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Original Research Article

Bacteriological Assessment of the Safety Quality of Frozen Chicken Commercially Dispensed in Rural-Urban Saki

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Abstract

Poultry meat products are highly desirable, palatable, digestible and nutritious for all ages hence the need for quality products of those that meet consumption fitness of consumers' wholesomeness. Twenty samples of frozen chicken which were randomly purchased from five different sellers at Sango market were analyzed to determine their bacteriological load. By using appropriate identification techniques including biochemical analysis for identification. All the frozen chicken samples from the five sellers examined were contaminated with some bacterial species. Some of the contaminants were: Bacillus cereus, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa. The total bacteria count for all the chicken examined from the different sellers was in the range of 0.7×10^2cfu/ml to 8.5×10^2cfu/ml and the coliform counts obtained for all the chicken samples ranged from 0.1×10^2 cfu/ml to 3.2×10^2cfu/ml. These finding suggest that most of the frozen chicken parts stored in the open market may constitute sources of bacterial food poisoning consequently of public health hazard. Statistically, it was seen that there was more likelihood of contamination of the frozen chicken from the sellers ($p<0.05$) than from the place of storage (refrigeration) of the chicken ($p>0.05$). However, the result revealed a high microbial of pathogenic bacteria obtained from samples kept in the open market. Conversely, a total of eight bacteria species were isolated from this study: Staphylococcus aureus (20), Staphylococcus epidermidis (8), Bacillus cereus (5), Escherichia coli (2), Proteus vulgaris (4), Klebsiella pneumonia (4) and Pseudomonas aeruginosa (5).

Keywords: Staphylococcus aureus, Chicken, Public health hazards, Frankfurters, Bologna and Sausages.

INTRODUCTION

Poultry meat products are highly desirable, palatable, digestible and nutritious for all ages. Poultry meat is comprised of about 20–23% protein, other are water and fat, phosphorus, iron and vitamins. Comminuted products, such as frankfurters, bologna and sausages typically contain about 17–20% protein, 0–20% fat, and 60–80% water [1].Quality products are those that meet some need or expectation of consumers and are safe and wholesome as well [2] .The microbiological safety and quality of poultry meat are equally important to producers, retailers and consumers. Two quite different groups of microorganisms are relevant: on the one hand certain foodborne pathogens, and, on the other, organisms that are generally harmless to human health, but, being psychro-trophic, are able to multiply on the product during chill storage. Spoilage results mainly from off-odour development, and product shelf-life is determined both by the number of spoilage organisms present initially and the temperature history of the product at all stages of production and subsequent storage and handling [3]. For chill- stored poultry, Viehweg *et al*. demonstrated that virtually all the odorous substances found at spoilage could be attributed to microbial growth and metabolism. Contamination of poultry meat with foodborne pathogens remains an important public health issue, because it can lead to illness if there are malpractices in handling, cooking or post-cooking storage of the product [4].

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Fresh (uncooked) foods such as chicken carries natural microflora that may contain organisms potentially harmful to humans. The microbial flora of table poultry is largely confined to the skin surface or visceral cavity. Isolates from poultry and poultry products could include members of the following general *Enterobacter, Alcaligenes, Escherichia, Bacillus, Flavobacterium, Micrococcus, Proteus, Pseudomonas, Staphylococcus, Coryne bacterium* and *Salmonella* [5].

Chicken consumption has considerably increase with an annual growth rate of 6% [6] and the global production of broiler meat increase from 73.1 million tonnes in 2008 to 8.31 tonnes in 2012 [7] which represent a major component of the human diet. Chicken is an important low cost source of animal protein because it offers several advantages over red meat making chicken account for an increasing trend in consumption; cut are easier to handle, the meat is associated to fewer religious restrictions and has relatively low fat and cholesterol contents; it is recognized as a healthiest food option. It can even remain for days before being sold, it was important to determine the presence of pathogenic bacteria such as *E. coli* and coliform bacterial which are direct indicators of excess human handling. Bacteriological quality of frozen chicken was also evaluated despite quality recorded risk and diseases being associated with chicken consumption is still limited. Surveillance for the presence of pathogenic organisms by these pathogens are up undetected [8, 9] in Nigeria. There is lack of qualitative data to assess hygienic and pathogenic presence along the food continuous and risk factors [10] in the study area. In some developing areas, food bore mortality in many children and having effects on children growth as well as on their physical development [10, 6] hence the need to determine the frozen chicken quality.

