

Mycological Quality of Fresh and Frozen Chicken Meat Retailed within Warri Metropolis, Delta State, Nigeria

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Received: March 26, 2017; Revised: September 22, 2017; Accepted: October 10, 2017

Abstract

Chicken meat continues to gain global acceptance. The unhygienic processing and retailing conditions, in most Nigerian States, expose the meat to microbial contaminations. Though, economically useful, most fungi are mycotoxigenic. The present study investigated the mycological quality of fresh and frozen chicken meat retailled in three major markets (Effurun, Ekpan, and Uborikoko) within Warri metropolis, Delta State, Nigeria. The spread plate technique was used to culture the samples on sterile potato dextrose agar (supplemented with antibiotics) at 28 ± 2 °C. Out of the 60 samples analyzed, 38 (63.3 %) yielded fungal growth, with 25 (65.7 %) and 13 (34.2 %) for fresh and frozen samples, respectively. The fungal loads ranged from $1.1 - 2.2 \times 10^4$ CFU/g and $1.3 - 4.0 \times 10^2$ CFU/g for the fresh and frozen samples, respectively. The fungal loads were not significantly different, except in frozen samples from Epkan market. *Penicillium* (20.8 -26.7 %), *Aspergillus* (20.0 - 22.9 %), *Cladosporium* (10.4- 23.3 %), *Mucor* (10.4-13.3 %), *Fusarium* (8.3 - 16.7 %), *Rhizopus* (0 - 12.5 %), *Alternaria* (0 - 8.3 %), and *Candida* spp. (0 - 6.3 %) were the major fungal isolates. The fresh chicken samples were more contaminated than the frozen samples, though not significantly different ($P > 0.05$). Poor processing environment and use of unhygienic retail equipments could be the possible contamination routes. The relatively high proportions of *Penicillium*, *Aspergillus*, *Cladosporium* and *Fusarium* spp. is of public health concern, and highlights the need for public education on good hygienic practices, proper environmental sanitations, and adequate thermal treatment of chicken meat before consumption.

Keywords: Warri metropolis, Chicken meat, Potato dextrose agar, Fungi.

1. Introduction

Globally, poultry sector has been recognized as a very significant and vital source of animal protein in the daily diet of an average household (Salawu *et al.*, 2014). Chicken has been generally reared for their meat and eggs. The meat is most widely accepted over beef or pork in Nigeria because of its excellent source of proteins, high digestibility, taste, low fat/cholesterol (Javadi and Safarmashaei, 2011) and without religious or health taboo. Nigeria's chicken population is about 150.682 million of which 25 % are commercially farmed, 15 % semi-commercially, and 60 % in backyards (Salawu *et al.*, 2014). Generally, the steps involved in processing chicken meat include slaughtering or bleeding, scalding,

defeathering and evisceration, which could be manually or mechanically done.

Microorganisms are ubiquitous and resident in wide varieties of foods of plants and animal origin. All foods possess a finite risk of microbiological contamination, but according to Roberts (1990), the highest risk factors include raw and animal foods. Chicken meat is one of such kind of products. Like every other animal, live chickens are hosts to diverse microorganisms residing on their skin, feathers and alimentary tract. These microorganisms can possibly contaminate the meat during processing chain, such as slaughtering, feather plucking, evisceration, and storage (Kozáčinski *et al.*, 2006; Bhaisare *et al.*, 2014). Moreover, when processed in unhygienic environments, others microorganisms present in the processing

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environment, equipment, and processors hands/apron can contaminate the final meat product.

Over the years, there have been outbreaks of infections associated with consumption of contaminated chicken products, and the predominant microorganisms isolated included *Salmonella* spp., *Campylobacter* spp., *Staphylococcus* spp., *Shigella* spp., *Escherichia* spp., *Listeria* spp., *Yersinia* spp., *Aeromonas* spp. and *Clostridium* spp. (De Boer *et al.*, 1991; Bhaisare *et al.*, 2014). Reports abound on the bacteriological quality of commercial poultry and other livestock's products (Akbar and Anal, 2013; Adeyanju and Ishola, 2014; Bhaisare *et al.*, 2014; Omorodion and Odu, 2014; Firildak *et al.*, 2015; Chuku *et al.*, 2016; Zakki *et al.*, 2017). Information on mycological quality of raw chicken meat is scanty. Fungi and their spores are ubiquitous in the environments. Some genera, such as *Aspergillus* spp., have been found to elaborate hazardous mycotoxins that are mutagenic, teratogenic, hepatotoxic, immunotoxic and nephrotoxic (Atanda *et al.*, 2013; Greco *et al.*, 2014; Żukiewicz-Sobczak, 2015; Adeyeye, 2016). The severity of fungal diseases ranges from superficial to deep-seated organ damages if not well managed. The presence of such fungi in edible food is of great public health importance. Considering the fact that the levels of acceptability and demand for chicken meat over other meats remain on the high side, it is pertinent to periodically assess them for fungal contamination and public health safety.

