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Research Article

ISOLATION AND IDENTIFICATION OF BACTERIA FROM FROZEN CHICKEN

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Abstract

A study was conducted on the Microbiological quality of Frozen chicken sold in Thiruvannamalai, Tamil Nadu, India. Samples were taken from the neighborhood market. We collected the frozen chicken and tested it for microbial contamination. Microorganisms were isolated and accurately characterized after being analyzed in batches on various parts. With the aid of mechanical components, samples were shaken in order to extract 0.9 g of the sample, which contained 10 ml of Peptone water for which a 10 folds dilution was prepared. The objective of the study was to isolate the bacteria present in the frozen chicken. *Staphylococcus aureus* was one of the microorganisms that was tested for growth in the 0.1 ml of each dilution that was obtained in various batches and introduced to various medium. These media were incubated for 24 hours at 37 °C. *Salmonella* sp., *Escherichia coli*, and within the poultry chicken sample, *Salmonella* sp. and *Pseudomonas* were found which is the permitted level of off bacteria present in frozen chicken. Between 1.4 and 2.4 × 10 was the typical upper limit for all bacteria in poultry microbial burdens. The samples examined as a result were microbiologically unsatisfactory. The isolates CFU/g and is between 101 and 10 CFU/g, which is outside the permissible limit. The four medications, Gentamicin, Ciprofloxacin, Erythromycin, and Amoxicillin/Clavulanate, were tested for antibacterial susceptibility. A few microbes were discovered to be antibiotic resistant, and the microbial load in the samples that were examined was microbiologically unsatisfactory. A competent sanitary method and storage arrangement thereby encourage the provision of safe poultry products. The outcome suggests that the majority of frozen chicken parts sold in open markets pose a serious risk to the public health and could be a source of bacterial food poisoning. A variety of additional harmful microorganisms as well as germs that can contaminate the food during cold storage may be present in frozen chicken. The current study set out to examine the distribution of antibiotic resistance among microbiological contaminants in frozen chicken. The infections in tainted chicken are excellent human carriers, which leads to economic losses brought on the associated danger to customers.

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1. Introduction

The primary dietary source for most meals each day is meat, which excellent benefits for human health. This makes it more susceptible to oxidation and exposes it to internal enzymatic activity. Due to the presence of essential growth ingredients such mineral elements a neutral pH and high

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moisture, meat provides an ideal environment for bacterial development and their production. During slaughter and cutting as well as more generally through blades, air, workers, carts, crates, and equipment, meat and meat products may become contaminated with microorganisms. Rich in nutrients and a delicacy, chicken meat is popular among wealthy families in particular. Microorganisms have traditionally been linked to food contamination by water, soil, processing. Equipment, contact surface, and food handlers. The majority of incidents of food borne illnesses and cross-contamination are caused by improper food handling. The development of spoilage bacteria causes product faults and may be the causes of an unfavorable, flavor, color, odor, texture or spoiling, and they can be caused by the creation of different metabolites such volatiles or exopolysaccharides. The original micro biota, which is created when bacteria contaminated and the numerous treatments used. Some bacteria population the selective for certain storage. In comparison to what was initially present, the micro biota diversity declines while the bacterial burden increase during storage (Chaillou *et al.*, 2015).

