Microbial Profile Of Chicken Meat Sold At Different Locations In Port Harcourt Metropolis

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Abstract: The microbiological quality of frozen chicken meat bought from Zartech cold room, Rumuola grocery retail market and Choba open market in Port Harcourt, Rivers state, was assessed. 5 samples were collected from each location, 15 samples collected in total. Total bacterial count (TBC), total *Staphylococus* count, total coliform count, total *Salmonella* count and fungal counts were done using Plate count agar, Mannitol salt agar, MacConckey agar, *Salmonella-Shigella* agar and Potato dextrose agar respectively. The result showed that the total bacterial count ranged from (5.9×10^6 cfu/g- 9.9×10^7cfu/g), total *Staphylococcus* count ranged from (2.5×10^4cfu/g – 7.2×10^5cfu/g), total coliform count ranged from (3.9×10^5cfu/g-1.6×10^6cfu/g), *Salmonella* count ranged from (2.5×10^4cfu/g – 3.1×10^5cfu/g). Fungal counts ranged from ($2.7x10^4$cfu/g - $5.9 x10^5$cfu/g). Biochemical tests were done to identify isolates; From the 28 bacterial isolates, 7 different organisms were identified; *Escherichia coli* (14.3%), *Salomonella sp* (17.9%), *Klebsiella sp* (3.6%), *Staphylococcus sp* (39.3%), *Serretia sp* (7.1%), *Shigella sp* (7.1%) *and Pseudomonas sp* (10.7%).The fungal isolates identified by microscopy and physical examination include; *Aspergillus fumigatus, Aspergillus niger, Penicillum sp and Mucor sp*. A sensitivity test was done using Mueler-Hinton agar and the results showed that *Staphylococcus sp* exhibited 50% resistance, *Salmonella sp*; 80%, *Klebsiella sp*; 40%, *Serretia sp*; 30% *Shigella sp; 20%*, *E.coli;* 40%, *Pseudomonas sp; 30%* resistance to the antibiotics used for the sensitivity. The presence of microorganisms in chicken meat is attributed to the conducive microbial environment it provides, as well as the poor hygienic practices during processing and selling especially in the open markets. Thus, proper storage and hygiene during processing and selling of chicken meat is of uttermost importance.
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Introduction

Poultry meat refers to the combination of muscle tissues, attached skin, connective tissues and edible organs of avian species commonly used as food which includes chicken, turkeys, geese, pheasants, pigeons, etc. Poultry meat constitutes an important food component for a large section of the world's population; they form part of the cheapest sources of staple animal protein together with other meats. Poultry is an important part of the animal food and the volume of their production, marketing and consumption is increasing to satisfy the public demand worldwide within the last decades (Bryan, 1980; Anand *et al.,* 1989; Mead 1997). Modern poultry processing requires a high rate of throughput to meet consumer demand, as poultry meat can easily be contaminated with microorganisms, due to many factors such as nutrients, high water activity and neutral pH, (Kabour, 2011). (Rumni et al., 2012) (Capita et al., 2002) (Sengupta etal 2011) However, healthy chickens entering slaughter processing might be highly contaminated by microorganisms, including food borne pathogens such as *Salmonella sp, Campylobacter sp*, *Escherichia sp, Staphylococcus sp* and these pathogens tend to disseminate in the processing plant (Mead *et al.,* 1994). They can be found on the surfaces of feet, feathers, skin and also in the intestines. During processing, a high proportion of these organisms will be removed, but further contamination can occur at any stage of the processing operation (Kabour, 2011) (Al-Groom Rania and Abu Shaqra Qasem 2014). The procedure for converting a live, healthy bird into a safe and wholesome poultry product provides many opportunities for micro-organisms to colonize on the surface of the carcasses. During the various processing operations, opportunities exist for the contamination of the carcass from the environment, the process in the plant itself, contamination via knives, equipment, the hands of workers and also by cross-contamination from carcass to carcass. Some processing operations increase contaminating micro-organisms or encourage their multiplication (Kabour, 2011). As a result, the microbial population changes from mainly Gram-positive rods and micrococci on the outside of the live chicken to Gram-negative micro-organisms on the finished product (Bryan, 1980; Thomas and McMeekin., 1980; Roberts, 1982; Banwart, 1989; Mead, 1989). Efforts should be made to prevent the build-up of contamination peaks during processing. Rinsing of the carcasses, especially during defeathering and evisceration is therefore of great

