

Import nation	TVC (cfu/g)		TCC(cfu/g)		Salmonella(cfu/g)		S. aureus(cfu/g)	
	Mean	Log	Mean	Log	Mean	Log	Mean	Log
USA	8.5x10 ⁵	5.93(±0.32)	4.0x10 ⁴	4.6(±0.26)	5.1x10 ⁴	4.71 (±0.13)	4.4x10 ⁴	4.64 (±0.18)
The Netherlands	9.5x10 ⁵	5.98(±0.39)	6.5x10 ⁴	4.81(±0.28)	1.7x10 ⁴	4.22 (±0.54)	3.2x10 ⁴	4.51 (±0.18)
Brazil	1.4x10 ⁶	6.14(±0.08)	7.6x10 ⁴	4.88(±0.17)	4.7x10 ⁴	4.67 (±0.26)	4.0x10 ⁴	4.59 (±0.36)

TVC: Total Viable Count, TCC: Total Coliform Count. Permissible levels of bacterial load log counts (TVC: ≤ 6.48, TCC: ≤ 4.0, SC: absence per gram, STC: ≤ 4.0, YMC: ≤ 5.3) [23-25]

Table 3 shows the mean TVC, TCC, *Salmonella* spp. and *Staphylococcus aureus* counts for chicken thighs, backs, and wings showing no significant differences ($P > 0.05$).

3.2 Bacteria Isolates

Table 4 shows the number of bacteria isolated from chicken meat products from the three import countries. Out of 45 chicken parts sampled, Brazil recorded 29 (49.2%) isolates whereas the USA recorded 13 (22.0%). *Staphylococcus aureus* (G+) was the highest (35.4%) isolate followed by *E. coli* (G-) (26.2%) and *Salmonella* spp. (G-) (24.6%).

Table 5 shows the number of bacteria isolated on chicken parts from the three countries of import. Chicken thighs had the highest numbers of isolates (46.2%), followed by backs (29.2%) and wings (24.6%).

3.3 Fungi Isolates

In Table 6, mean log cfu/g for fungal counts for chicken products were 2.03, 2.06 and 1.97 for the USA, the Netherlands and Brazil respectively.

In Table 7, of twenty-five (25) fungal growths, four fungi genera were identified including 33.3% (11) *Aspergillus* spp, 15.2% [26] *Cladosporium* spp, 24.2 % (8) *Penicillin* spp and *Rhizopus* spp 27.3% (9). Out of the 45 chicken sampled, a total of 25 (55.56%) had fungal growth of which 28%,

32% and 44% were isolated from the USA, the Netherlands and Brazil respectively.

4. DISCUSSION

4.1 Microbiological Quality

In this study, means total aerobic plate count of $>5 \log_{10}\text{cfu/g}$ were reported for imported frozen chicken parts from the USA, the Netherlands and Brazil consistent with previous studies in Ghana and the Czech Republic [27,28]. Meanwhile, Kunadu et al. [29] and Pesewu et al., [15] reported higher bacteria counts for chicken meats at informal live bird markets and supermarkets in Ghana [15,29]. On the other hand, higher counts of $8.44 \log_{10}\text{cfu/g}$ and $10.17 \log_{10}\text{cfu/g}$ were reported for market chicken meats in Ouagadougou, Burkina Faso [30] and Nepal [31] respectively.

Mean total coliform counts and *Salmonella* counts of $>4 \log_{10}\text{cfu/g}$ were reported in this study consistent with $4.97 \log_{10}\text{cfu/g}$ and $6.5 \log_{10}\text{cfu/g}$ reported in previous studies [32,33]. The means counts in our study were higher than 3.14 to $3.80 \log_{10}\text{cfu/g}$ reported for chicken meat products from supermarkets in Ghana [28]. Higher coliform counts of $7.0 \log_{10}\text{cfu/g}$ and $32.2 \log_{10}\text{cfu/g}$ were also reported in previous studies contrary to this study [34,35]. In this study, total *Staphylococci* spp. of $>4 \log_{10}\text{cfu/g}$ were reported in agreement with previous studies [35,36] but higher than $1.99 \log_{10}\text{cfu/g}$ reported in market meat elsewhere [37].

