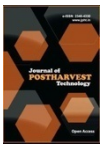

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RESEARCH ARTICLE

Analysis of raw chicken meat for microbiological quality

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ABSTRACT

A study was conducted with a forty numbers of isolates from twenty fresh chicken meat samples of which 16 (40.00 percent) and 24 (60.00 percent) isolates were used in study. A total of 13 (32.50 percent) strains of coagulase negative *Staphylococci*, 5 (12.50 per cent) strains of *Aeromonas* spp. and 22 (55.00 per cent) strains of *Salmonella* spp. were isolated. The microbiological quality of chicken meat as observed by TVC was in the range of 4.70 to 8.60 $\log_{10}$ cfu/g with average TVC of 6.36 ± 1.19 to 6.92 ± 0.35 $\log_{10}$ cfu/g. The analysis of microbes in chickens muscle, liver, heart, and gizzard samples revealed the TVC ($\log_{10}$ cfu±SE) in the range of 6.02 to 7.80 (average 6.91± 2.13), 5.40 to 7.50 (average 6.45±1.27), 4.70 to 7.80 (average 6.36±1.19) and 4.95 to 8.60 (average 6.92 ±0.35), respectively**.** The investigation revealed that prevalence of *Aeromonas* spp. and coagulase negative *Staphylococcus* in muscle, liver, heart, gizzard were found to be 20 percent, 20 per cent 18 per cent and 60 per cent and that of coagulase negative *Staphylococcus* was 23.06 percent, 23.06 per cent 53.88 per cent and 0 per cent. The investigation also revealed the prevalence of *Salmonella* spp. present in muscle (31.88%), liver (31.86%), heart (0.0%) and gizzard (36.24%)**.** The results recorded that 88.88% isolates of *Salmonela* spp. showed resistance towards Amoxycillin (30mcg) and Ampicillin (10mcg) and 71.42% isolates of *Aeromonas* spp. showed resistance towards Amoxycillin (30mcg) and Ampicilin (10mcg). Streptomycin (10mcg) showed 100% resistance to *Staphylococcus* isolates.

Keywords: Chicken**,** *Aeromonas*, *Staphylococcus* , *Salmonella,* food safety

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INTRODUCTION

Chicken meat is more popular in the consumer market due to easy digestibility and acceptance by the majority of people (Yasoda et al., 2001). In India, only 4% of poultry processing in India is carried out in modern processing plant and remaining 96% are processed in small poultry slaughtering and dressing units involving 300- 500 birds / day where prevailing hygienic and sanitary conditions are far from satisfactory (Anon, 1994). It serves an excellent medium for the growth of pathogens viz. *Staphylococcus* spp.*, Salmonella* spp. and *Aeromonas* spp. The broiler chicken production in the year 2022 is projected at 5 million tones. The live bird market sales of broiler still constituted more than 90-95 percent of total volume of sales. The processed chicken meat segment comprises only about 5 percent of total production (National action Plan for Egg and Poultry, 2022).

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Poultry meat is comprised of about 20–23% protein of which comminuted meat products, such as frankfurters, bologna and sausages typically contain about 17–20% protein, 0–20% fat, and 60–80% water (Smith, 2001).Food safety problems pose a great threat to the health of consumers with the greatest burden in developing countries. Street-vended foods play a key role in providing many urban dwellers with cheap, nutritious, and accessible food which is available with comparatively low prices (Mohan et al., .2022). The lack of adequate food service equipment hampers effective implementation of safe food storage practices at street food vending sites (Muyanja et al., 2011).

The presence and absence of bacterial flora and index organism provide useful guidelines for determination of wholesomeness of raw and processed chicken meat as well as safety to the public health. The presence or change in microbial load in stored meat products is influenced by many factors like temperature, nutrient presence in meat, pH, water activity, humidity and packaging system that affects the growth and multiplication of bacteria (Forest et al.1975). Due to the chemical composition and biological characteristics, meats are highly perishable foods providing an excellent source of nutrients for the growth of several hazardous microorganisms that can cause infection in humans, resulting in spoilage of the meat and, therefore, economic loss. By-products of slaughterhouse may be defined as everything from the abattoir or normal bucher's shop that is not sold directly as food (Gracey 1986).

Salmonella spp. is gaining an increase important as a food borne bacterial pathogens. *Salmonella* infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease (Crump et al.2004). They are Gram negative motile bacilli. The presence of Salmonellosis is a worldwide problem causing substantial loss in health and production. It is endemic in most of the countries resulting in heavy economic loss (CADRAP, 2006). In the United States of America (USA), Campylobacter jejuni has been reported as the most common pathogen causing foodborne illnesses, followed by Listeria and Salmonella and the Foodborne Diseases Active Surveillance Network (FoodNet) of the Centre for Disease Control (CDC) reported that the incidence of foodborne attributed hospitalization caused by campylobacteriosis and salmonellosis was increasing over the years(CDC, 2009).

