



(RESEARCH ARTICLE)



## Bacteriological quality of frozen chicken sold in the capital cities of the South Eastern States of Nigeria

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### Abstract

**Aims:** To determine and compare the bacteriological quality of frozen chicken sold in the Capital cities of the South Eastern States of Nigeria.

**Method:** A total of 150 samples (frozen chicken) were collected from different Capital cities (Abakaliki, Awka, Enugu, Owerri and Umuahia) of the five (5) South Eastern States of Nigeria, which were 30 samples each State. The Aerobic Plate Counts (APC), Total Coliform Counts (TCC), Psychrotrophic Plate Counts (PPC), *Escherichia coli* counts, *Staphyococcus aureus* counts and the presence of *Salmonella spp* were determined by plate count technique using the appropriate media. Colony counts were taken from the plates with the specified number of colonies.

**Results:** For the Capital cities which are Abakaliki, Awka, Enugu, Owerri and Umuahia, the samples mean values for the Aerobic Plate Counts (APC) were  $9.61 \times 10^6$ ,  $8.50 \times 10^6$ ,  $6.12 \times 10^6$ ,  $8.10 \times 10^6$  and  $6.46 \times 10^6$  respectively, the Total Coliform Counts (TCC) were  $4.80 \times 10^3$ ,  $5.59 \times 10^3$ ,  $2.46 \times 10^3$ ,  $2.62 \times 10^3$  and  $3.72 \times 10^3$  respectively, the Psychrotrophic Plate Counts (PPC) were  $5.71 \times 10^6$ ,  $4.57 \times 10^6$ ,  $4.22 \times 10^6$ ,  $5.53 \times 10^6$  and  $4.41 \times 10^6$  respectively, the *Escherichia coli* counts were  $2.33 \times 10^3$ ,  $2.68 \times 10^3$ ,  $1.56 \times 10^3$ ,  $1.80 \times 10^3$  and  $2.12 \times 10^3$  respectively, the *Staphyococcus aureus* counts were  $5.58 \times 10^3$ ,  $3.93 \times 10^3$ ,  $4.36 \times 10^3$ ,  $4.16 \times 10^3$  and  $4.11 \times 10^3$  respectively and the incidence of *Salmonella spp* were 19, 13, 6, 15 and 16 respectively. Abakaliki with the highest mean values counts and highest incidence was the Capital with the highest bacterial load/contamination. Awka was the Capital with second most bacterial load. Enugu was the Capital with the lowest bacterial load. Owerri and Umuahia were contaminated but had bacterial load lower than those of Abakaliki and Awka samples. The bacterial load of Owerri and Umuahia tends to be equivalent. The mean values of the Capital cities exceed the international permissible limits and there were presence of *Salmonella spp*

**Conclusion:** The frozen chicken meats sold in the Capital cities of South Eastern States of Nigeria are highly contaminated and can cause food poisoning when eaten raw or under cooked. Therefore, proper cooking should be done before consumption.

**Keywords:** Frozen chicken; Aerobic Plate Counts; Total Coliform Counts; Psychrotrophic Plate Counts; *Escherichia coli* counts; *Staphyococcus aureus* counts; *Salmonella spp*

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## 1. Introduction

The poultry sector can be considered one of the most developed and modernized in the world of agriculture. Advances in genetics, together with the development of nutrition, health and management techniques, resulted in the current poultry farming, with high efficiency and organization to produce animal protein of high biological value for human consumption, at a low cost [1].

Food-borne diseases associated with the consumption of poultry meat and its processed products are of public health significance worldwide [2]. The most frequent source of primary micro flora of meat are skin and feathers, in addition to digestive and respiratory organs. The microbial flora of table poultry largely confined to the skin surface or visceral cavity isolates from poultry and poultry products could include members of the following genera *Enterobacter*, *Alcaligenes*, *Escherichia*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Corynebacterium* and *Salmonella*. [3]. Poultry and poultry products are frequently contaminated with several types of microorganisms. This problem is even more severe under temperature-abused conditions as well as improper or inefficient refrigeration commonly observed in retail chicken sold in open markets. Poultry can be kept in good condition for months if freezing is prompt and rapid and the storage temperature is low enough. Therefore, microbiological quality of processed carcasses mostly depends on a healthy condition and external micro flora of an animal and the hygienic conditions during slaughtering and processing [4, 5].

