ISSN : 2321-9602

# Indo-American Journal of Agricultural and Veterinary Sciences




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*Indo-Am. J. Agric. & Vet. Sci., 2022*

*ISSN 2321–9602 www.iajavs.com*
*Vol. 15, Issuse 1 March 2022*

Indo-American Journal of Agricultural and Veterinary Sciences

# Analysis of Bacterial Isolates and Their Antibiotic Resistance Profiles in Commercially Available Frozen Chicken Products

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**ABSTRACT**

Frozen chicken products contribute a large proportion of the protein needs of the average Nigerian family. Although chicken products are safe when frozen continuously and properly stored, they are easily prone to microbial contamination. However, the majority of the frozen chicken products sold in open markets are smuggled into the country in very unhygienic circumstances that make them susceptible to microbial contamination thus compromising their quality. This study, therefore, evaluated the microbiological quality of frozen chicken parts sold in Port Harcourt metropolis, Rivers State, Nigeria. The objective was to isolate the microorganism(s) if any, present in the frozen chicken samples, characterize and identify them and then determine the antibiotic susceptibility profiles of the isolates. A total of two hundred chicken samples were collected from different pppmarkets and evaluated for microbial contamination using standard microbiological.

*Keywords: Frozen chicken; standard limit; antibiotic susceptibility and open market.*

## 1. INTRODUCTION

Fresh poultry products are known to be prone to deterioration due to microbial action as well as physical and chemical changes. In normal handling and storage of poultry products, these deteriorating changes are attributed to microbiological activity. Like all fresh (uncooked) foods chicken carries natural microflora that may contain potentially harmful organisms to humans [1-4]. The type of organisms isolated depend on where the samples are taken from and on the stage of processing [5]. Both poultry muscle and skin are excellent substrates that support the growth of a wide variety of microorganisms [6].

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consumed on a large scale in Nigeria. It is estimated that over 70% of the country's poultry needs is imported, sotme of them smuggled into the country in very unhygienic circumstances [10]. Improper storage and preservation due to inadequate power supply have also compromised the quality of the locally produced poultry products. Seizure and destruction of imported poultry products as a deterrent to their importers by the Immigrations Services is yet to fully achieve the desired objective. Besides the high frequency of consumption of imported frozen chicken and its attendant negative impact on local poultry production, there are claims that they are preserved with chemicals that predispose consumers in the long run to serious ailments such as cancer [11-13]. The use of antimicrobial agents in poultry and animal husbandry has also been established to contribute to the problem of antimicrobial resistance. There is thus a possibility of increased risk of infection and change in the susceptibilities of the pathogens that contaminate these products and which could adversely affect the lives of consumers [3,6,9]. According to the Center for Disease Control and Prevention (CDC), over a million people suffer from salmonella infections in the United States of America annually resulting in nearly 20,000.00 hospitalizations and 380 deaths [10]. Although similar statistics regarding the number of people affected by infections due to food and poultry borne pathogens may not be readily available here, the quality of some of the poultry products being displayed for sale in our markets clearly falls short of the acceptable standards [14]. This study therefore set out to isolate, evaluate, characterize and identify the different microorganisms that may be present in frozen chicken and to determine their susceptibilities to commonly available antimicrobial agents. This will help to determine the impact of this massive consumption of frozen chicken products on the problem of antimicrobial resistance and public health in general.

## 2. MATERIALS AND METHODS

### 2.1 Reagents

Peptone water, 1% tetramethyl p- phenylenediamine dihydrochloride, crystal violet, safranin red, iodine, hydrogen peroxide and Kovac's reagent.

### 2.2 Culture Media

Macconkey agar, cetrimide agar, salmonella- shigella agar, nutrient agar, nutrient broth, mannitol salt agar, Mueller-Hinton agar and Sabouraud dextrose agar.

### 2.3 Sample Collection

A total of 50 samples were collected from the markets in each location making a grand total of
200 samples of frozen chicken parts collected from the different locations listed below.

**Location A:** Rumuomoi, Obiwali and Nkpolu market.
**Location B:** Rumuokoro and Choba market.
**Location C:** Mile 1 and Mile 3 open markets
**Location D:** Oil mill market

A total of fifty samples were randomly collected from each of the markets visited. The samples were collected in small batches of ten to twenty samples and quickly taken to the laboratory for processing on each day of sampling.

The chicken parts were aseptically collected with sterile gloves. They were wrapped in sterile foils, placed in a cooler containing ice to maintain the temperature and immediately transported to the laboratory. 10g of each of the various chicken parts were weighed (sensitive weighing balance, HCK, Dispel, India) and placed in 10 ml of 0.1% peptone water (Titan Biotech, 43955-1.Bhiwadi India, Exp Date, 7/19). This was afterwards shaken with the aid of a mechanical shaker for 15 minutes. Serial dilutions of the slurry obtained were subsequently made in sterile universal bottles containing 9 ml of sterile 0.1% peptone water up to dilution [7].