The present study, therefore, investigates the fungal quality of fresh and frozen chicken meat sold in Warri metropolis with a view to ascertaining their mycological portability and public health safety.

2. Materials and Methods

2.1. Study Location

The study area was within Warri, a major city in Delta State, South-South Nigeria (Figure 1). Geographically, it is located at coordinates 5°31'N 5°45' E and 5.517 °N 5.750°E. It is one of the major hubs of Petroleum activities and businesses in Southern Nigeria, with an estimated population of about 7,056,289 (Anon, 2017). It shares boundaries with Ughelli/Agbarho, Sapele, Okpe, Udu and Uvwie, although most of these places, notably Udu, Okpe and Uvwie, have been integrated to the larger cosmopolitan Warri. Effurun serves as the gateway to and the economic nerve of the city.

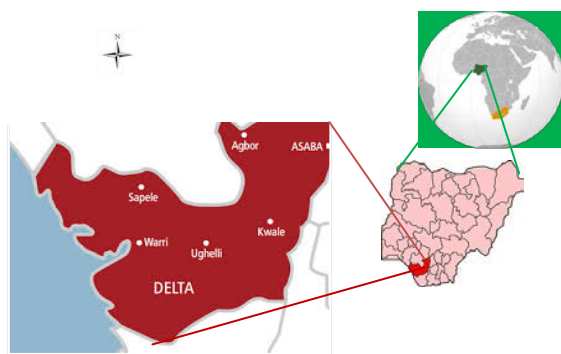


Figure 1. Map showing Warri in Delta State, Nigeria, West Africa.

2.2. Collection of Sample

Three major markets located within Warri metropolis, namely Effurun, Ekpan, and Uborikoko, served as sample collection centres. A total of 60 fresh and frozen chicken thigh samples, processed and retailed with the selected main markets were randomly sourced, purchased and labelled appropriately. The collected samples were placed in sterile ice-packed containers and conveyed to the Laboratory for analysis within 2 h. The sampling regime was between April – June, 2016.

2.3. Preparation of Sample

Twenty-five grams (25 g) of each chicken thigh sample were mixed with 225 mL of sterile (0.1%) peptone water in sterile beaker and thoroughly homogenized under aseptic conditions. Thereafter, the homogenized samples were serially diluted to 10^6 as described by APHA (2001).

2.4. Determination of Fungal Load in Collected Samples

The fungal load for each sample was determined using the streak plate technique (APHA, 2001) on sterile Potato Dextrose Agar (PDA) (Hi-media), previously prepared according to the manufacturer's specifications. The antibiotic, streptomycin (100 mg/L) was added to the culture media to make it more selective for fungal growth. From the dilutions, particularly (10^2 , 10^4 and 10^6), made above, 0.1 mL aliquot was taken and aseptically inoculated onto the pre-set antibiotic-supplemented PDA, before spreading evenly with a sterile glass spreader. The inoculated agar plates were incubated on previously disinfected work bench at 28 ± 2 °C for 3-5 days. The observed colonies were enumerated mechanically. The fungal loads were determined using the formula below, and results were expressed as CFU/g of sample:

$$\text{Total fungal count (CFU/g)} = \frac{\text{Average plate count}}{\text{Volume Cultured} \times \text{dilution factor}}$$

2.5. Isolation and Characterization of Isolates

The prominent fungal colonies on the culture media plates were sub-cultured by inoculating them onto fresh sterile PDA media for further characterization. The growth pattern, pigmentation, and size of colonies were observed and recorded during the incubation period to aid identification of the organisms. The colonial morphology was examined using lactophenol (LP) cotton blue stain. A drop of lactophenol was placed on a clean microscopic slide. A small portion of each fungal isolate was taken using a sterile needle and placed in the drop of lactophenol. A clean cover glass was gently placed over the suspension and observed microscopically. The observed cultural and microscopic morphological characteristics for each stained isolates were compared with standard reference keys and atlas for their probable identities (Alexopoulos and Mims, 1979; Fawole and Oso, 1988; Jay, 1992; De Hoog *et al.*, 2000).

2.6. Statistical Analysis

Data were analyzed using the descriptive statistic SPSS (version 20). Differences in mean of analyzed data were considered significant at $P < 0.05$.