Chicken is one of the most widely used meats in the world largely because its protein is of excellent quality and contains all the essential amino acids needed by man. Chicken consumption has considerably increased with an annual growth rate of 6% and the global production of broiler meat increased from 73.1 million tons in 2008 to 83.1 million tons in 2012 [6], since it represents a major component of the human diet and chicken is an important low cost source of animal protein [7]. It offers several advantages over red meat that account for an increasing trend in chicken consumption; cuts are easier to handle, the meat is associated with fewer religious restrictions and has relatively low fat and cholesterol contents; it is recognized as a healthier food option [8,9]. However, chicken is not only highly susceptible to spoilage, but also frequently implicated in the spread of foodborne illnesses. During the various stages of slaughter and processing, all potential edible tissues are subjected to contamination from a variety of sources within and outside the animal [10, 11, 12] and also from the environment, equipment and operators [13]. Over 30 genera of microorganisms including seven pathogens (*Campylobacter*, *Salmonella*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli O157: H7*), are known to contaminate poultry products. Since poultry meat itself offers an excellent medium for the multiplication of most bacteria, including those that are not inhibited by low temperatures, storage of processed poultry meat is vital and therefore considered only under circumstances which inhibit the multiplication of the initial load of bacteria [14]. Generally, the microbiological quality of meat products including chicken as purchased by consumers depend on factors such as the quality of the raw products and other materials used or added during processing operations to the products.

The Aerobic plate Count (APC) is considered as an index of food quality, which gives an idea about the hygienic measures during processing and help in assessing the keeping quality of such food item [15]. During the slaughter of poultry birds, there can be fecal contamination of the carcasses from the gut of these birds which means bacteria present in the spilled gut content is passed on as contaminants. Of importance is the *Salmonella* and coliforms especially *Escherichia coli*. *Collibacillosis* and *Salmonellosis* have been described as the leading causes of food-borne illnesses worldwide [16], therefore, it becomes important that ensuring consumer health concerns the greater involvement of the health sector. Contamination of chicken carcasses with enterotoxigenic *Staphylococcus* isolates of human origin often occurs at processing [17].

Freezing is considered an excellent method for keeping quality of chicken meat for long period (9-12month) at temperature below -18 °C, as during the freezing, growth of many types of microorganisms will be ceased due to metabolic injury while others especially psychrotrophic bacteria can grow until the medium freezes [18]. Psychrotrophic bacteria are responsible for many undesirable changes in flavor, odor texture and color of the food products. Deterioration of chicken meat caused by chemical and/or physical factors can occur depending on the microbiological conditions of poultry carcasses which are directly affected by slaughter, sanitization and storage conditions [19]. All perishable foods are liable to early spoilage within a day or two days when not preserved. The shelf-life of these foods are improved by storage mainly under a very low temperature by refrigeration (4.4 °C) or freezing (-17°C). Perishable food of protein nature is of easy degradation and spoilage by microorganisms. This is because protein permits the rapid growth of these organisms [20].

*Staphylococcus aureus* is considered one of the most important Staphylococci species and had been ranked in the third place as one of the most important foodborne diseases worldwide [21]. It is used as heat treatment sufficiency indicator, hygienic conditions during food processing, production and preparation [22].

Frozen chicken is a proteinous food of poultry origin which are stored at a lower temperature to improve its shelf life and to decrease microbial degradation, growth and metabolism. Chicken permits the growth of different groups of microorganisms such as bacteria and fungi. Therefore, it is required that they are kept at low temperature in a frozen form to decrease the free water, optimum for microbial growth. The preservation of meat by chilling and freezing is an alternative to keep the chemical, organoleptic, and nutritional characteristics of product as close as possible to the initial conditions and further prevent an unfavorable action of microorganisms and enzymes [23].

Freezing is usually the method of choice for long term preservation of meat, and is known to cause 1 to 2 Log CFU/g reductions in microbiota, especially during slow freezing [20, 24]. However, freezing can give a false sense of quality and safety if the cold chain is not properly maintained. The microbiology of chicken meat at the point of sale depends on several factors including method of slaughter, sanitation during processing, packaging and dispatch, hygienic handling at retail, and maintenance of the cold chain from slaughter to retail [14]. An efficacious way of preventing poultry borne human diseases is to monitor the microbiological quality of poultry meat and meat products during production, transportation, storage and distribution [25].