Table 3. Mean bacterial load counts of chicken parts

Chicken Parts	TVC (cfu/g)		TCC(cfu/g)		<i>Salmonella</i> (cfu/g)		<i>S. aureus</i> (cfu/g)	
	Mean	Log	Mean	Log	Mean	Log	Mean	Log
Thighs	1.8×10^6	6.25	3.5×10^4	4.55	5.4×10^4	4.73	3.5×10^4	4.54
Backs	6.2×10^5	5.79	6.3×10^4	4.80	4.7×10^4	4.67	2.9×10^4	4.46
Wings	1.0×10^6	6.01	8.5×10^4	4.93	1.6×10^4	4.21	5.5×10^4	4.74

TVC: Total Viable Count, TCC: Total Coliform Count. Permissible levels of bacterial load log counts (TVC: ≤ 6.48 , TCC: ≤ 4.0 , SC: absence per gram, STC: ≤ 4.0 , YMC: ≤ 5.3) [23-25]

Table 4. Prevalence of Bacteria Isolates per Import country

Import Nations	USA (*n / %)	The Netherlands (*n / %)	Brazil (*n / %)	Total (N/%)
Bacterial Isolates				
<i>Escherichia coli</i>	3(20.0)	6(40.0)	8(53.3)	17(26.2)
<i>Salmonella species</i>	2(13.3)	5(33.3)	9(60)	16(24.6)
<i>Klebsiella species</i>	2(13.3)	4(26.7)	3(20.0)	9(13.8)
<i>Staphylococcus aureus</i>	6(40.0)	8(53.3)	9(60%)	23(35.4)
Total (%)	13(22.0)	23(28.8)	29(49.2)	65(100)

*n = 15; *N=65; Not significantly different ($P > 0.05$)

Table 5. Frozen chicken parts per bacteria isolates

Frozen chicken parts	Thighs (*n/%)	Wings (*n/%)	Back (*n/%)	Total (N/%)
Bacterial Isolates				
<i>Escherichia coli</i>	7(46.7)	4(26.7)	6(40.0)	17(26.2)
<i>Salmonella</i> species	6(40.0)	6(40.0)	4(26.7)	16(24.6)
<i>Klebsiella</i> species	4(26.7)	2(13.3)	3(20.0)	9(13.8)
<i>Staphylococcus aureus</i>	13(86.7)	4(26.7)	6(40.0)	23(35.4)
Total (%)	30(46.2)	16(24.6)	19(29.2)	65(100)

*n = 15; *N=65; No significantly different (P>0.05)

Table 6. Plate count for fungi

Countries of import	Thighs	Wings	Back	Total	Mean Log ₁₀ CFU/g
USA	2.04	2.01	2.04	6.09	2.03
The Netherlands	2.03	2.08	2.06	6.17	2.06
Brazil	1.98	1.92	2.02	5.92	1.97

Permissible levels of bacterial load log counts (TVC: ≤ 6.48, TCC: ≤ 4.0, SC: absence per gram, STC: ≤ 4.0, YMC: ≤ 5.3) [23-25]

Table 7. Fungal genera Identified per import country

Import Nation	<i>Cladosporium</i>	<i>Aspergillus</i>	<i>Penicillin</i>	<i>Rhizopus</i>	Total (%)
USA	2*	3*	2*	3*	30.3
The Netherlands	1*	5*	3*	3*	36.4
Brazil	2*	3*	3*	3*	33.3
Total (%)	15.2	33.3	24.2	27.3	100

Permissible levels of bacterial load log counts (TVC: ≤ 6.48, TCC: ≤ 4.0, SC: absence per gram, STC: ≤ 4.0, YMC: ≤ 5.3) [23-25]

We suspect that the differences in bacteria counts for chicken meats in this study and previous studies might be linked to technologies used, hygienic conditions during slaughtering, processing, packaging, storage and distribution [37-40]. A study using ISO 22000:2005 certified poultry processing plant reported remarkable reductions in bacteria counts in the four sequential processing steps [37,39]. The final washing, freezing and packaging are the critical control point identified to control microbial contaminations [37,39]. These processes might differ for poultry processing plants for each import country and hence the differences.

4.2 Bacteria Isolates

Klebsiella spp., *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli* were microbial contaminants isolated in this study. These bacteria are common contaminants of poultry meats [37]. Microbial contaminations occurred largely at evisceration due to faecal material from the cloaca of chicken carcasses [41]. Moreover, frozen chicken meat products imported into Ghana are subjected to several freeze-thaw cycles during transportation over long distances,

through transit from ports to cold storage facilities providing favourable conditions for microbial proliferation. The presence of these bacteria is an index of the hygiene quality of the chicken meat. Several studies also reported similar microbial contaminants consistent with this study [42,43].