Majority of research have focused on the bacteria that predominated in spoilt food, while others have utilized the criterion of microbiology acceptability (total viable counts exceeding 7 log CFU/g) to determination spoiling (Zhang *et al.*, 2012). The locations and stages of sample collection affect the sorts of organisms that are isolated (Frazier *et al.*,1985). Fresh poultry items, such as flesh are known to deteriorate quickly. Poultry microbial flora is mostly restricted to the skin surface or visceral cavity isolates from poultry products may contain bacteria from the following genera: *Enterobacter, Alcaligenes, Escherichia, Bacillus, Flavobacterium, Micrococcus, Proteus, Pseudomonas, Staphylococcus, Corynebacterium* and *Salmonella* (Frazier *et al.*, 1988). There are a number of *Salmonellosis* transmission pathways, but the majority of cases in human result from eating contaminated foods, especially those that are of animal origin (Carrasco *et al.,* 2012). Particularly variety of foods the most of significant source of human *Salmonella* infection, such as *S. typhimurium* and *S. choleraesus* are a variety of food product, especially poultry meat (Yang *et al.*, 2011). For the detection of various *Salmonella* species, 25 molecular methods like Polymerase Chain Reaction (PCR), particularly when combined with selective broth culture, have shown to be quite useful (Oliveira *et al*, 2003). Multiplex PCR (MPOR) can be used to simultaneously detect mutable pathogenic bacteria when multiple target genes need to be amplified (Soumet *et al.*, 1999).

2. Materials and Methods

Collection of Samples

The frozen chicken samples were collected from Thiruvannamalai market places, Tamil Nadu, India. The samples were processed immediately to the laboratory and stored in Refrigerator.

Isolation of Bacteria

The bacteria in sample was identified by Spread plate method. One ml of crushed frozen chicken was taken into a 10^{-1} tube containing 9 ml of saline. From this, 1 ml of sample was serially diluted 10^{-2}, 10^{-3}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} & 10^{-8}. One ml was taken from each tube and inoculated into Nutrient agar plates and spread using L-rod. Plates were incubated at 37 ºC for 24 hours. Colony morphology was observed and the isolated colonies were inoculated into Nutrient broth and incubated at 37 ºC for 24 hours. The test sample was further used to identify the morphological and biochemical characteristics of the organisms.

Identification of Morphological Characteristics by Gram staining

A thin smear was made from the colonies of agar plate and heat fixed. The smear was covered with 2 - 3 drops of Crystal violet for a minute. The slide was washed with water and then covered with Gram iodine for one minute. Again, the smear was washed to decolorize the slide gently by adding acetone/alcohol till it destains the Gram`s iodine. Then, the slide was counter stained

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with safranin for 30 seconds. Once again, the slide was washed with water blot dried with tissue paper and viewed under the Oil immersion microscope.

Biochemical Tests

Biochemical test based on Indole test, Methyl Red test, Vogues Proskauer test, Citrate utilization test, Triple Sugar Iron test, Oxidase test, Catalase test and Urease test.

Selective media for *Staphylococcus aureus*

The Mannitol Salt agar was prepared and poured in petriplates and allowed to get solidify. The samples were streaked on the plates and incubated at 37 °C for 24 hrs. After incubation, the plates were observed for *Staphylococcus aureus* growth and formation of Golden yellow colonies.

Selective media for *Escherichia coli*

The Eosin Methylene blue (EMB) agar was prepared and poured in petriplates and allowed to solidify. The samples were streaked on the plates and incubated at 37 °C for 24 hrs. After incubation, the plates were observed for *Escherichia coli* growth and formation of Green metallic sheen colonies.

Selective media for *Salmonella* sp.

The *Salmonella – Shigella* agar (SSA) was prepared and poured in petriplates and allowed to solidify. The samples were streaked on the plates and incubated at 37 °C for 24 hrs. After incubation, the plates were observed for *Salmonella* growth and formation of black coloured colonies.

Selective media for *Pseudomonas* sp.

The Cetrimide agar was prepared and poured into petriplates and allowed to get solidify. The samples were streaked on the agar plates and incubate at 37 °C for 24 hrs. After incubation, the plates were observed for *Pseudomonas* growth and formation of fluorescent green color colonies.

Determination of Antibacterial activity

Antibiotic Susceptibility Test

The Mueller Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving, it was allowed to cool in a water bath, before being poured into plastic flat bottomed petridishes on a level horizontal surface. The MHA medium was then allowed to cool to room temperature and stored in a refrigerator. At least three to five well isolated colonies of the same morphologically were selected from an agar plate culture. The growth was then transferred into a tube containing 4 - 5 ml of Nutrient broth. The Nutrient broth was then incubated until it achieves or exceeds the turbidity of the McFarland standard solution.