Due to the high demand and consumption of poultry products, chicken has been consumed without regarding if it passes through the formal inspection processes.

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## **2. Material and methods**

### **2.1. Sources of materials**

A total of 150 samples were randomly collected from different cold rooms in the State Capital of Abia, Anambra, Ebonyi, Enugu and Imo States which are Umuahia, Awka Abakaliki, Enugu and Owerri respectively. These are the states that makes up the South Eastern Nigeria. Therefore, 30 samples were obtained from each state Capital and the frozen chicken parts collected were wings, drumsticks and thighs. The shelf life of the samples were maintained by keeping in ice boxes until they reach the laboratory for analysis.

### **2.2. Preparation of homogenate chicken sample**

Aseptic sampling was done, with glove hands and no two samples were collected with the same gloves. 25 g of the examined chicken recommended cuts samples were transferred to a septic blender jar and 225 ml of 0.1% sterile peptone water were aseptically added to the content of jar. Each sample was then homogenized in the stomacher at 2000 r.p.m for 1-2 minutes to provide a homogenate.

### **2.3. Serial dilution**

Tenth-fold serial dilutions were prepared (by adding 1ml from food homogenate to 9ml of 0.1% sterile peptone water tube then take 1ml from this tube to another one containing 9ml of 0.1% sterile peptone water and so on). This was done to decrease bacteria concentration and to get a proper acceptable count.

### **2.4. Aerobic Plate Count**

Aerobic Plate Count (APC) was determined following FAO [26] with standard plate count agar using pour plate technique. Aerobic plate count was calculated on plates with 25-250 colonies

### **2.5. Total Coliform Count**

Total Coliform Count (TCC) was determined following ISO [27] with Violet Red Bile agar using pour plate technique. Total coliform was calculated on plates with 25-250 colonies.

### **2.6. Psychotropic Plate Count**

Psychotropic Plate Count (PPC) was determined following Tatini and Kauppi [28] with Standard Plate Count agar using pour plate technique. Psychotropic plate count was calculated on plates with 30-300 colonies.

### 2.7. Isolation and Enumeration of *Escherichia coli*

*Escherichia coli* was detected following ISO [29] with Trypton Bile X-glucuronide agar using pour plate technique. *Escherichia coli* produced greenish-bluish colonies with bluish halo zone. *Escherichia coli* count was calculated on plates with 15-300 colonies.

### 2.8. Isolation and Enumeration of *Staphylococcus aureus*

*Staphylococcus aureus* was isolated and enumerated following FDA [30] with Braid Parker agar using pour plate technique. *Staphylococcus aureus* count was calculated on plates with 20-200 colonies.

### 2.9. Detection of *Salmonella spp*

*Salmonella spp* was detected following FDA [31] using Bismuth Sulphite agar. *Salmonella* produced gray to black shiny, convex colonies.

### 2.10. Identification of Isolates

The pure culture of the bacterial isolates (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella spp*) were obtained and were further identified by Gram staining technique and biochemical tests such as catalase, coagulase, oxidase, indole, citrate, urease, motility and hydrogen sulphide.

**Table 1** Identification of Isolates

| Shape | GS | CA | CO | OX | IN | CI | UR | MO | H2S | Organism                     |
|-------|----|----|----|----|----|----|----|----|-----|------------------------------|
| Rod   | -  | +  | +  | -  | +  | -  | -  | +  | -   | <i>Escherichia coli</i>      |
| Cocci | +  | +  | +  | -  | -  | +  | +  | -  | -   | <i>Staphylococcus aureus</i> |
| Cocci | -  | +  | -  | -  | -  | -  | -  | +  | +   | <i>Salmonella spp</i>        |

Key: + = Positive, - = Negative, GS = Gram stain, CA = Catalase, CO = Coagulase, OX = Oxidase, IN = Indole, CI = Citrate, UR = Urease, MO = Motility, H2S = Hydrogen sulphide

### 2.11. Statistical analysis

The data were analysed using IBM® SPSS® statistics version 21. Differences in mean values of analysed data were considered significant at P=.05.