3. Results and Discussion

Food-borne pathogens have continued to be a major threat to food safety, especially in developing countries where proper hygiene and sanitation facilities are often

poor. The global incidence of food borne infections has greatly increased in recent years due to gross neglect of set food safety standards (EFSA, 2016). Millions of people throughout the world have been reported to die annually as a result of illness traced to food-borne pathogens (CDC, 2013). One of such foods with global epidemiological reports as important sources of human food-borne ailments is poultry products (EFSA, 2007; Arora *et al.*, 2015).

In the present study, fresh and frozen chicken samples retailed within three major markets in Warri, Delta State, were analyzed for their fungal concentration and quality. Out of the 60 chicken thighs sampled, 38 (63.3 %) yielded significant fungal growth, with 25 (65.7 %) and 13 (34.2 %) for the fresh and frozen samples, respectively (Figure 2). This is an indication that the fresh chicken samples were probably more contaminated by fungi within the processing, retailing and or storage equipment. The mean fungal counts for fresh chicken samples ranged from $1.1 - 2.2 \times 10^4$ CFU/g (Figure 3). The highest fungal load was recorded from Effurun market samples, while the lowest was found in Ekpam samples. For the frozen chicken samples, the highest mean fungal load (4.1×10^2 CFU/g) was found in Epkan samples, while the least load was from Iborikoko market (1.3×10^2 CFU/g) (Figure 4). Statistical analysis of the results revealed that the contamination of the fresh samples were not significantly ($P > 0.05$) higher than the frozen samples. These findings were comparable to the previous reports. An earlier study reported a fungal contamination of $0 - 8.0 \times 10^4$ CFU/g in chicken meat retailed in three different markets (Creek road market, Mile 3 market and Rumokoro market) in Port Harcourt, Rivers State (Omorodion and Odu, 2014). The fungal load reported for fried ready-to-eat chicken meat sold in two selected motor park points within Abakaliki, Ebonyi State, Nigeria, ranged from 0.25×10^5 to 0.25×10^4 CFU/mL. Vural *et al.* (2013) reported a fungal contamination level of $0 - 2.2 \times 10^4$ CFU/mL in frozen turkey meat sold in Diyarbakir, Turkey. Stagnitta *et al.* (2006) reported a mould and yeast counts of $10^3 - 10^5$ CFU/g for processed meat food samples from retail stores located in San Luis city, Argentina. The fungal loads obtained in the present study were significantly lower than those ($5.787 \times 10^5 - 1.840 \times 10^6$ CFU/g) reported for fresh fish retailed in Benin City, Edo State, Nigeria (Udochukwu *et al.*, 2016). However, relatively higher fungal contaminations were reported for non-chicken meat (Ajiboye *et al.*, 2011; Haleem *et al.*, 2013; Ehigiator *et al.*, 2014).

The presence of fungi in more than half of the total chicken samples possibly suggests environmental contamination, since fungi are ubiquitous in soil, water, air, feeds and processing materials (Greco *et al.*, 2014). Additionally, earlier studies reported fungal spores to be abundant in air and dust particles around waste dumpsites (Igborgbor and Ogu, 2015), and, thus, are easily carried by wind from the wastes dumpsites to exposed meat products in their vicinities. All the markets from which the samples were collected had huge waste dumpsites at various locations within and around the markets. This could be the reason for the lack of statistical differences between the levels of fungal load in the three markets. However, the observed variations could be attributed to differences in the levels of storage/retail facilities and handling practices in various markets/shops. The frozen samples were

expected to yield insignificant levels of fungal counts, but this study suggested otherwise. Freezing/refrigeration is a common preservation method for meat and vegetables. The detection of relatively high proportion of psychrotrophic fungi in the frozen chicken samples, despite the relatively low temperature of the storage facilities, is of public health significance. This finding is in concordance with previous study (Altunatmaz *et al.*, 2013; Vural *et al.*, 2013; Oranusi *et al.*, 2014). It further underscores the need to constantly maintain the recommended storage temperature of ≤ 4 °C and adequate thermal treatment of frozen meats before consumption to prevent mycotoxicoses.