Inoculation of Test Plates

The swab was dipped into the inoculums suspension 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 inoculums. Drug impregnated disks were then placed on the inoculate Mueller Hinton agar plates after being exposed for about 3 minutes. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and placed in an incubator set to 35 °C.

Preparation of Stock Solutions for Antibiotic Susceptibility Testing using Drugs available in the Market

Amoxicillin/Clavulanate

Amoksiklav Forte 312.5 mg/5 ml was used in the testing of the susceptibility of the microorganisms. This was diluted to obtain the working concentration. Five ml of the suspension said to contain 312.5 mg of the drug was measured and transferred into a sterile bottle; the paper discs meant for the disc diffusion test were then dipped into the suspension, individually.

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Ciprofloxacin

The concentration of the infusion used was 2 mg/ml this was diluted to obtain the working concentration, using a sterile pair of forceps each paper disc was dipped into the infusion.

Erythromycin

Miral Erythromycin Suspension was used in the testing of the susceptibility of the microorganisms this was diluted to obtain the working concentration. Five ml of the suspension was measured and transferred into a sterile bottle; the paper discs meant for the disc diffusion test were then dipped into the suspension, individually.

Gentamicin

The concentration of the drug in an ampoule is about 280 mg/2 ml, this was diluted to obtain the working concentration. The prepared paper disc was dipped into 2 ml of the infusion individually and this was used for the disc diffusion test.

Preparation of Dried Filter Paper Discs

Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter with the aid of a whole puncher, which were placed in a petridish and sterilized in the oven. A pair of forceps was then used to pick the discs which were individually dipped into the different antibiotics until they were properly soaked. Drug impregnated disks were then placed on the inoculated Mueller Hinton agar plates after being exposed for about 3 minutes. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and placed in an incubator set to 35 °C.

Standardization of the Antibiotic Discs

The prepared antibiotics were tested for their efficacy using the Kirby-Bauer disc diffusion method and were checked if the diameter of the zone of inhibition was between the ranges for sensitivity of the organisms. Test organisms such as *Escherichia coli* and *Staphylococcus aureus* were used. They were sub-cultured before the sensitivity testing. The inoculums were prepared from the cultures and were matched for turbidity with 0.5 McFarland solutions. The prepared antibiotic discs were placed on the inoculated agar plate along with the commercially available discs for comparison of the efficacy of the prepared discs. The plates were then incubated at 37 °C overnight. After incubation, the zones of inhibition were measured for each of the antibiotic discs and was seen if they were within the sensitivity range of the organisms.

Characteristics of the Medium

The main characteristics of the medium (Muller Hinton Agar medium) was to support the growth of the organisms normally tested and not obtained antagonist of antibacterial activity. Twenty four hours old culture of selected bacteria was mixed in Nutrient broth and turbidity was observed after 24 hours of incubation.

Antibacterial activity using Disc Diffusion Method

The bacterial strain was swabbed on Muller Hinton agar plates. The disc was placed on the MHA plates on different dilutions. Twenty ml of dilutions were added on each disc. The plates were incubation at 37 °C for 24 hours. The zone of inhibition was measured and mentioned as mm in dm.

3. Results and Discussion

Isolation of *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas* sp. were done by several method. First step of dilution done up 10^{-1} to10^{-8} for frozen chicken sample shown. A frozen chicken sample crushed with peptone water shown in *Staphylococcus aureus*, *Pseudomonas, Escherichia coli, Salmonella* sp. Isolation from Selective media from colonies that was suspected as *Escherichia coli, Salmonella* sp., *Staphylococcus aures* and *Pseudomonas* sp. that are purification replicates culture on Mannitol salt agar, EMB agar, SSA agar, and Cetrimide agar in order to get organisms. Pure isolates and pure

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culture the agar slant obtained isolates were used as for identification of microorganisms in frozen chicken in Biochemical and morphological characteristics (Table - 1). The antibiotic sensitivity result was given in Table – 2.