## 3. Results

Table 2 shows the mean in CFU/g of the Aerobic Plate Counts (APC) of the frozen chicken samples of different South Eastern Nigeria States Capital. Abakaliki has the highest mean value of  $9.61 \times 10^6$ . Umuahia and Enugu has the lowest mean values of  $6.46 \times 10^6$  and  $6.12 \times 10^6$  respectively. While Awka and Owerri have mean values of  $8.50 \times 10^6$  and  $8.10 \times 10^6$  respectively. Abakaliki showed statistical significant difference from other places at  $p = .05$ . Awka and Owerri showed statistical significant difference when compared to Umuahia and Enugu ( $p = .05$ ). There was a significant increase of Aerobic Plate Count (APC) of Abakaliki when compared to other places. There was no significant difference of Aerobic Plate Count (APC) between Awka and Owerri. No significant difference of Aerobic Plate Count (APC) between Umuahia and Enugu at  $p = .05$ .

**Table 2** Mean values of the Aerobic Plate Counts (CFU/g) of frozen chicken sold in Capital cities of South Eastern States of Nigeria (n=30)

| Samples source | Min.              | Max.              | Mean $\pm$ SE                           |
|----------------|-------------------|-------------------|---|
| Abakaliki      | $4.2 \times 10^6$ | $1.1 \times 10^7$ | $9.61 \times 10^6 \pm 0.97 \times 10^6$ |
| Awka           | $3.0 \times 10^6$ | $9.4 \times 10^6$ | $8.50 \times 10^6 \pm 1.12 \times 10^6$ |
| Enugu          | $2.5 \times 10^6$ | $8.4 \times 10^6$ | $6.12 \times 10^6 \pm 0.53 \times 10^6$ |
| Owerri         | $2.7 \times 10^6$ | $9.0 \times 10^6$ | $8.10 \times 10^6 \pm 1.04 \times 10^6$ |
| Umuahia        | $3.0 \times 10^6$ | $8.0 \times 10^6$ | $6.46 \times 10^6 \pm 0.51 \times 10^6$ |

Key: S.E = Standard Error

Table 3 shows the mean in CFU/g of the Total Coliform Counts (TCC) of the frozen chicken samples of different South Eastern Nigeria States. Awka has the highest mean value of  $5.59 \times 10^3$ . Abakaliki has the second highest mean value of  $4.80 \times 10^3$ , next being Umuahia with a mean value of  $3.72 \times 10^3$ . Owerri and Enugu has the lowest mean values of  $2.62 \times 10^3$  and  $2.46 \times 10^3$  respectively. There was a significant increase in Total Coliform Counts (TCC) of Awka and Abakaliki when compared to Umuahia, Owerri and Enugu. The Capitals showed significant difference at  $p=.05$  with exception of Owerri and Enugu which showed no significant difference between them.

**Table 3** Mean values of the Total Coliform Counts (CFU/g) of frozen chicken sold in Capital cities of South Eastern States of Nigeria (n=30)

| Samples source | Min.              | Max.              | Mean $\pm$ SE                           |
|----------------|-------------------|-------------------|---|
| Abakaliki      | $2.2 \times 10^3$ | $6.3 \times 10^3$ | $4.80 \times 10^3 \pm 0.52 \times 10^3$ |
| Awka           | $1.8 \times 10^3$ | $4.9 \times 10^3$ | $5.59 \times 10^3 \pm 0.46 \times 10^3$ |
| Enugu          | $1.0 \times 10^3$ | $4.8 \times 10^3$ | $2.46 \times 10^3 \pm 0.26 \times 10^3$ |
| Owerri         | $1.9 \times 10^3$ | $6.2 \times 10^3$ | $2.62 \times 10^3 \pm 0.29 \times 10^3$ |
| Umuahia        | $1.0 \times 10^3$ | $5.1 \times 10^3$ | $3.72 \times 10^3 \pm 0.44 \times 10^3$ |

Key: S.E = Standard Error

Table 4 shows the mean in CFU/g of the Psychrotrophic Plate Counts (PPC) of the frozen chicken samples of different South Eastern Nigeria States. Abakaliki has the highest mean value of  $5.71 \times 10^6$ . Owerri has the second highest mean value of  $5.53 \times 10^6$ . Awka, Umuahia and Enugu have mean values of  $4.57 \times 10^6$ ,  $4.41 \times 10^6$  and  $4.22 \times 10^6$  respectively. Abakaliki and Owerri showed statistical significant difference when compared to Awka, Umuahia and Enugu ( $p=.05$ ).