Chicken products from Brazil recorded the highest bacterial isolates relative to the Netherlands and USA. We suspect the differences could result from differences in technologies employed in poultry husbandry and processing of the meats [40]. Also, the animal health, rearing conditions, quality of slaughtering, processing, packaging and storage conditions [44,45]. Although large-scale slaughterhouses is a rapid and highly automated process, notwithstanding, considerable opportunities exist for contamination [46,47]. Hence, the performance of the slaughter process is very critical and has a direct consequence on the microbial quality of the chicken meat products [48]. This could have accounted for the observed differences in numbers of the bacteria isolates.

We obtained 26.2% and 24.6% prevalence for *E. coli* and *Salmonella* of which more than 50%

were recorded for frozen chicken meat products from Brazil. These values are much lower than 66.7% and 91.7% reported for frozen chicken meat at retail outlets in Ghana [16]. These values in our study were fairly consistent with 27.82% and 13.64% obtained for *Escherichia coli* and *Salmonella* spp. but inconsistent with 18.52% and 1.84% for *Staphylococcus aureus* and *Klebsiella* spp. reported in Nigeria [43]. The presence of 35.4% *S. aureus* in chicken meat products of this study is inconsistent with 17.9% obtained by Kozačinski and colleagues in Croatia [32]. The presence of 24.6% *Salmonella* of this study is fairly similar to 20% reported in broiler chicken [49] but much lower than 91.7% in chicken drumstick [16].

The presence of *S. aureus*, *E. coli* and *Salmonella* points to possible contamination at the plants coupled with transportation and storage challenges [50] and raises microbial safety standard credibility. The mere presence of these bacteria in raw meat may not necessarily pose a health threat to consumers since the meats would be subjected to cooking. Compared with the permissible limits stipulated by [23-25] for coliforms counts, *S. aureus*, *E. coli* and *Salmonella* spp. counts for chicken breast, backs and thigh samples examined were slightly higher and unacceptable.

4.3 Fungal Isolates

Fungal contaminants, *Aspergillus*, *Penicillium*, *Cladosporium* and *Rhizopus* were isolated, consistent with previous studies [50]. *Aspergillus* and *Rhizopus* species were reported in chicken meat retailed in Lahore City, Pakistan [51] in agreement with this study. *Candida* and *Cryptococcus* spp. reported in poultry meat (drumstick and breast) retailed in Local Iraqi Markets [52] were not reported in our study. The presence of 33.3 % *Aspergillus* spp. and 24.2 % of *Penicillin* spp. in our study are in agreement with 35.7 % and 21.4% reported for *Aspergillus* spp. and *Penicillin* spp. for processed chicken meat retailed in Abakaliki, Ebonyi State, Nigeria [53]. However, *Neocosmospora* spp. and *Mucor* spp. reported by [53] were not found in this study.

Fungal contaminations in chicken meat products may occur due to improper processing and temperature fluctuation during storage and transportation [22,50]. The presence of fungal contaminants in chicken meat products poses a health threat to consumers. Some fungi under

certain environmental conditions release secondary metabolites such as mycotoxins which could cause serious disorders in humans and animals [54]. Mycotoxins such as aflatoxins, ochratoxins, etc. produced by fungi like *Aspergillus*, *Fusarium* and *Penicillium* [55,56] in food could cause severe health problems such as allergic reactions, cancer, and organ damage [57]. Although, there is a paucity of information on the acceptable limits for fungal contaminants in water and food, the presence of mycotoxigenic fungi pose public health concerns.

5. CONCLUSION

The results of this study highlighted the microbial quality of pre-cut imported frozen chicken meats into the country stored in major cold stores in the Kumasi metropolis. The presence of potential foodborne pathogens is linked to possible contaminations at slaughterhouses and transportation from ports to cold storage facilities over long distances with several freeze-thaw cycles. Therefore regular monitoring by regulatory authorities together with stakeholders is critical to ensure frozen chicken imported into the country are safe for public health good.

AVAILABILITY OF SUPPORTING DATA

All data used for this manuscript are available upon a reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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