Due to the frequency of consumption of frozen chicken in the Nigerian populace there is possibility of increased risk of infection change in the susceptibilities of different micro-organisms that could be isolated from these products which could have a negative impact on lives of the consumers. They are basically classified based on their mechanism of action as wall inhibitor cell membrane inhibitor nuclei acid inhibitors protein synthesis inhibitors were selected; amoxicillin Base on these floor antibiotic gentamycin and ciprofloxacin.

This study might give an insight to the possible contaminations areas that could be investigated in cases of food poisoning. Data from this study will also be of use to hygiene officers and food handlers in improving and strengthening hygienic production of frozen chicken in order to avoid bacterial food contaminations [4, 11].

MATERIALS AND METHODS

Study Area

The research was carried out in the microbiology laboratory of the Oke-Ogun Polytechnic Saki Oyo State, Nigeria.

Sample Collection

Twenty samples of frozen chicken were purchased from five different sellers at Sango market in Saki. The samples collected were wrapped aseptically in sterile polythene bags and labeled appropriately. All the samples were transported immediately to the laboratory for analysis.

Preparation of Homogenate of Chicken Samples

Using a pair of sterile plastic gloves, the various chicken samples were cut away from the bone with the aid of sterile knives. Twenty grams of each of the chicken samples were separately grounded in a sterile mechanical blender and 10 ml of sterile water was added. The chicken meat was blended at medium speed and a slurry was obtained [11]. Serial dilutions of each slurry samples was carried out and then subsequently made into each sterile test tube up to 10^{-10} dilution. From an appropriate dilution 10^{-2}, 0.1 ml was then inoculated on the following media as follows:

1. **Nutrient Agar:** This was used for the enumeration of total bacteria isolates from the samples. The plates were incubated at 37ºC for 24 – 48 hours.
2. **MacConkey Agar:** This was used for the enumeration of coliforms in the samples. The plates were incubated at 35ºC for 24 – 48hours.

Identification of Isolates

The various microorganisms were subjected to morphological and biochemical tests for their identification according to the combined specification of [12], [13]. Gram staining procedures were carried out for preliminary identification of the bacterial isolates and to supplement this method, standard biochemical tests which include catalase test, Mannitol test, citrate utilization test, coagulase test and Methyl Red – Voges Proskauer (MR-VP) test were carried out [14].

Gram Staining

Gram staining is a method used to differentiate bacteria into two major groups, Gram positive and Gram negative. It is based on the chemical and physical properties of the bacterial cell wall. It primarily detects the peptidoglycan layer which is present and thick in Gram positive bacteria and relatively absent or thin in Gram negative bacteria resulting in a purple/blue colouration for Gram positive bacteria and red/pink colouration for Gram negative bacteria due to the reaction with the applied dye. A smear of the inoculum was made on a clean grease free slide using a properly sterilized wire loop, two drops of crystal violet were applied after the slide was heat-fixed over an open flame. The slide was rinsed

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after 45 seconds and Grams Iodine was applied afterwards and allowed to stay for 45 seconds, then decolourized with alcohol and rinsed with water after another 45 seconds. The slide was then counter stained with safranin dye, rinsed with water after 45 seconds and was examined under the microscope starting from the lowest objectives (x40) up to oil immersion. The test was performed according to [12].

Catalase Test
Catalase test is a test for the production of an enzyme catalase which breaks down hydrogen peroxide into water and oxygen. It is commonly used to differentiate *Staphylococcus spp.* from *Streptococcus spp*. According to [13], few drops of Hydrogen peroxide H_2O_2 were added to a smear of the test organism and the observation of effervescence was an indication of catalase positive organisms.