**Table 4** Mean values of the Psychrotrophic Plate Counts (CFU/g) of frozen chicken sold in Capital cities of South Eastern States of Nigeria (n=30)

| Samples source | Min.              | Max.              | Mean $\pm$ SE                           |
|----------------|-------------------|-------------------|---|
| Abakaliki      | $1.9 \times 10^6$ | $6.8 \times 10^6$ | $5.71 \times 10^6 \pm 0.59 \times 10^6$ |
| Awka           | $1.8 \times 10^6$ | $6.0 \times 10^6$ | $4.57 \times 10^6 \pm 0.45 \times 10^6$ |
| Enugu          | $1.7 \times 10^6$ | $5.5 \times 10^6$ | $4.22 \times 10^6 \pm 0.48 \times 10^6$ |
| Owerri         | $1.8 \times 10^6$ | $6.6 \times 10^6$ | $5.53 \times 10^6 \pm 0.75 \times 10^6$ |
| Umuahia        | $1.4 \times 10^6$ | $5.9 \times 10^6$ | $4.41 \times 10^6 \pm 0.50 \times 10^6$ |

Key: S.E = Standard Error

Table 5 shows the *Escherichia coli* counts in CFU/g of the frozen chicken samples of the Capital cities of South Eastern Nigeria States. Awka had the highest mean value of  $2.68 \times 10^3$ . Abakaliki had the second highest mean value of  $2.33 \times 10^3$  followed by Umuahia which mean value was  $2.12 \times 10^3$ . Enugu had the lowest mean value of  $1.56 \times 10^3$ . Owerri had the mean value of  $1.80 \times 10^3$ . Awka showed a significant increase when compared to Owerri and Enugu but showed no significant difference with Abakaliki and Umuahia at  $p=.05$ .

**Table 5** Mean values of the *Escherichia coli* counts (CFU/g) of frozen chicken sold in Capital cities of South Eastern States of Nigeria (n=30)

| Samples source | Min.             | Max.              | Mean $\pm$ SE                           |
|----------------|------------------|-------------------|---|
| Abakaliki      | $<1 \times 10^2$ | $6.3 \times 10^3$ | $2.33 \times 10^3 \pm 0.17 \times 10^3$ |
| Awka           | $<1 \times 10^2$ | $4.9 \times 10^3$ | $2.68 \times 10^3 \pm 0.14 \times 10^3$ |
| Enugu          | $<1 \times 10^2$ | $4.8 \times 10^3$ | $1.56 \times 10^3 \pm 0.10 \times 10^3$ |
| Owerri         | $<1 \times 10^2$ | $6.2 \times 10^3$ | $1.80 \times 10^3 \pm 0.26 \times 10^3$ |
| Umuahia        | $<1 \times 10^2$ | $5.1 \times 10^3$ | $2.12 \times 10^3 \pm 0.12 \times 10^3$ |

Key: S.E = Standard Error

Table 6 shows the *Staphylococcus aureus* counts in CFU/g of the frozen chicken samples of the Capital cities of South Eastern Nigeria States. Abakaliki has the highest mean value of  $5.58 \times 10^3$ . Enugu, Owerri and Umuahia have mean values of  $4.36 \times 10^3$ ,  $4.16 \times 10^3$  and  $4.11 \times 10^3$  respectively. Awka has the lowest mean value of  $3.91 \times 10^3$ . Abakaliki showed significant increase when compared to others, which showed no significant difference between them ( $p = .05$ ).

**Table 6** Mean values of the *Staphylococcus aureus* counts (CFU/g) of frozen chicken sold in Capital cities of South Eastern States of Nigeria (n=30)

| Samples source | Min.             | Max.              | Mean $\pm$ SE                           |
|----------------|------------------|-------------------|---|
| Abakaliki      | $<1 \times 10^2$ | $2.2 \times 10^4$ | $5.58 \times 10^3 \pm 0.55 \times 10^3$ |
| Awka           | $<1 \times 10^2$ | $1.1 \times 10^4$ | $3.93 \times 10^3 \pm 0.39 \times 10^3$ |
| Enugu          | $<1 \times 10^2$ | $1.6 \times 10^4$ | $4.36 \times 10^3 \pm 0.44 \times 10^3$ |
| Owerri         | $<1 \times 10^2$ | $1.4 \times 10^4$ | $4.16 \times 10^3 \pm 0.43 \times 10^3$ |
| Umuahia        | $<1 \times 10^2$ | $1.4 \times 10^4$ | $4.11 \times 10^3 \pm 0.44 \times 10^3$ |