Staphylococcus spp. is considered to be one of the leading causes of food borne illness. They are Gram positive cocci arranged in grape like clusters. Meats, dairy products and meats are often contaminated with entero- toxigenic strains of the bacterium. The number of *S. aureus* strains that exhibits antimicrobial resistance properties has also increased.

There is evidence that *Aeromonas* spp. is also involved in the etiology of gasterointestinal illness in human.(Parveen,2012). The dominant species of ground chicken meat were *A. hydrophila* and *A. caviae*. The present study was undertaken on the occurance of bacteriological pathogens viz. *Aeromonas*, *Staphylococus* and *Salmonella* spp. in the muscle of leg, heart, gizzard and liver in the raw chicken meat, its drug sensitivity test and bacteriological load of its isolates.

MATERIALS AND METHODS

A total of forty numbers of isolates from twenty fresh chicken meat samples of which 16 (40.00 per cent) isolates were from the Food Processing Unit, University of Science and Technology, Meghalaya and 24(60.00 per cent) were from Local Retail Shop at 9th Mile, Guwahati in Assam, India (Table : 1). Muscle, liver , heart and gizzard were collected aseptically and minced in pestle and mortar.

Table 1: Bacteriological status of chicken meat samples from different source

Source	Numbers of Samples Tested	Number of bacteriologically positive	Numbers of isolates
Food Processing Unit, USTM, Meghalaya	8 (20.00)	8 (20.00)	16 (40.00)
Local Retail Shop, 9th Mile, Guwahati	12 (30.00)	12 (30.00)	24 (60.00)
Total	20 (50.00)	20 (50.00)	40 (100.00)

Media

Liquid and solid media and chemicals for biochemical test purchased from Hi- media. All media were prepared using standard procedure. Media were sterilized at 121°C for 15 minutes and check for sterility at 37°C for 24 hours before use.

Glassware

Glassware used were from Borosil Company, which were calibrated and standardized.

Processing of materials

The chicken were kept off fed for 6 hours before slaughtered and dressed in the Food Processing unit at Department of Applied Biology, University of Science and Technology, Meghalaya - 793101. After dressing the carcass manually meat were kept in the deep freeze (20 ±2°C). The pieces of meat were minced in pestle and morter. Isolation of *Aeromonas* spp., coagulase negative *Staphylococci* spp. and *Salmonella* spp. were prepared with standard procedure. The overnight nutrient broth culture of test organism spread over the plate with a sterile cotton swab is taken. Selective media were used for the isolation of *Aeromonas* spp., coagulase negative *Staphylococci* spp. and *Salmonella* spp. After getting pure culture growth, morphological and biochemical reaction were studied.

Total Plate Count

Eleven grams of meat samples were thoroughly mixed in 99ml sterile 0.1 per cent peptone normal saline diluents to obtain 1:10 (w/v) diluents for which further 10 fold dilutions were made. The dilution were then inoculated by pour plate method on total plate count agar for enumeration for the total aerobic plate count. One ml inoculum were transferred to petri dish to which melted media maintain at 45°C was poured and mixed with inoculum by gentle rotating movements and allow to saolidify. Duplicate plates were inoculated for each dilution and incubated at 37 °C for 24 hours in the inverted position. The plates with the dilution containing approximately between 30 and 300 colony forming units (cfu) were selected and their numbers counted and were expressed as $\log_{10}$ numbers / grams of sample. The average counts of the duplicate plates were taken into account.

Isolation of *Salmonella* spp., coagulase negative *Staphylococci and* Aeromonas spp. in meat samples

Identification of bacteria was done on the basis of cultural, morphological and biochemical reactions as per standard procedure (America Public Health Association, 1966 and ISO, 2002).

Isolation of Salmonella spp.

Raw meat samples of 10 g each weighed, homogenized in 90 ml buffered peptone water, and incubated at 24 hours for selective enrichment for morphological and cultural characteristic (ISO,2002).

Isolation of Staphylococcus spp.

About 10 g of raw meat samples homogenized in 90 ml peptone water and 10 ml of sample were used to prepare serial dilutions. In a sterile pipette, 0.1 ml of the appropriate sample test dilutions was transferred in duplicate onto the Brain Heart infusion agar. The plates were then incubated at 35-37°C for 24 hours and then re-incubated for further hours. Observation ensued for typical colonies appearing black or grey, shining and convex, and 1-1.5 mm in diameter after 24 hours. (ISO, 2002).