Coagulase Test
Coagulase test is a test used to identify *Staphylococcus aureus* from other *Staphylococcus* isolates. Coagulase is an enzyme produced by organisms that enable the conversion of fibrinogen to fibrin which results in the clotting of blood. According to Westly *et al*., a drop of blood plasma (anti-coagulated with EDTA) was placed on an inoculated saline drop and clumping after 10 seconds of gentle rocking read as coagulase positive organisms [14].

Urease Test
Some bacteria produce urease; an enzyme that hydrolyzes urea, a common metabolic waste product of vertebrates that contain nitrogen and is excreted in urine. Urease spills urea into ammonia and carbon dioxide making the two products available for bacterial use. The test organisms were inoculated in Urease broth medium these were then incubated at 37^{o}C for 24 hours, a positive result for urease utilization changes the medium from golden yellow to purple.

Mannitol Test
Mannitol Salt Agar (MSA) is a selective and differential medium. The high concentration of salt (7.5%) selects for members of the genus *Staphylococcus*, since they can tolerate high saline levels. Organisms from other genera may grow, but they grow very weakly. Inoculum from the pure culture bottles were streaked on Mannitol Salt Agar (MSA), these were incubated at 37^{o}C for 24 hours. After incubation, growth of yellow colonies gives a positive test for *Staphylococcus aureus* [15].

Eosin Methylene Blue Test
Eosin methylene blue agar (EMB) is a selective and differential medium used to isolate faecal coliforms. Eosin Y and methylene blue are pH indicator dyes which combine to form a dark purple precipitate at low pH; they also serve to inhibit the growth of most gram positive organisms. Sucrose and lactose serve as fermentable carbohydrate sources which encourage the growth of faecal coliforms and provide a means of differentiating them. Inoculum from the pure culture bottles were streaked on Eosin Methylene Blue Agar these wee incubated at 37^{o}C for 24 hours. After incubation a dark mucoid metallic green sheen gives a positive test for *E. coli*.

Citrate Utilization Test
This test detects the ability of an organism to utilize citrate, as the sole source of carbon and energy. According to [13], the bacteria was inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue, this medium also contained inorganic ammonium salts, which was utilized as sole source of nitrogen. A positive diagnosis rested on the generation of alkaline products by the usage of citrate hence an increase in the pH medium and a subsequent colour change from green to blue.

Indole Test
Some bacteria can produce indole from amino acid tryptophan using enzyme tryptophan. The production of indole is detected using Kovac's reagent, indole reacts with the aldehyde in the reagent to give a red colour as a ring at the top. The test organisms were inoculated in peptone water which contained amino acid tryptophan and incubated overnight at 37ºC. After incubation, few drops of Kovac's reagent were added. Formation of a red or pink coloured ring at the top was taken as positive.

Oxidase Test
Using the Kovac's oxidase reagent, the sterile swab sticks were dipped in the oxidase reagent and thereafter used to swab the test organisms. A purple colour change indicates that the organism is oxidase positive while the ones without colour change indicate a negative result.

Methyl –Red (MR)
This is to detect the ability of an organism to produce and maintain stable acid end-products from glucose fermentation that they overcome during the buffering action of the system. Methyl Red is a pH indicator, which remains red in colour at pH of 4.4 or less. The bacterium was inoculated into a glucose phosphate broth which contains glucose and a phosphate buffer this was incubated at 37^{o}C for 48 hours. During this period the mixed-acid producing organism must

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produce sufficient acid to overcome the phosphate buffer and remain acid. The pH of the medium was tested by the addition of 5 drops of MR reagent. Development of red colour is taken as positive. MR negative organism produce yellow colour.

Voges-Proscauer

While MR test is useful in detecting mixed acid producers, VP test detects butylene glycol producers. Acety-methyl carbinol (acetoin) is an intermediate in the production of butylene glycol. In this test two reagents. 40% KOH and alpha naphthol are added to test broth after incubation and exposed to atmospheric oxygen. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl producing a red colour that indicates a positive test for VP [15].