## 2.4 Culture Methods

From each appropriate dilution, 0.1ml was inoculated onto nutrient agar (Titan Biotech, M4D2AP01, Bhiwadi India, 12/18) and MacConkey agar (Titan Biotech, 71863-1, Bhiwadi India, 12/18) to be used for the enumeration of total bacteria isolates and coliform bacteria respectively and both were incubated at 37°C for 24 hours. Sabouraud Dextrose Agar (Lab M, Uk,3/19) was also inoculated with 0.1 ml of the diluted slurry to be used for the enumeration of yeast isolates in the samples. The plates were incubated at 25°C for 5-7 days [8]. Colonies from the incubated MacConkey agar and nutrient agar plates were picked and sub cultured onto salmonella-shigella agar, cetrimide and mannitol salt agar plates and the plates were then incubated at 37°C for 24 hours. The resultant colonies were observed and characterized. All microorganisms once isolated were preserved in agar slants and kept in the incubator at 37°C.

## 2.5 Morphological and Biochemical Tests

Several biochemical tests including Gram staining, indole test, catalase test, oxidase test and coagulase test were carried out [15].

## 2.6 Antibiotic Susceptibility Test

Antibiotic susceptibility tests were carried using the modified Kirby- Bauer method [16].

Mueller-Hinton agar was prepared according to the manufacturer's instructions.

Immediately after autoclaving it was allowed to cool in a water bath, before being poured into flat-bottomed Petri dishes on a level horizontal surface. The agar medium was then allowed to cool to room temperature and stored in a refrigerator.

Pure colonies were selected from an agar plate and transferred into a tube containing 5 ml of nutrient broth (Lab M, 134472/241, Uk, 12/18) The nutrient broth was then incubated until it achieved the turbidity of the McFarland standard solution.

## 2.7 Inoculation of Test Plates

A sterile swab was dipped into the inoculum suspensions and then streaked on the dried

surface of the Mueller-Hinton agar plate. The surface was streaked two more times rotating the plate approximately 60˚ each time to ensure an even distribution of the inoculum. Antibiotic sensitivity discs containing different antibiotics were gently placed on the inoculated Mueller- Hinton agar plates and allowed to stand for ten minutes to allow for diffusion. Each disc was pressed down to ensure complete contact with the agar surface. The plates were then incubated in an inverted position at 35°C [17]. After incubation, the zones of inhibition of susceptible organisms were observed and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines for sensitivity [1].

## 3. RESULTS AND DISCUSSION

The isolates were identified based on their morphological and biochemical characteristics as shown in Table 1. The aerobic mesophilic count (total plate count) from each location was determined after subculturing under optimum conditions. For each location, the average total number of microbial isolates were calculated per serial dilution and tabulated. The total plate count for each location was more than the coliform count and the cell count reduced as the serial dilution increased as shown in Table 2.

The percentage of each microorganism in the entire population was calculated, based on the results in Table 2 which show the average cell count of each microorganism per location. The percentages were as follows: *Escherichia coli* (27.82%), *salmonella sp* (13.64%), *Shigella sp* (4.88%), *Staphylococcus aureus* (18.52%), *Staphylococcus sp* (2.92%), *Bacillus subtilis (*17.83%), *Enterobacter sp* (4.15%), *Micrococcus sp* (1.61%), *Klebsiella sp* (1.84%), *Proteus sp* (3.07%) and *Citrobacter sp* (3.65%).

The susceptibility and resistance patterns of the microorganisms to the antibiotics were noted after the diffusion test was carried out. From the Table 3, it could be observed that different antibiotics had different actions on the microorganisms. *Staphylococcus aureus* was

**Table 1. Morphological and Biochemical Characteristics of Isolates from the frozen chicken parts**

| Colony morphology | Cell character | Gram staining | Indole test | Catalase test | Oxidase test | Probable identity | Locations |
|---|---|---|---|---|---|---|---|
| Mucoid | Short rod | - | + | + | - | *Escherichia coli* | A, B, C |
| Large white mucoids | Rods arranged in chains. | + | - | + | + | *Bacillus subtilis* | A, B, C And D |
| Yellow, small and irregular | Cocci | + | - | + | - | *Staphylococcus aureus Proteus* | A, B, C And D |
| Small white mucoid | Rods | - | + | + | - | *sp* | A, B |
| Large white and mucoid on nutrient agar, black centres observed in selective media | Rods | - | - | + | - | *Salmonella sp* | A, B, C And D |
| Pale almost translucent colonies Small and raised | Rods | - | - | + | - | *Shigella sp* | B, C, D |
| | Rods | - | - | + | - | *Klebsiella sp* | A, B |
| Pale pink colonies | Rod | - | - | + | - | *Enterobacter* | A, C and D |
| Light red small colonies | Cocci | + | - | + | - | *Micrococcus sp* | A C |
| Moist, low, smooth and translucent | Rod | - | - | + | - | *Citrobacter sp* | B, C and D |
| Small white colonies | Cocci | + | - | + | - | *Staphylococcus sp* | A, C and D |