Isolation of Aeromonas spp.

1gm of minced meat were diluted in the peptone water to 10 fold dilution of which 0.1ml of the dilution inoculated in liquid and selective media for morphological and cultural chara cteristi. Colonies thus formed in the selective media were closely observed. Isolation of distinct colonies has been performed in nutrient agar as slant which was used as stock culture. On Mcconkey lactose agar, the colonies observed were circular with a smooth surface. On Bismuth sulphide agar jet black colonies were obtained after incubated for 24 hours. In nutrient it showed uniform turbidity after 24 hours (AOAC, 2012 and ISO, 2002).

Grams reaction

Prepared a heat fixed smear from an 18-24 h culture. Stained with crystal violet for 1-2 minutes. Rinsed off with Grams iodine and allowed the iodine to act for 1 minute. Poured off the iodine, dried and washed the slides with 95 per cent alcohol for only 5 -10 seconds. Rinsed under the tap water and stained with dilute carbol fuchsine solution for 10 seconds. The slide were vanished with water and dried (AOAC, 2012)

Biochemical test

Catalase test, oxidative – fermentive test, indole test, Methyl- red test were performed as per standard procedure (Paniker et al., 1986).

Antibiogram

Antibiogram of the isolates of *Aeromonas* spp., coagulase negative *Staphylococci* spp. and *Salmonella* spp. from raw chicken meat were performed using the standard disk diffusion method (CLS, 2012).

RESULTS AND DISCUSSION

Total viable count

It was observed that the range of 4.70 to 8.60 $\log_{10}$ cfu/g with average TVC of 6.36 ± 1.19 to 6.92 ± 0.35 $\log_{10}$cfu/g (Table 2), which is comparable to the previous reports of Asmara et al. (1994) who reported TVC in raw chicken meat in the range of 6.55-7.15.$\log_{10}$cfu/g. The recommended microbiological standard for raw meat was in the range of 10^5 – 10^7 $\log_{10}$cfu/g. The results

of the study are in agreement with Chaiba et al. (2007) who reported the presence of *Staphylococcus* spp. and *Salmonella* spp. in raw chicken meat. The presence of such high microbial counts can be attributed to improper handling of raw chicken products and inadequate storage conditions (Jay, 2005).

Table 2: Total Viable Count collected from different chicken meat samples

Samples	Total Nos. of Samples analyzed	Nos. of samples in Acceptable range	Total viable count ((log 0 Cfu/g range Min.	Max.	Average viable count (log) Cfu/g SE
Muscle	08	6	6.02	7.80	6.91 ± 2.13
Liver	02	1	5.40	7.50	6.45 ± 1. 27
Heart	02	1	4.70	7.80	6.36 ± 1.19
Gizzard	08	6	4.95	8.60	6.92 ± 0.35

Total viable count in chicken muscle, liver, heart and gizzard samples

The analysis of bacteria in chicken muscle, liver, heart and gizzard samples revealed the TVC (log_{10} cfu/g ±SE) in the range of 6.02 to 7.80 (average 6.91 ±2.13), 5.40 to 7.50 (average 6.45±1.27), 4.70 to 7.80 (average 6.36 ±1.19) and 4.95 to 8.60 (average 6.92 ±0.35) respectively. A total of 13 (32.50 per cent) of coagulase negative *Staphylococci*, 5 (12.50 per cent) of *Aeromonas* and 22 (55.00 per cent) strains of *Salmonella* spp. could be isolated which is shown in Table 3. This was closely associated with Borah et al. (1992). Their presence is attributable to poor hygiene and unsanitary facilities on the vending site (Sandel, 2004). The investigation revealed that prevalence of isolates of *Aeromonas* spp. and coagulase negative *Staphylococcus* in muscle, liver, heart, gizzard which were found to be 20 percent, 20 per cent 0 per cent and 60 per cent and that of coagulase negative *Staphylococcus* was 23.06 percent, 23.06 per cent 53.88 per cent and 0 per cent which is similar to the reports by Sharma et al. (2009). The investigation also revealed the prevalence of *Salmonella* spp. present in muscle (31.88%), liver (31.86%), heart (0.0%) and gizzard (36.24%) which is similar to the reports by Hayes and Freeman (1945) (Table 4 and 5). The presence of *Staphylococcus* counts in chicken meat indicated the presence of poor hygienic and food handling practices as well as cross contamination (Tesfaye et al., 2016).