The frozen chicken parts that were bacteria species, of which 4 were Gram positive and when ingested, these organisms can cause both adults and children to experience diarrhea and/or gastrointestinal disturbances. This is in line with findings from prior studies on frozen chicken stored in various environments. *Escherichia coli* were isolated in the samples obtained from each frozen chicken have high amount of *Escherichia coli* isolated, with an average of about 150 Coliform cells counted on the organisms. The quality of chicken meat is at its best right after processing, and keeping it at a level that is acceptable depends on the initial microbial levels and the precautions taken to prevent organism development. The two main issues are minimizing a health danger and controlling spoilage organisms.

It is clear from the results in that all of the samples of chicken that were analyzed were organoleptically acceptable, unlike 3 (8.6 %) out of 35 samples of raw chicken meat and just 1 (2.9 %) out of 35 samples of frozen chicken burger. Results showed that all of the Investigated samples of raw chicken flesh had an aerobic plate count of 100 %. The examined samples of frozen chicken was high aerobic bacterial count, which suggests that the product may have been Contaminated due to poor sanitation practices during handling, processing, or distribution. Additionally, the spices may have increased the bacterial count whereas the low count in cooking procedures. Chicken may have resulted from when present in large quantities, the Enterobacteriaceae are regarded as spoiling Agents and could pose risks to consumer health. The data showed that *Staphylococcus aureus* counts were higher in frozen chicken burgers than those found in raw chicken meat, which may be attributed to contamination from various sources during processing stages. In contrast, low counts of the bacteria were found in chicken sandwiches, which may have resulted from human.

Table – 1: Identification of Bacterial isolates

Biochemical tests	*Escherichia coli*	*Salmonella* sp.	*Staphylococcus aureus*	*Pseudomonas* sp.
Gram Staining	-ve	-ve	+ve	-ve
Shape	Rod	Bacilli	Cocci	Rod
Motility	+ve	+ve	-ve	+ve
Indole	+ve	-ve	-ve	-ve
Methyl Red	+ve	+ve	-ve	+ve
Voges Proskauer	-ve	-ve	+ve	-ve
Citrate Utilization	-ve	-ve	-ve	+ve
Triple Sugar Iron	+ve	k\a	-ve	-ve
Urease	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve	+ve
Colony Morphology	Green metallic sheen	Black colonies	Yellow small irregular colonies	Large opaque blusih green colonies with smooth edges.

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Table – 2: Antibiotic Susceptibility Test

Organism	*Escherichia coli*		*Salmonella* sp.		*Staphylococcus aureus*		*Pseudomonas* sp.	
Antibiotic	P (in mm)	C (in mm)	P (in mm)	C (in mm)	P (in mm)	C (in mm)	P (in mm)	C (in mm)
Amoxicillin	18	16	20	18	19	19	10	12
Erythromycin	-	-	12	10	20	18	18	16
Gentamycin	23	21	15	20	23	21	20	7
Ciprofloxacin	32	20	16	14	25	23	12	11

4. Conclusion

The chicken can be an excellent environment for bacterial development. The relatively high bacterial load of poultry meat in the fresh sample could be a result of improper handling and environmental contamination during microbial load, washing the meat, transportation, and storage. The present study to isolate the organism in frozen chickens. The resistance profile of the isolated microorganisms was collected, and they were not only quantified but also defined the identified microorganism present in frozen chicken. The contaminated chicken act as an excellent carrier of pathogens to the humans, which results in financial losses that prepared by related risk to the consumers. The microorganism identified by using Gram stain and biochemical tests etc., Further analysis by selective media to isolate a specific organism *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas* sp. To determination of antimicrobial activity (Antibiotic susceptibility test) as exhibited using drugs are standardization of the antibiotic disc used sterile Amoxicillin, Ciprofloxacin, Erythromycin, Gentamycin. Antibacterial activity using disc diffusion method to analysis the zone of inhibition.

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