Key: S.E = Standard Error

Table 7 shows the incidence of the target organisms (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella spp*) in the Capital cities of the South East States of Nigeria. Of the 30 samples collected from each State Capital, 150 samples in total and examined, Awka showed the highest incidence (18) of *Escherichia coli*, followed by Abakaliki and Umuahia which there were 12 incidence each. Enugu has the lowest incidence (6). That of Owerri was 9. The total incidence of *Escherichia coli* was 57 which was 38% of the total number of samples examined. The incidence of *Staphylococcus aureus* in the Capital cities of the South East States of Nigeria. Of the 30 samples collected from each State Capital, 150 samples in and examined, Abakaliki showed the highest incidence (27) of *Staphylococcus aureus*, followed by Enugu which there were 18. Awka and Umuahia have the lowest incidence which were 9 each. That of Owerri was 12. The total incidence of *Staphylococcus aureus* was 75 which was 50% of the total number of samples examined. The incidence of *Salmonella spp* in the Capital cities of the South East States of Nigeria. Of the 30 samples collected from each State Capital, 150 samples in total and examined, Abakaliki showed the highest incidence (19) of *Salmonella spp*, followed by Umuahia which there was 16. Enugu has the lowest incidence which was 6. Those of Owerri and Awka were 15 and 13 respectively. The total incidence of *Salmonella spp* was 69 which was 46% of the total number of samples examined.

**Table 7** Percentage incidence of the target organisms (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella spp*) (n=30)

| <i>Escherichia coli</i> |          |          | <i>Staphylococcus aureus</i> |          |          | <i>Salmonella spp</i> |          |          |
|-------------------------|----------|----------|------------------------------|----------|----------|-----------------------|----------|----------|
| Samples source          | Positive | Negative | Samples source               | Positive | Negative | Samples source        | Positive | Negative |
| Abakaliki               | 12       | 18       | Abakaliki                    | 27       | 3        | Abakaliki             | 19       | 11       |
| Awka                    | 18       | 12       | Awka                         | 9        | 21       | Awka                  | 13       | 17       |
| Enugu                   | 6        | 24       | Enugu                        | 18       | 12       | Enugu                 | 6        | 24       |
| Owerri                  | 9        | 21       | Owerri                       | 12       | 18       | Owerri                | 15       | 15       |
| Umuahia                 | 12       | 18       | Umuahia                      | 9        | 21       | Umuahia               | 16       | 14       |
| Total (%)               | 57 (38)  | 93 (62)  | Total (%)                    | 75 (50)  | 75 (50)  | Total (%)             | 69 (46)  | 81 (54)  |

Table 8 shows The percentage (%) number of isolates per capital. From the 150 samples (30 each Capital) examined, Abakaliki has the highest bacterial incidence which was 48 (23.88%) followed by Umuahia and Awka which was 42 (20.90%) each. Enugu has the lowest being 30 (14.93%) while that of Owerri was 39 (19.40%).

**Table 8** Percentage number of all isolates per Capital (n=30)

| Organisms                    | Abakaliki  | Awka       | Enugu      | Owerri     | Umuahia    |
|------------------------------|------------|------------|------------|------------|------------|
| <i>Escherichia coli</i>      | 12         | 18         | 6          | 9          | 12         |
| <i>Staphylococcus aureus</i> | 27         | 9          | 18         | 12         | 9          |
| <i>Salmonella spp</i>        | 19         | 13         | 6          | 15         | 16         |
| Total (%)                    | 58 (28.86) | 40 (19.90) | 30 (14.93) | 36 (17.91) | 37 (18.41) |

Table 9 shows the percentage (%) number of isolates (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella spp*) in each frozen chicken sample parts. 50 samples each of wings, drumsticks and thighs were examined, the bacteria isolates were isolated 60 times from the wings, 63 times from the drumsticks and 78 times from the thighs. These gave a percentage occurrence of 29.85% for the wings, 31.34% for the drumsticks and 38.81% for the thighs.

