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COMPARATIVE BACTERIOLOGICAL ANALYSIS OF FROZENAND FRESH CHICKEN MEATS SOLD AROUNDOLD SITEBAYERO UNIVERSITYKANO

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ABSTRACT
Foodborne pathogens are serious public health problems that greatly affect the cost of food production and healthcare while poultry are often associated with foodborne disease outbreaks. This study aimed at investigating the occurrence and distribution of bacterial pathogens associated with chicken meats sold around Bayero University, Kano old site. A total of twenty (20) meat samples comprised of 10 frozen and ten freshly slaughtered chicken were purchased from different selling outlets around the study area. The samples were processed using standard cultural and biochemical procedures. The results of the study showed the mean aerobic mesophilic bacterial count of 5.93 x 10^6 CFU/g and 3.12 x 10^6 CFU/g for frozen and freshly slaughtered chicken meat samples respectively. The coliform counts were 420.1 and 347.7 CFU/100g for frozen and freshly slaughtered chicken meat samples respectively. The distribution of different bacterial species revealed E. coli (26.0%), Klebsiella spp (4.0%), Salmonella spp (19.5%), Campylobacter spp. (14.6%) and Staphylococcus aureus (30%) in frozen chicken meat samples while freshly slaughtered chicken meat samples revealed E. coli (21.0%), Klebsiella spp (8.9%), Salmonella spp (30%), Campylobacter spp. (15.0%) and Staphylococcus aureus (24.0%). This showed that frozen chicken meats were more contaminated than freshly slaughtered chicken meats which might be associated with poor handling and storage conditions.
Keywords: Chicken meat, Contamination, Bacteria, Pathogens, Kano

INTRODUCTION

Foods consumed by humans and animals are excellent media for rapid growth of microorganisms because of the abundant organic matter, sufficient water content and a neutral or slightly acidic P^H (Yang and Chen, 1993). Meats are rich sources of nutrients and hence excellent environments for bacterial growth. Microbial growth is controlled by factors related to the food itself (intrinsic factors) and also to the environment where the food is being stored (extrinsic factors). Food composition is a critical intrinsic factor that influences microbial growth. If a food consists primarily of carbohydrates, spoilage does not result in major odours (Allen and Hamilton, 1989).

Chicken meat is a rich source of protein and is a delicacy particularly among well to do families. The presence of microorganisms in food has always been attributed to contamination through water, soil, processing equipment, contact surfaces, and the food handlers (Kawo and Abdulmumin, 2009; Vihavainen, 2007). Improper handling of food is responsible for most cases of food borne diseases and cross contamination (Bukar *et al.,* 2009; Veluz, *et al.,* 2012).Growth of spoilage bacteria lead to defects in the products and can be responsible for unwanted taste, color, odor, texture, or aspect. There are multiple spoilage mechanisms, and they can result from the production of various metabolites such as volatiles or exopolysaccharides. Once bacteria contaminate meat and constitute the initial microbiota, the storage conditions and the various treatments applied shape the fate of this microbiota. The storage temperature as well as the nature and concentration of the gas useding as mixtures for packaging are selective for some bacterial populations. Storage at low temperature favors the growth of psychrotrophic and psychrophilic bacteria while CO_2 has an inhibitory effect on *Pseudomonas spp*. During storage, the bacterial load increases but the microbiota diversity decreases compared with that initially present (Chaillou, *et al.,* 2015; Holl, *et al.,* 2016). Microbial spoilage occurs as a consequence of the growth and metabolic activities of spoiling bacteria.

In most studies, the bacteria that dominate spoiled food have been considered those responsible for spoilage and, in some studies, the criterion of microbiological acceptability (total viable counts reaching 7 log CFU/g) has been used to define spoilage (Zhang, *et al.,*2012; Holl, *et al.,* 2016).This study was aimed at determining the bacteriological quality of chicken meats sold around Bayero University Kano old site.

MATERIALS AND METHODS

Sample Collection

Chicken meat samples were collected in sterile polythene bags directly from the sellers and immediately taken to the laboratory for analysis.

Determination of Aerobic mesophilic bacterial count

Each sample was processed according to method described by Food and Agricultural Organization (FAO, 1979) and adopted by Nwachukwu and Chukwu (2013), twenty-five grams (25 g) of each sample was aseptically collected and placed in a sterile blender to which 225 ml of buffered peptone water was added and homogenized for 2 min at normal speed. Serial dilution was carried out according to procedure described by Madigan *et al.*(2012). Bacterial count (Aerobic plate count) was achieved using pour-plate method by pipetting 1ml of serially diluted sample into a sterile Petri plate followed by the addition of molten nutrient agar (about 45^0C) and mixing well by gently swirling the plate on the bench top. The molten were allowed to solidify and the plates were then incubated at 37^0C for 24 hours. Colonies on a plate (30-300) were counted and recorded (Madigan *et al.,* 2012). Number of colonies were multiplied by the inverse of the dilution factor.