importance (Mead, 1982; Anand *et al.,* 1989; Mead, 1989). Spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin. One such system is hazard analysis and critical control point (HACCP), a systematic, science based approach designed to prevent, reduce or eliminate identified hazards in food products, (Kukay *et al*, 1996). It is generally accepted that the HACCP approach, is the most effective way of reducing or eliminating contamination during food processing (NACMCF, 1998). Chicken meat is the commonest poultry meat sold in Nigeria and the most important as it is sold in every market and indispensable in a standard restaurant menu, it provides staple amount of protein with low fat. Because of the increasing demand for chicken meat, Nigerians have taken up chicken rearing in order to make ends meet. Agriculturists and nutritionists are generally agreed that developing the poultry industry of Nigeria is the fastest means of bridging the protein-deficiency gap presently prevailing in the country (food and agriculture organisation of the United Nations (Akinwunmi et al., 1979). In addition, when compared to other livestock, poultry has by far, the quickest and highest rate of turn over. Estimates from consumption and demand surveys in Nigeria indicate that the consumption of poultry is gradually outstripping most other kinds of meat except beef. It is therefore not surprising that funds invested in poultry production are recouped faster than any other livestock enterprise. The poultry industry, if properly harnessed can also serve as a source of foreign earnings complementing crude oil which at present constitutes the main source of foreign earnings in Nigeria, (The poultry site news 2009). In poultry production, small scale poultry production represents one of the few opportunities for saving, investment and security against risks. It accounts for approximately 90% of total poultry production (Branckaert, 1999). Despite the acknowledged importance of poultry production, Akanni (2007) opined that it is characterized by low production level due to limited finance for the procurement of basic poultry equipment and materials. The result of this is that many of the small-scale poultry farmers are not encouraged to increase their productivity; thereby moving from small-scale production to a large scale production by small–scale poultry farmers encountered hindrances in the poultry industry which could be detrimental to increase poultry production. This study was aimed at:

- Evaluating the bacteriology and mycological quality of raw chicken meat.
- Compare the level of contamination of chicken carcasses from three (3) locations;

i) The cold rooms ii) The retail markets iii) open markets.

To check the susceptibility of isolates to given antibiotics

Materials And Methods

Microbiological analysis of chicken meat

25g of the thawed sample was taken aseptically by a sterile scalpel and placed in a sterile stomacher bag containing 225 ml of peptone water and placed inside a homogeniser. The samples were homogenized for 2-3 minutes giving the 1:10 emulsion. This was transferred into a different 500ml conical flask and served as stock solution at the 10^{-1} dilution.

Serial dilution and plating

1ml of the solution was transferred into the first test tube containing 9ml of the diluent (peptone water), which gave a 10^{-2} dilution, which was further serially diluted from (10^{-1} -10^{-5}). For each dilution, a new syringe was used to avoid errors and contamination. Aliquots (0.1ml) of each dilution were transferred to the respective media in duplicates and spread uniformly using a hockey stick. The plates were incubated aerobically at 37°c for 18-24 hours.

Table 1. The various media used and their purposes

Media		Purpose
Plate count agar		Total viable count
Mannitol salt agar		Staphylococcus count
MacConckey agar		Total coliform count
Salmonella-shigella agar		Salmonella count
Potato dextrose agar		Fungal count
Mueler-Hinton agar		Sensitivity

Subculture

Various colonies were sub cultured on freshly prepared plate count agar. It was done by streaking a colony in plates containing medium using a sterilized (flamed) wire loop. This was aimed at isolating pure cultures for further identification. The incubation period for subculture was 18-24 hours at 37°C.

Identification and characteristics of isolates

Colony morphology

Here special features such as colour, surface area, edge, elevation, opacity, are observed and recorded Gram staining, Biochemical tests: **Indole production**, Methyl red test, Voges-Proskauer (VP) Test, Citrate utilization test. Triple Sugar Ion (TSI), Catalase test, Motility test, Oxidase test, Sugar Fermentation Test.