RESULTS

Table-1 shows the average coliform forming units per milliliter (cfu/ml) calculated from each sample per seller for both the total bacteria count and total coliform count. It can be seen that sample B from seller 3 had the highest total bacteria count of 10.3 x 10^2cfu/ml while sample B from seller 1 had the lowest total bacteria count of 0.7 x 10^2cfu/ml. It was also observed that sample C from seller 5 had the highest total coliform countof3.2 x 10^2cfu/ml while sample B and D from seller 1 had the lowest total coliform count of 0.1 x 10^2cfu/ml.

Table 1: Average Colony Forming Unit/Ml

Samples	A	B	C	D	A	B	C	D
Seller 1	0.8	0.7	0.8	1.2	0.2	0.1	0.5	0.1
Seller 2	6.6	6.4	4.0	4.1	1.1	0.9	2.8	1.9
Seller 3	10.1	10.3	8.9	3.0	1.6	2.4	1.2	0.5
Seller 4	9.3	7.3	4.0	3.6	2.7	2.4	1.8	1.1
Seller 5	8.5	6.1	7.3	4.5	2.9	1.1	3.2	2.2
Total Bacteria Count (10^2)					Total Coliform Count (10^2)			

Table-2 shows the number of isolates per sample from each seller, giving a total of 48 isolates. It can be seen that seller 1 had the lowest number of isolates with six (6) isolates while the rest sellers had quite a high number of isolates with 10 and 11 isolates.

Table-2: Pure Culture Isolated From Each Sample

Sample	A	B	C	D	Total Isolate Per Seller
Seller 1	01	02	01	02	06
Seller 2	02	02	04	02	10
Seller 3	04	03	01	02	10
Seller 4	02	02	04	03	11
Seller 5	03	03	0 3	02	11
Total					48 x 10

Table-3 shows each organism isolated per sample from each seller. It can be seen that *Staphylococcus aureus* was isolated from all the samples from the five (5) sellers. *Staphylococcus epidermidis* was also isolated from almost all the samples except that of seller three (3). The frequency of occurrence of these organisms is thus; *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus* and *Pseudomonas aeruginosa, Proteus vulgaris* and *Klebsiella pneumonia* with *Escherichia coli* as the least occurring.

More of these two organisms (20 *Staphylococcus aureus* and 8 *Staphylococcus epidermidis*) were isolated compared to others

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Table-3: Organisms Isolated From Each Sample

Samples	A	B	C	D	Total Isolate Per Seller
Seller 1	*S. aureus*	*S. aureus* *S. epidermidis*	*S. aureus*	*S. aureus* *P. aeruginosa*	06 (4:1:1)
Seller 2	*S. aureus* *K. pneumonia*	*S. aureus* *S. epidermidis*	*S. aureus* *S. epidermidis* *K. pneumoniae* *P. aeruginosa*	*S. aureus* *S. epidermidis*	10 (4:2:3:1)
Seller 3	*S. aureus* *B. cereus* *K. pneumoniae* *P. aeruginosa*	*S. aureus* *B. cereus* *K. pneumoniae*	*S. aureus*	*S. aureus* *B. cereus*	10 (4:3:2:1)
Seller 4	*S. aureus* *S. epidermidis*	*S. aureus* *S. epidermidis*	*S. aureus* *S. epidermidis* *B. cereus* *E. coli*	*S. aureus* *E. coli* *B.cereus*	11(4:3:1:2:1)
Seller 5	*S.aureus* *P. vulgaris* *P. aeruginosa*	*S. aureus* *P. aeruginosa* *P. vulgaris*	*S. aureus* *S.epidermidis* *P. vulgaris*	*S. aureus* *P. vulgaris*	11 (4:4:2:1)
					48 (20:8:5:2:4:4:5)