A total of seventy-eight fungi belonging to eight genera were isolated from the fresh and frozen samples, and included *Aspergillus*, *Mucor*, *Fusarium*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhizopus* and *Candida* species (Table 1). The frequencies of fungal isolates in the fresh chicken samples were *Aspergillus* 11 (22.9 %), *Penicillium* 10 (20.8 %), *Rhizopus* 6 (12.5 %), *Cladosporium* 5 (10.0 %), *Mucor* 5 (10.0 %), *Fusarium* 4 (8.3 %), *Alternaria* 5 (8.3 %), and *Candida* 3 (6.3 %) (Figure 5). For the frozen sample, the frequencies of isolation were *Penicillium* 8 (26.7 %), *Aspergillus* 6 (20.0 %), *Cladosporium* 7 (23.3 %), *Fusarium* 5 (16.7 %), and *Mucor* sp. 4 (13.3 %) (Figure 6). Previous studies have reported the presence of some of the fungi in various commercial poultry meat. *Candida* and *Cryptococcus* spp. were reported in poultry meat (drumstick and breast) retailed in Local Iraqi Markets (Haleem *et al.*, 2013). Only *Aspergillus* spp. was reported by Oranusi *et al.* (2014) for chicken meat retailed in Ogun State, Nigeria. The genera, *Penicillium* spp. 3 (21.4 %), *Aspergillus* spp. 5 (35.7 %), *Neocosmospora* spp. 2 (14.2 %) and *Mucor* spp. 4 (28.5 %) were reportedly isolated from processed chicken meat retailed in two selected motor park points in Abakaliki, Ebonyi State, Nigeria (Jerry *et al.*, 2015). Ajiboye *et al.* (2011) isolated *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp. and *Rhizopus* sp. from dried meat samples retailed in Oja-Oba market in Ilorin, Nigeria. Recently, *Aspergillus niger*, *A. fumigates*, *A. flavus*, *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Fusarium equiseti* and *F. avenaceum* were reported in chicken meat retailed in Lahore City, Pakistan (Zakki *et al.*, 2017). Our findings were in agreement with the previous reports, except for the presence of *Cladosporium*, *Mucor* and *Alternaria* spp. Differences in fungal distribution from one environment to another could be attributed to the observed variations. The spores of the moulds isolated in the present study are abundantly distributed in soil, water and air and can easily contaminate exposed and poorly processed food (Lange, 2014; Żukiewicz-Sobczak *et al.*, 2015).

Some fungi, under certain environmental conditions, release secondary metabolites, generally known as mycotoxins. Mycotoxins were reported to cause serious disorders in plants, humans and animals (Sule *et al.*, 2015). Different types of mycotoxins have been reported, but the agro-medically important types include aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids (Zain, 2011). Some of the major mycotoxigenic fungi are distributed among the genera *Aspergillus*, *Fusarium* and *Penicillium* (Zain, 2011; Ismaiel and Papenbrock, 2015). Prolong exposure to

food contaminated by mycotoxin-producing moulds in food have been reported to cause severe health hazards, particularly among which are allergic reactions, cancer, and organ damages (Tasic and Tasic, 2007; Atanda *et al.*, 2013; Greco *et al.*, 2014; Żukiewicz-Sobczak *et al.*, 2015; Wigmann *et al.*, 2015). Although, there is paucity of information on the acceptable limit for fungal contaminants in water and food, the presence of mycotoxigenic fungi could be of concern to the public health. It is, however, important to point out that the presence of mould is not a direct indication of mycotoxin contamination, because mycotoxin production depends on the type of fungal species and extent of growth, substrate components, aeration, relative humidity, temperature and storage environment (Ashiq, 2015). Moreover, previous studies have shown that relatively high temperature and humid conditions majorly favours fungal proliferation and secretion of mycotoxins (Atanda *et al.*, 2013, Ashiq, 2015). The detection of relatively high number of potential mycotoxigenic fungi in the present study calls for improved sanitary, processing and storage facilities by the chicken processors or retailers and consumers alike to prevent impending dangers of ingesting toxins of fungi.

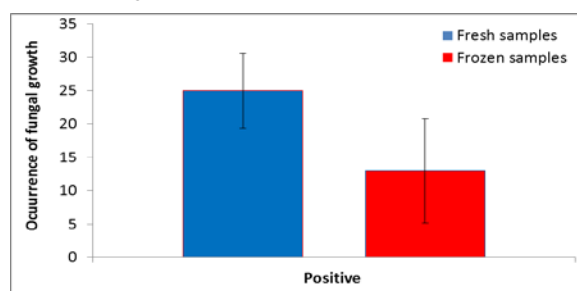


Figure 2. Occurrence of fungal growth in chicken meat samples from markets within Warri metropolis, Delta State