Table 3: Distribution of different isolates from chicken meat

Isolates	Number of isolates from Food Processing Unit, USTM, Meghalaya	Number of isolates from Local Retail Shop, 9th Mile, Guwahati	Total number of isolates(per cent)
coagulase negative *Staphylococci*	04 (10.00)	09 (22.50)	13 (32.50)
Aeromonas	02 (05.00)	03 (07.50)	05 (12.50)
Salmonella	10 (25.00)	12 (30.00)	22 (55.00)
	16 (40.00)	24 (60.00)	40 (100.00)

Table 4: Biochemical characteristic of *Salmonella* spp. isolate from chicken meat

Isolates	Numbers of isolates tested	Number of isolates showing Positive results to			
		Methyl Red test	Voges- Proskauer test	Citrate utilization test	Hydrogen sulphide production test
22	22	22 (100.00)	22 (100.00)	22 (100.00)	22 (100.00)

Table 5: Prevalence of *Aeromonas* spp., coagulase negative *staphylococci* and *Salmonella* spp. in meat samples

Samples	Total Nos. of samples analysed	Number of samples for microorganism		
		Aeromonas spp.	coagulase negative staphylococci	Salmonella spp.
Muscle	10	1(20)	3 (23.06)	7(31.88)
Liver	10	19 (20)	3 (23.06)	7 (31.88)
Heart	10	0 (0)	7 (53.88)	0 (0.00)
Gizzard	10	3 (60)	3 (60.00)	8 (36.24)

Cultural and biochemical characteristic of coagulase negative S*taphylococci* spp. and *Salmonella* spp. and *Aeromonas* spp in meat

On McConkey lactose agar, the *Salmonella* spp. colonies were circular with a smooth surface whereous in Bismuths sulfide agar jet black colonies were obtained after incubation of 24 hours which is closely reported by Asmara et al. 1994. 13 numbers of isolates of coagulase negative *Staphylococus* showing fermentation to oxidative- fermentative test. Only one (07.00 per cent) isolates showed yellow pigment while twelve (93.00 per cent) isolates showed white pigments (Table 6). This was in close association with Chaiba et al. (2007). Haemolysis of blood agar could be performed by only in one (07.00 per cent) isolates. Mannitol was fermented and gelatin liquefaction also showed positive results along with Methyl red test, Voges- Proskeaur, Citrate utilization test and Hydrogen Sulphide production test (Table 6).

Table 6: Biochemical characteristic of coagulase negative *staphylococci* isolates from chicken meat

Isolates	Number of isolates tested	Number of isolates showing Positive results to						
		Production of Pigments		Haemolysis of blood agar	Coagulase Production	Mannitol Fermentation	Gelatin liquefaction	Oxidative – fermentative test
		Yellow	White					
1	13 (100.00)	1 (07,00)	12 (93.00)	1 (97.00)	0	13 (100.00)	13 (100.00)	13 (100.00)

Antibiogram

The study showed 88.88% isolates of *Salmonella* spp. had resistance towards Amoxycillin (30mcg) and Ampicillin (10mcg) and 71.42% isolates of *Aeromonas* spp.shown resistance towards Amoxycillin (30mcg) and Ampicilin (10mcg) and Streptomycin (10mcg) showed 100% resistance to *Staphylococcus* isolates (Table 7). This is similar to the reports given by Sachan and Agarwal (2000). The emergence of antibiotic-resistant foodborne pathogens has raised the concern of the public as these pathogens are more virulent, causing an increase in the mortality rate of infected patients (Chiu et al.2002).

Table 7: Antibiogram for *Salmonella*, *Aeroromonas* and *Staphylococcus*

Antimicrobials	*Salmonella* spp.			*Aeroromonas* spp.			*Staphylococci* spp.		
	R	S	VS	R	S	VS	R	S	VS
Amoxycillin (30mcg)	06	01	0	05	02	0	07	0	0
Ampicillin (10mcg)	06	01	0	04	02	01	07	0	0
Streptomycin (10mcg)	0	0	7	05	02	0	07	0	0

R: Resistance S: Sensitivity VS: Very sensitivity

CONCLUSION

Poultry meat is a vehicle of bacterial transfer and plays an important role in disease transmission**.** This study has been an attempted to provide Good Hygiene Practice in chicken meat production and consumption for prevention of disease. The results of this study could help effective planning and implementation of food safety system for the control and prevention of food related pathogenic bacteria. Contamination of chicken meat and giblets is indicative of bad microbiological quality of retail chicken outlet which may be done to prevent contamination during processing and distribution. Thus, personal hygiene and education of butchers would play a significant role towards supplying safe and potentially non- hazardous meat to the society.

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