Sensitivity test

The media was prepared according to the manufacturer's instructions. The isolates were inoculated into the media using a sterile wire loop and then incubated for 24 hours. After 24 hours, the sensitivity discs were placed on the media and further incubated. The zones of inhibitions were checked and measured after 24 hours. No zone of inhibition indicated resistant organisms to that labelled antibiotic, while large zones of inhibition, indicated susceptibility to the labelled antibiotic.

Result

From the results obtained, the total bacterial count on plate count agar ranged from 5.9×10^6 - 9.9×10^7, total *Staphylococcal* count on Mannitol salt agar ranged from:

2.5×10^4 –7.2×10^5, total coliform count on MacConckey agar ranged from 3.9×10^5 - 1.6×10^6, *Salmonella* count on *Salmonella-Shigella* agar ranged from 2.5×10^4 – 3.1×10^5. Fungal counts ranged from 2.7×10^5 cfu/g - 5.9×10^5cfu/g. From the 28 bacterial isolates, 7 different organisms; *Escherichia coli, Salomonella sp, Klebsiella sp, Staphylococcus sp, Serretia sp, Shigella sp and Pseudomonas sp* were identified using biochemical tests which is summarized in Table 3.9, the fungal isolates were identified by microscopy and physical examination and 4 different fungi were identified namely *Aspergillus fumigatus, Aspergillus flavus, Penicillum sp and Mucor* as summarised in Table 3.

The sensitivity test showed that some isolated organisms were susceptible to the tested antibiotics while others were resistant; *staphylococcus sp* exhibited 50% resistance, *salmonella sp;* 80%, *Klebsiella sp;* 40%, *Serretia sp;* 30% *Shigella sp;* 20%, *E.coli;* 40%, *Pseudomonas sp; 30%*.

Table 2. **Results from Cold Room Samples**

Sample code	TBC	Staph count	Salmonella count	Total coliform count
CRTI	$5.9X10^6$	$3.2X10^4$	$2.5X10^4$	$4.1X10^5$
CRT2	$6.2X10^6$	$2.5X10^4$	$3.3X10^4$	$3.9X10^5$
CRT 3	$9.4X10^6$	$4.1X10^4$	$2.7X10^4$	$5.5X10^5$
CRW1	$1.2x10^7$	$3.9x10^4$	$3.9x10^4$	$6.1x10^5$
CRW2	$1.4x10^7$	$4.7x10^4$	$3.6x10^4$	$5.8x10^5$

Key
CRT= Cold room thigh
CRW = Cold room Wing

Table 3. Results from Retail Store Sample

Sample code	TBC	Staph count	Salmonella count	Total coliform count
RST1	$1.1x10^7$	$4.3x10^4$	$4.7x10^4$	$5.1x10^5$
RST2	$9.6x10^6$	$3.7x10^4$	$3.8x10^4$	$5.9x10^5$
RST3	$1.0x10^7$	$4.0x10^4$	$4.9x10^4$	$5.5x10^5$
RSW1	$1.2x10^7$	$4.5x10^4$	$5.5x10^4$	$6.7x10^5$
RSW2	$1.4x10^7$	$4.4x10^4$	$4.2x10^4$	$6.0x10^5$

Key
RST = Retail Store thigh
RSW = Retail store wings

Table 4. **Results from open market samples**

Sample code	TBC	Staph count	Salmonella count	Total coliform count
OMT1	4.310^7	$5.0x10^4$	$6.7x10^4$	$1.2x10^6$
OMT2	$6.5x10^7$	$4.8x10^4$	$5.9x10^4$	$1.5x10^6$
OMT3	$7.9x10^7$	$5.6x10^4$	$6.3x10^4$	$1.3x10^6$
OMW1	$2.1x10^7$	$7.2x10^4$	$3.1x10^5$	$1.6x10^6$
0MW2	$1.9x10^7$	$6.3x10^4$	$9.1x10^4$	$1.6x10^6$

Key
OMT = Open market Thigh
OMW = Open market Wing

Table 5. **Average cell counts**

Location	Average TBC cfu/g	Average STC cfu/g	Average SAC cfu/g	Average TCC cfu/g
Cold room	$9.5x10^6$	$3.7x10^4$	$3.2x10^4$	$5.1x10^4$
Retail store	$1.1x10^7$	$4.2x10^4$	$4.6x10^4$	$5.8x10^6$
Open market	$8.7x10^7$	$5.8x10^4$	$1.8x10^5$	$1.4x10^7$

TBC; Total bacterial count, STC; *Staphylococcus sp* count, SAC; *salmonella* sp count, TCC; Total coliform count.