**Table 9** Percentage (%) number of isolates in each frozen chicken part (n=50)

| Organisms                    | Wings      | Drumsticks | Thighs     |
|------------------------------|------------|------------|------------|
| <i>Escherichia coli</i>      | 17         | 18         | 22         |
| <i>Staphylococcus aureus</i> | 22         | 24         | 29         |
| <i>Salmonella spp</i>        | 21         | 21         | 27         |
| Total (%)                    | 60 (29.85) | 63 (31.34) | 78 (38.81) |

#### 4. Discussion

In the study, the bacteriological quality of frozen chicken sold in the different Capital cities of South Eastern States of Nigeria were determined. Samples were collected from the Capital cities of the five (5) South Eastern States of Nigeria. The Aerobic Plate Count (APC), Total Coliform Count (TCC), Psychotropic Plate Count (PPC), *Staphylococcus aureus* count, *Escherichia coli* count and the presence of *Salmonella spp* were of study interest.

The range of Aerobic plate counts ( $9.61 \times 10^6$  -  $6.12 \times 10^6$ ) in this study is higher than that obtained by Noah et al [32] which ranges from  $5.7 \times 10^5$  to  $3.2 \times 10^5$  and that obtained by Daoud et al [33] which ranged from  $2.70 \times 10^3$  to  $2.10 \times 10^3$ . The range of Total Coliform Count ( $5.59 \times 10^3$ - $3.72 \times 10^3$ ) is in accordance with Mohamed [34] which ranged from  $2.61 \times 10^3$  to  $2.07 \times 10^3$  and higher than Daoud [35] which range from  $6.9 \times 10^2$  to  $6.4 \times 10^2$ . The range of Psychrotrophic Plate Count ( $5.71 \times 10^6$ -  $4.22 \times 10^6$ ) in this study is higher than that obtained Hassan et al [36] which ranges from  $7.58 \times 10^4$  to  $8.17 \times 10^3$  and that obtained by Mohamed [34] which was  $1.06 \times 10^6$ .

The percentage incidence of each of the target organisms showed that *Staphylococcus aureus* has the highest incidence of the three target organisms. This was in accordance with the study carried out by Denis et al [37] and Adesiji et al [38]. This can be due to the organism's survival and resistance to extremely cold temperature, as also described by ICMSF [39] and Stewart [40]. *Salmonella spp* has the second highest percentage incidence. *Escherichia coli* has the lowest percentage incidence. The incidence of the target organisms in the samples showed a percentage incidence of *Escherichia coli* to be 38% which was higher than that reported by Marwan-Heba [41] and Ali et al [42] which were 26.9% and 25% incidence respectively and is lower than that reported by Parvin et al [43] which was 76.10%. The percentage incidence of *Staphylococcus aureus* (50%) was higher than that reported by Afifi-Dina [44] and Fatin et al [45] which were 34.3% and 21.0% respectively and in accordance with that reported by Abdalrahman et al [46] which was (53%). The percentage incidence of *Salmonella spp* (46%) is higher than that reported by Amira et al [47] and Abdu and Abubakar [48] which were 7.8% and 19.5% respectively and in accordance with that reported by Doaa [49] which was 40%. The incidence of *Escherichia coli* for frozen chicken is an indication that the chicken is fecal contaminated directly or indirectly through the water used from the environment or personnel during processing as also described by Paulsen et al [50]. The incidence of *Staphylococcus aureus* in frozen chicken is due to poor hygiene of workers and the technique used in eviscerating the chicken carcasses during processing as also described by Adams and Mead [17]. The incidence of *Salmonella* for frozen chicken sample might be as a result of the bacterial load from fresh sample as also described by Cornelia et al [51]. If the frozen chicken samples were stored at an adequate freezing temperature, there would be no room for the survival leading to high incidence of these organisms.

The evaluation of the percentage number of isolates per State Capital shows that Abakaliki has the highest number of the target bacteria followed by Awka and Umuahia. Owerri and Enugu has the least number of isolates.

The evaluation of percentage number of isolates of the target bacteria in frozen chicken parts (wings, drumsticks and thighs) showed that the thighs has the highest number of the target bacteria. This was in accordance with the work carried out by Denis et al [37]. This implies that the thighs are the most carrier of isolates. It could promote the growth of many organisms due to its high flesh content as also described by Kozačinski et al [12].