Coliform Count

This was carried out according to the method described by Atlas (1997). In this method, a set up consisting of nine (9) test tubes each containing 9ml of lactose broth and inverted Durham tube, were autoclaved to expel air and to sterilize. Inoculation was made from the serially diluted samples from the 1:10 to 1:1000 dilutions. All the 9 test tubes were incubated at 37^oC for 24 hours and another 24 hours in the absence of gas. Following 24 or 48 hours of incubation the tubes were observed for gas production and the number of gas positive tubes was compared with the most probable number (MPN) table to estimate the most probable number of coliforms per gram of the sample.

Isolation and identification of pathogenic bacteria

One (1) mlof each homogenized sample was added into a test tube containing 9mls sterile distilled water and serially diluted to 10^{-5}.One ml aliquot each was poured into eight separate petridishes followed by adding warm prepared mannitol salt agar, MacConkey agar, *Salmonella-Shigella* agar and blood agar respectively into duplicate petridishes. The petridishes were mixed by swirling and incubated at 37^oC for 24hours.After incubation, the plates were examined for bacterial growth including colony size, elevation, edge, colour, odour and pigmentation. Discrete colonies were subcultured on similar media and incubated at 37^oC for 24hours. They were subjected to Gram's staining and biochemical tests for identification (Bukar *et al.,* 2009).

Gram's staining

Gram staining was carried out on the isolate to differentiate the gram negative bacteria from the gram positive bacteria (Cheesbrough, 2006).

Biochemical Tests

Catalase Test:

A drop of distilled water was added onto a clean glass slide and a colony of the test bacteria was smeared on it using a wooden stick and 2-3 drops of hydrogen peroxide was added on to the smeared and observed for change (Karen, 2010). Positive – bubbleswhile Negative – No bubbles

Coagulase Test

- A drop of distilled water was placed on two clean glass slides
- A colony of the test bacteria was then emulsified on the slides
- A loop full (not more) of plasma was added to one of the suspensions and was mixed gently. And was checked for clumping of the organisms within 10 seconds and no plasma is added to the second suspension and observation was made for positive result (Cheesbrough, 2006).

  Positive – clumping within 10 seconds while Negative – No clumping within 10 seconds.

Simon's Citrate Test

A slope of citrate agar medium was prepared in bijou bottle. Using a sterile wire loop, a colony of the test bacteria was inoculated on the slope then stab the butt and incubated at 35^oC for 48 hours. The set up was checked for positive result (Cheesbrough, 2006). Positive – Blue while Negative – Green.

Methyl Red test

The medium was incubated lightly from a young culture of the organism, and incubated at 30^oC for two days. Five drops of the indicator will be added to the culture. A red color indicates a positive acid reaction (Cheesbrough, 2006).

Voges Proskauer (VP)

About 2mo of the medium was inoculated to the test organism and incubated at 37°C, then 1ml of 10% KOH was added and leave at room temperature for 1hr. Pink color indicates positive. No color change Negative (Cappuccino and Sherman, 2002).

Indole Test

The test bacteria were inoculated in a test tube containing 3ml of sterile peptone water, and were incubated at 37°C for 48hrs, then 0.5ml of Kovac's reagent was added to the test tube containing the test bacteria and was shake gently. Positive – Red color on the surface within 10 minutes while Negative – Yellow ring on the surface(Cheesbrough, 2006).

Urease Test

Urea agar was prepared and inoculated with the test bacteria and incubated at 37°C for 48hours and observed for color change. Positive – pink color while Negative – Yellow color(Cheesbrough, 2006).

Triple Sugar Iron Test

Using a straight wire loop, the butt of the TSI in a test tube was stabbed and then incubated for 24hours at 37°C; the test tube was then observed and recorded. Hydrogen sulphide production is indicated by the darkening of the medium while gas production is indicated by the formation of bubbles (Cheesbrough, 2006).

RESULTS

Aerobic mesophilic bacterial count of chicken meat samples showed that frozen chicken had higher mean bacterial count of 5.93 x 10^6 CFU/g while freshly slaughtered chicken meat had lower mean bacterial count of 3.11 x 10^6 CFU/g as shown in Table 1.The P-value is 0.344154 shows that there is significant difference between the two samples, for the coliform count. The mean coliform count of frozen chicken meat was higher (420.1) than that of freshly slaughtered chicken meat (347.1) Table 2.