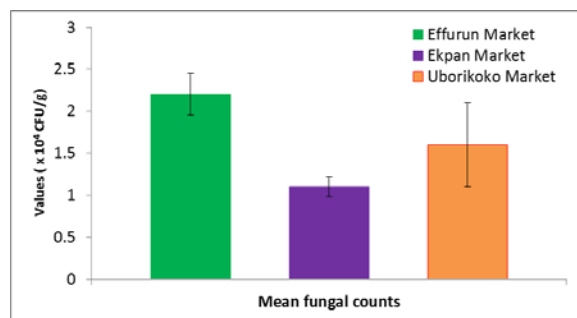


Figure 3. Mean fungal count for fresh chicken meat samples

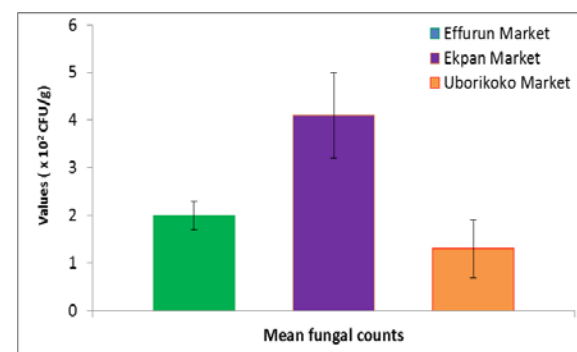


Figure 4. Mean fungal count for frozen chicken meat samples

Table 1. Characteristics and identity of fungal isolates from chicken sample

Isolate code	Description of fungal isolates	Fungal identity
1	The colony has black filaments at its centre which was surrounded by whitish and hairy edge. The reverse of the plate was yellowish.	<i>Aspergillus</i> sp.
2	The colony has blue-green centre surrounded by white hyphae. The reverse was greenish yellow	<i>Penicillium</i> sp.
3	The colony was whitish, loose, fluffy, cotton like and filamentous mould with reverse being whitish or light cream	<i>Mucor</i> sp.
4	The colony was a white, loose filamentous mould with black spores. Hyphae spread to cover the whole plate. The reverse was whitish. The fungus resembles cotton wool in its appearance.	<i>Rhizopus</i> sp.
5	White thick mycelium and white colour at bottom of plate	<i>Fusarium</i> sp.
6	Flat white cottony growth on plate, erect conidiophores, septate hyphae with cylindrical conidia	<i>Alternaria</i> sp.
7	Medium-size hyphae, white with loose filaments, reverse side on plate is white when young.	<i>Cladosporium</i> sp.
8	White cream smooth colonies, spherical, budding	<i>Candida</i> sp.

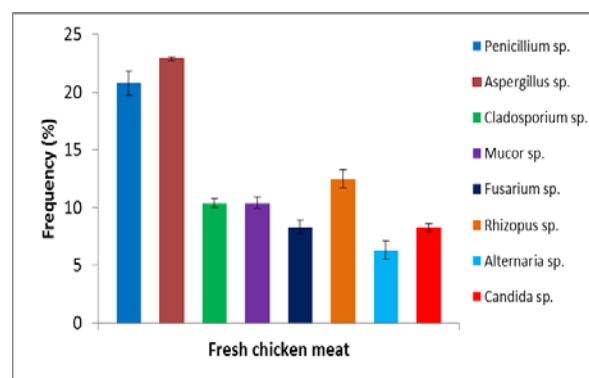


Figure 5. Frequency of fungal isolates from fresh chicken meat samples

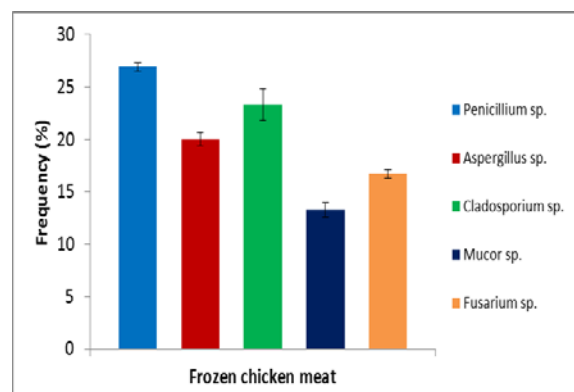


Figure 6. Frequency of fungal isolates from frozen chicken meat samples

4. Conclusion

Commercial fresh and frozen chicken meat samples in major markets within Warri metropolis were found to be contaminated by diverse levels of opportunistic, pathogenic, and saprophytic moulds and yeasts. The fresh chicken meat samples were more contaminated than the frozen samples, though not significantly different ($P > 0.05$). Considering the occurrence of relatively high proportions of *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium* species in both frozen and fresh chicken samples, and their potential health hazards, regular environmental sanitation, good handling practices, proper storage temperatures and adequate thermal treatment of fresh and frozen chicken meat before consumption are recommended.

Acknowledgements

Special appreciation to the Director and Staff of Light Path Medical Laboratory, Warri, Delta State for provision of equipment/reagents and technical support during the study.

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