Table 6. **Fungal counts on Potato Dextrose Agar**

Sample code	Fungal counts (cfu/ml)	Average count
CRT1	$3.0x10^5$	
CRT2	$2.7x10^5$	
CRT3	$3.3x10^5$	$3.2x10^5$
CRW1	$3.7x10^5$	
CRW2	$3.5x10^5$	
RST1	$4.1x10^5$	
RST2	$3.9x10^5$	
RST3	$4.5x10^5$	$4.4x10^5$
RSW1	$4.4x10^5$	
RSW2	$4.9x10^5$	
OMT2	$4.9x10^5$	
OMT3	$5.6x10^5$	$5.5x10^5$
OMW1	$6.3x10^5$	
OMW2	$5.9x10^5$	

Table 7. **Comparison of bacterial counts on chicken thighs and wings.**

Location	Bacterial counts on thigh	Bacterial counts on wings
Cold room	**7.2×10^6**	**1.3×10^7**
Retail stores	**1.0×10^7**	**$2.4x 10^7$**
Open markets	**3.2×10^7**	**1.0×10^8**

Table 8. **Comparison of fungal counts on chicken thighs and wings.**

Location	Fungal counts on thigh	Fungal counts on wings
Cold room	**3.0×10^5**	**3.6×10^5**
Retail stores	**4.2×10^5**	**$4.7x 10^5$**
Open markets	**5.3×10^5**	**6.1×10^5**

Discussion

Chicken meat is generally accepted worldwide, by various culture and nationalities as it is rich in nutrients and easily digestible. Variations in microbial load of chicken meat can be attributed to efficacy of storage as well as handling and processing of the meat. Chicken meat cannot be found naturally sterile as the natural environment of the live birds and their natural flora also influence the microbiological quality of the meat (Mead, 2000). However, the methods of handling and storage may worsen or improve its natural state. Unfortunately large numbers of chicken carcasses sold in our markets in Nigeria are not properly stored and handled and still represent a considerable hazard. Means of cross contamination include using dirty utensils and equipment, transfer of bacteria from hand, fingers and flies. These contaminations can be avoided if vendors wash their hands after any action that could contaminate hands such as visiting the toilet; as this is a major source of faecal contaminants. The isolated organisms in the course of this research which can constitute public health hazards if ingested in large quantities are described as follows; *Salmonella sp* can lead to salmonellosis which is characterised by mild to severe nausea, abdominal cramps, diarrhoea, fever, malaise, mucous membrane congestion, *Salmonella typhi* and *Salmonella paratyphi* are septicaemic and produce typhoid symptoms. *Escherichia coli* may produce verocytotoxins which can cause diarrhoea and haemorrhagic colitis in humans and can lead to life threatening sequels, such as haemolyticureamic syndrome and thrombocytopaenic purpura, (Salmon *et al*, 1989).

Presence of *E.coli* indicates faecal contamination possibly from unwashed hands of the vendors and workers. *Shigella sp* the causative agent of shigellosis or "bacillary dysentery" has been increasingly