DISCUSSION

According to international microbiological standards (Amon, 1974 and Refai, 1979), recommended limits of bacterial contaminants for foods are in the range of $10^1 – 10^2$cfu/g of food for coliform organisms and less than 10^5cfu/g of food for total bacteria plate counts. The present study as shown in Table 1, however revealed that all the frozen chicken under the different designated retailers/sellers were within the acceptable range for total bacteria count i.e. seller 1 gave a range of $0.7\times 10^2 – 1.2 \times 10^2$cfu/g, seller 2 gave a range of 4.0×10^2cfu/g – 6.6×10^2cfu/g, seller 3 gave a range of 3.0×10^2cfu/g – 10.3×10^2cfu/g, seller 4 gave a range of $3.6\times 10^2 – 9.3 \times 10^2$cfu/g and seller 5 gave a range of $4.5 \times 10^2 – 8.5 \times 10^2$cfu/g. For the coliform count only the frozen chicken from seller 1 was within the acceptable range of $0.1 \times 10^2 – 0.5 \times 10^2$cfu/g while the frozen chicken from the other sellers were above the acceptable range i.e seller 2 gave a range of $0.9 \times 10^2 – 2.8 \times 10^2$cfu/g, seller 3 gave a range of $0.5 \times 10^2 – 2.4 \times 10^2$cfu/g, seller 4 gave a range of $1.1 \times 10^2 – 3.4 \times 10^2$cfu/g and seller 5 gave a range of $1.1 \times 10^2 – 3.2 \times 10^2$cfu/g. This is in agreement with the findings of other workers [16, 17] concerning frozen chicken stored under different conditions giving a range of $1.4 \times 10^2 – 3.1\times 10^2$ for total bacteria count and 1.2×10^1 - 3.2×10^1 for total coliform count of the isolates from chicken freezer depots, $1.5\times 10^3 – 6 \times 10^6$ total bacteria count and 1.2×10^2 - 8.5×10^2 for total coliform count of the isolates from open markets and 1.1×10^1 - 1.5×10^1 for total bacteria count and 1.2×10^2 - 1.5×10^2 for total coliform count of the isolates from cold rooms. However, the result revealed a high microbial of pathogenic bacteria obtained from samples kept in the open market. This might have been as a result of contaminations resulting from poor hygiene on the part of the sellers, from various contaminating insects and during evisceration of the chicken during processing.

Table-3 showed a total of 8 bacteria species were isolated from the examined frozen chicken samples i.e *Staphylococcus aureus* (20), *Staphylococcus epidermidis* (8), *Bacillus cereus* (5), *Escherichia coli* (2), *Proteus vulgaris* (4), *Klebsiella pneumonia* (4) and *Pseudomonas aeruginosa* (5). The presence of *Staphylococcus aureus* and *Staphylococcus epidermidis* was high in the frozen chicken because they are frequently found on the human skin since they form part of the skin flora and they are easily transferred to the frozen chicken from the seller especially in the absence of hand gloves. They can also be spread through contaminated surfaces. Meanwhile the enteric organisms which are *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* are present in the frozen chicken as a result of faecal contamination and the presence of *Bacillus cereus* is as a result of soil contamination because they are soil dwelling. The presence of these organisms are also sources of diarrhea and or gastro intestinal disturbance to both adult and children when consumed and may lead to food intoxication.

Statistically, it was seen that there was more likelihood of contamination of the frozen chicken from the sellers ($p<0.05$) than from the place of storage (refrigeration) of the chicken ($p>0.05$).

From the studies, low microbial count was however experienced from seller 1 as a result of proper hygiene and the use of hand gloves which reduces the risk of contamination and also proper refrigeration of the frozen chicken in cold room which maintained a very low temperature making it difficult for majority of the microorganisms to survive. The slightly high microbial count as observed in the other sellers might have been due to poor hygiene on the part of the workers and might have been as a result of the constant exposure of the chicken parts to the open environment. Other

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factors can be poor sanitary conditions, open tables, perching by flies and other organisms, spores of bacteria from the open environment, knives and tables on which these chicken parts have been placed might not be totally ruled out.

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