**Table 2. Average cell count of each Microorganism per Location**

| Microorganism | Location A (Average cell count) | Location B (Average cell count) | Location C (Average cell count) | Location D (Average cell count) | Total average cell count in all locations per microorganism |
|---|---|---|---|---|---|
| *Escherichia coli* | 150 | 180 | 200 | 195 | 725 |
| *Salmonella sp* | 80 | 60 | 95 | 120 | 355 |
| *Shigella sp* | - | 0 | 35 | 72 | 127 |
| *Staphylococcus aureus* | 102 | 98 | 122 | 160 | 482 |
| *Staphylococcus sp* | 28 | - | 36 | 12 | 76 |
| *Bacillus subtilis* | 68 | 44 | 202 | 150 | 464 |
| *Enterobacter sp* | 22 | - | 36 | 50 | 108 |
| *Micrococcus sp* | 14 | - | 28 | - | 42 |
| *Klebsiella sp* | 12 | 36 | - | - | 48 |
| *Proteus sp* | 20 | 60 | - | - | 80 |
| *Citrobacter sp* | - | - | 50 | 45 | 95 |
| | | | | | Total= 2602 |

**Table 3. Antibiotic susceptibility test results showing zones of inhibition for the antibiotics used**

| Organism | *Escherichia coli* | *Staphylococcus aureus* |
|---|---|---|
| Antibiotics | IZD (mm) | IZD (mm) |
| Amoxicillin/clavulanate | 18 | 18 |
| Erythromycin | -- | 20 |
| Gentamicin | 22 | 30 |
| Ciprofloxacin | 30 | 24 |

**Table 4. Antibiotic susceptibility of isolated pathogens**

| Microorganism | Amoxicillin/Clavulanate | Erythromycin | Gentamicin | Ciprofloxacin |
|---|---|---|---|---|
| *Bacillus subtilis* | S | S | R | S |
| *Staphylococcus aureus* | R | R | R | R |
| *Staphylococcus sp* | S | S | R | S |
| *Citrobacter sp* | R | S | S | S |
| *Enterobacter sp* | R | R | S | S |
| *Escherichia coli* | R | R | S | S |
| *Micrococcus sp* | S | R | S | R |
| *Salmonella sp* | S | R | S | S |
| *Shigella sp* | S | S | S | S |
| *Proteus sp* | R | S | R | S |
| *Klebsiella sp* | R | S | S | S |

*S= Susceptible; I= Intermediate; R = Resistance*

completely resistant to all antibiotics and *Klebsiella sp* was susceptible to all antibiotics used [18].

A total of 11 bacteria species from the various frozen chicken parts examined were isolated four of which were Gram-positive and seven Gram- negative. Some of these organisms have been implicated in diarrheal and gastro intestinal diseases in both adults and children. This is in agreement with the findings of other studies [7, 19-21]. *Escherichia coli* was present in the samples collected from the various locations. Location C had the highest number of *Escherichia coli* isolated, while location A had the least number as seen in Table 2. Presence of *E. coli* may be linked to faecal contamination. In this study, *E. coli* was more prevalent than other organisms. This is in contrast to another study which found *Staphylococcus aureus* to be predominant [22]. However, the findings of our study appear to be in agreement with theirs with respect to the Gram-negative isolates being more that the Gram-positive ones.

*Citrobacter* species were not isolated from the frozen chicken samples analyzed from the first two locations. Compared to *Escherichia coli, salmonella, Staphylococcus aureus*, *Bacillus subtilis,* other staphylococcal species, Enterobacter, Micrococcus, Klebsiella, Proteus and Citrobacter were isolated but in minute amounts. The limit of microorganisms
permissible in poultry products falls within $10^1$ - $10^2$ CFU/g as recommended by International Microbiological Standards for ready - to - eat poultry products [6,23]. From the results of our study, the microbial load in raw poultry ranged between 1.4 $x10^3$ and 2.4x $10^3$CFU/ g. Although it may be argued that the microbial load could be reduced upon cooking and fall within acceptable limits, this may not necessarily be so and these values are therefore microbiologically unacceptable. Chicken is also used in preparing the local roasted meat known as ''suya'' and some parts of the meat may not be properly roasted and thus may harbour microorganisms In general, the risk of foodborne illness may be reduced by applying the principles of Hazard Analysis and Critical Control Points (HACCP) [15]. It is a preventive food safety system in which every step in the manufacture, storage and distribution of a food product is scientifically analyzed for microbiological, physical and chemical hazards.

## 4. CONCLUSION

The findings of our study revealed the presence of dangerous pathogens such as Salmonella, Shigella, *E. coli* and *S. aureus* some of which are not permitted to be present in fresh products such as chicken. They have been implicated in various food borne diseases and diarrheal illnesses. In order to safeguard public health, public enlightenment should be sustained to create awareness on the proper cooking, packaging and storage of poultry products. The illegal importation of poultry products should be curbed by the creation of right policies and implementation of existing laws. Adequate provision of power is also advocated to enable local livestock producers to preserve their products at appropriate temperatures and prevent spoilage due to microbial contamination. In conclusion, frozen chicken products sold in Port Harcourt were contaminated by different bacteria beyond acceptable load. The bacteria isolates were resistant to some of the antibiotics tested.

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