Staphylococcus aureus had the highest rate of occurrence (30.0%) with the least bacterial specie being *Klebsiella spp* (4.0%) in frozen chicken meat while *Salmonella spp* had the highest rate of occurrence (30.0%) with the least bacterial specie being *Klebsiella spp* (8.9%) in freshly slaughtered chicken meat (Table 3).

Table 1: Aerobic mesophilic bacteria count of chicken meat sold around old site Bayero University Kano

S. No	Frozen (X10^6 CFU/g)	Fresh (X10^6 CFU/g)
1	1.80	2.89
2	6.87	3.65
3	2.73	5.11
4	1.27	7.50
5	7.37	3.32
6	2.80	3.45
7	2.79	1.16
8	2.58	1.12
9	2.10	5.23
10	4.44	1.11
Mean	**5.93**	**3.11**

Table 2: Coliform count of chicken meat sold around old siteBayero University Kano

S. No	Frozen (MPN/100g)	Fresh (MPN/100g)
1	120	64
2	2400	14
3	28	2400
4	150	43
5	210	75
6	15	150
7	75	93
8	64	460
9	1100	28
10	39	150
Mean	**420.1**	**347.1**

Table 3: Distribution of isolated bacteria in chicken meat around old site Bayero University Kano

Organisms	Frozen (n=10) Number (%)	Fresh (n=10) Number (%)
E. coli	26.0	21.0
Klebsiella spp	4.0	8.9
Campylobacter spp	14.6	15.0
Salmonella spp	19.5	30.0
Staphylococcus aureus	30.0	24.0

DISCUSSION

It is obvious from the study that the frozen chicken is highly contaminated than the freshly slaughtered.The mean variable bacteria count of the isolated bacteria were 5.9 x $10^6$and 3.11 x$10^6$for both frozen and freshly slaughtered chicken respectively. The frozen chicken also had the highest number of mean coliform count of 420.1 than the freshly slaughtered chicken which had 347.7. This high bacterial load observed in frozen chicken could be attributed to contamination from the environment and personnel or from materials used including water during processing, transportation and storage. The finding of this work is in agreement with that of Farkas (2010), which revealed that frozen chicken contained higher bacterial load than freshly slaughtered chicken.The following organisms; *E. coli*, *Staphylococcus aureus,Salmonella, Campylobacter* and *Klebsiella* were isolated and identified. The isolated organisms were identified to be part of the normal body flora and environment associated bacteria. This suggest that the environment (soil, air and water) is the major source of bacterial contamination of chicken meat. The result of the current work is in agreement with that of Sackey (2010) and Authority (2015) who also isolated similar bacteria genera.The presence of *Salmonella* in chicken meat may be attributed to the health status of the living bird which carries *Salmonella*, poor hygienic conditions during slaughtering, cross contamination either from the birds, instruments, machines, workers, scalding tanks, defeathering machines, manual evisceration and intestinal contents can spill and contaminate the muscle and organs of the chicken, which is an important source of *Salmonella* in chicken meat (Akkaya*et al.,* 2006). The high occurrence of *Staphylococcus aureus* (30.9%) as observed in frozen chicken meat could be due to poor personal hygiene of workers and the technique used in eviscerating the chicken carcasses during processing. The relatively high percentage of occurrence observed in *E. coli* 21% and 26.9% for freshly slaughtered and frozen chicken respectively is an indication that the chicken meat is faecally contaminated directly or indirectly through the water used from the environment or the personnel during processing. This finding is also in agreement with that of Abd El-Alim (2017). The *Klebsiella* isolated from both freshly slaughtered and frozen chicken meats could have originated from the intestinal contents of the birds being process according to Jimenez *et al.* (2006).

CONCLUSIONAND RECOMMENDATION

This study revealed that frozen chicken had the highest number of aerobic mesophilic count of 5.93 x 10^6 and coliform count of 420.1 than the freshly slaughtered chicken with 3.11 x 10^6 and 347.7 for aerobic mesophilic and coliform counts respectively. Therefore there is high level of contamination in the chicken meat. The isolation of enteric pathogens (*E. coli*, *Salmonella, Klebsiella, Campylobacter* and *Staphylococcus aureus*) in chicken meat in this study shows that they harbor foodborne pathogens which could play a role in the spread of foodborne illnesses.The high prevalence of potential foodborne pathogens is of great concern. It is recommended that regulatory authorities work closely with stakeholders in the frozen chicken trade to set performance objectives at different stages in the value chain and employ control measures that will effectively preserve chicken meat until use.

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