involved in foodborne outbreaks. According to the Centers for Disease Control and Prevention's Emerging Infections Program, Foodborne Diseases Active Surveillance Network (FoodNet), *Shigella sp* was the third most reported foodborne bacterial pathogen in 2002. Foods are most commonly contaminated with *Shigella* by an infected food handler who practices poor personal hygiene. *Shigella sp* is acid resistant, salt tolerant, and can survive at infective levels in many types of foods such as meats, fruits and vegetables, low pH foods, prepared foods, and foods held in modified atmosphere or vacuum packaging. Survival is often increased when food is held at refrigerated temperatures. *Pseudomonas sp* is commonly associated with spoilage of meats and the presence may be as a result of spoilage of the chicken carcasses in temperature abused environments which can lead to untimely deterioration of chicken carcasses as the required low freezing temperature during storage is needed to elicit bacteriostatic effect on pathogens and spoilage organisms. *Staphylococcus sp* was the highest occurring pathogens (39.3%) which could be as a result of its presence on both the skin of the live birds and on the skin of humans as well as the air and water. *Staphylococcus sp* can cause food intoxication; the heat resistant enterotoxin may lead to nausea, vomiting, cramps, chills and weak pulse. From the resultwe can see that the averaged total bacterial counts were highest in the open markets which can be as a result of the abused temperatures and cross contamination by vendors, buyers and flies since the chicken carcasses were displayed on the tables in the open markets which is similar to the findings of Odetunde and Lawal, 2011 which stated that the total bacterial counts for all the parts examined sold in open markets in Ibadan ranged from 3.3×10^6-6.9×10^7, the bacterial counts in the retail stores was in the range of 3.1×10^5 to 6.0×10^6 cfu/g and the ones stored in the cold room had a range of 1.5×10^4cfu/g to 1.5×10^6 cfu/g. The coliform counts obtained for all the chicken parts ranged from 1.2×10^4 cfu/g to 3.2×10^4 cfu/g, 1.2×10^4 cfu/g to 7.2×10^4 cfu/g and 1.1×10^5 cfu/g to 1.4×10^5 cfu/g for chicken parts stored in the cold rooms, retail store and open market respectively, while the mould and yeasts count gave a range of 1.4×10^1 cfu/g to 1.5×10^2 cfu/g, 1.2×10^2 cfu/g to 7.2×10^3 cfu/g and 1.3×10^3 to 1.5×10^4 cfu/g for cold room, retail stores and open market respectively. The cold rooms had the lowest averaged total bacterial counts (TBC) which was as a result of attempted adherence to the required storage temperature as well as increased hygiene consciousness in the cold rooms. According to the World health organization directives on microbial limits, Total bacterial count shall not exceed 5×10^5 colonies per gram of sample; Coliform count shall not exceed 5×10^3 colonies per gram of sample. *Staphylococcal* count shall not exceed 1×10^2 colonies per gram of sample *Salmonella sp* should be absent in 25 gram of chicken meat sample. The present study however revealed that the total bacterial count ranged from 5.9×10^6 - 9.9×10^7, total *Staphylococcal* count ranged from 2.5×10^4 – 7.2×10^5, total coliform count on ranged from 3.9×10^6 - 1.6×10^7, *Salmonella* count ranged from 2.5×10^4 – 3.1×10^5. Unfortunately, none of the results aligned with the set microbiological limit which shows negligence on the part meat inspection agencies in Nigeria. The chicken wings had considerably higher values of total bacterial and fungal counts than the chicken thighs which could be as a result of the bulk of the defeathering process carried out more on the wings than in the thigh. In the sensitivity test carried out, *Staphylococcus sp* exhibited 50% resistance, *Salmonella sp;*80%, *Klebsiella sp;* 40%, *Serretia sp*; 30%, *Shigella sp;* 20%, *E.coli;* 40% and *Pseudomonas sp;20%* resistance. Resistant organisms in poultry meat could be as a result of the use of the misuse of antibiotics during poultry rearing, (Threfall et al 2003). Over the years *Salmonella sp* has exhibited significantly high levels of resistance as well as *Escherichia coli and Staphylococcus aureus,* (Caroline *et al,* 2013). In producing high quality chicken meat that will have long shelf life and protect consumers from food-borne infections, it is essential that the microbial level of any processed product be kept as low as possible. This is because the microbial load is a measure of food quality of the food (Frazier *et al*, 1995).

Regulatory agencies should be set up to ensure the enforcement of microbiological safety of meats by providing documents containing microbiological limits clearly specified to be used for assessment of safety and for monitoring the nature and quality of meats;

The principles of HACCP for the production and handling of fresh chicken meat as well as establishing legal prosecution for enforcing microbiological standards should be Formulated and standardised; Public should be adequately enlightened on the importance of hand washing and hygiene consciousness.

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