In determining and comparing the bacterial load in CFU/g for all Capitals; Abakaliki with the mean values of  $9.61 \times 10^6$ ,  $4.80 \times 10^3$ ,  $5.71 \times 10^6$ ,  $2.33 \times 10^3$  and  $5.58 \times 10^3$  for Aerobic Plate Count (APC), Total Coliform Count (TCC), Psychrotrophic Plate count (PPC), *Escherichia coli* and *Staphylococcus aureus* respectively was the Capital with the highest bacterial load. Awka has mean values of  $8.50 \times 10^6$ ,  $5.59 \times 10^3$ ,  $4.57 \times 10^6$ ,  $2.68 \times 10^3$  and  $3.93 \times 10^3$  for Aerobic Plate Count (APC), Total Coliform Count (TCC), Psychrotrophic Plate count (PPC)  $\times 10^3$  *Escherichia coli* and *Staphylococcus aureus* respectively was the Capital with the second highest bacteria load. Enugu with the mean values of  $6.12 \times 10^6$ ,  $2.46 \times 10^3$ ,  $4.22 \times 10^6$ ,  $1.56 \times 10^3$  and  $4.36 \times 10^3$  for Aerobic Plate Count (APC), Total Coliform Count (TCC), Psychrotrophic Plate count (PPC) *Escherichia coli* and *Staphylococcus aureus* respectively was the Capital with the lowest bacterial load. Owerri has mean values of  $8.10 \times 10^6$ ,  $2.62 \times 10^3$ ,  $4.57 \times 10^6$ ,  $1.80 \times 10^3$  and  $3.93 \times 10^3$  for Aerobic Plate Count (APC), Total Coliform Count (TCC), Psychrotrophic Plate count (PPC), *Escherichia coli* and *Staphylococcus aureus* respectively. Umuahia has mean values of  $6.46 \times 10^6$ ,  $3.72 \times 10^3$ ,  $4.41 \times 10^6$ ,  $2.12 \times 10^3$  and  $4.11 \times 10^3$  for Aerobic Plate Count (APC), Total Coliform Count (TCC), Psychrotrophic Plate count (PPC), *Escherichia coli* and *Staphylococcus aureus* respectively.

According to ICMSF [52] and FDA [53] recommendation; it states that the number of microbial count for poultry meats (frozen) must not exceed  $5 \times 10^5$  for Aerobic plate count,  $5 \times 10^2$  for *Escherichia coli*,  $5 \times 10^2$  for *Staphylococci* and absence of *Salmonella* in 25g of the frozen poultry meat. Comparing this with the mean values of the Areobic Plate Count (APC) of each Capital city, the frozen chicken sold in these Capitals exceed the satisfactory and acceptable levels for consumption. The raw frozen chicken sold in the Capitals having higher *Staphylococci* count, higher *Escherichia coli* count and presence of *Salmonella* in some of the samples constitute high risk if causing food poisoning and are not acceptable for consumption unless thoroughly cooked. This implies that the processing practices such as slaughtering, washing and personal hygiene of handlers of frozen chicken in Capital cities of South Eastern States of Nigeria are not up to standard and there is a need for external regulation for appropriate checks and getting quality products.

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## 5. Conclusion

From the results, it can be concluded that the raw frozen chicken sold in the Capital cities of the five (5) South Eastern States of Nigeria are not satisfactory for consumption especially in raw form like giving to domestic animals such as dogs, cats and so on. It can pose a serious health treat and implications. The relative high bacterial load of frozen chicken could be reflection of the poor handling of the meat, contamination from the environment during slaughtering, washing, dressing of the meat, transportation and storage, unhygienic habit of the personnel observed during processing process. The contaminated chicken could be a vehicle for the transmission of pathogens among the people which could be of a great public health risk to the consumers. Strict hygienic measures should be taken during processing of both fresh and frozen chicken. The frozen chicken samples should be thoroughly cooked before consumption in order to kill bacteria and make them good for consumption. Regulatory body in food industry should extend their search light on to the market were fresh meat are process for sanction were necessary to enhance Good Manufacturing Practices (GMP) and Good Handling Practices (GHP).

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No competing interests exist.



*Authors Contributions*

This work was carried out in collaboration between the authors, Authors PCC and AGA designed the study, and wrote the protocol. Authors HON and PCI wrote the first draft of the manuscript. Authors CAO and CA carried out the statistical analysis. Authors ROU, TOO, OSE and SCI helped with the analysis of the work. The final manuscript was read and approved by the authors.

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