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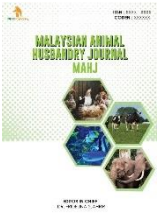

RESEARCH ARTICLE

# MICROBIOLOGICAL ASSESSMENT OF BROILER CHICKEN MEAT FROM DIFFERENT SLAUGHTERHOUSES OF POKHARA VALLEY

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**ABSTRACT**

Chicken meat is a nutritious food consuming all over the world having low cost and fat content which is highly perishable with short shelf life even when refrigerated in low temperature. Microbiological assessment was done by taking samples from different slaughtered houses of different wards of Pokhara and was evaluated in the lab. To assess the hygiene quality of chicken meat slaughtered in the slaughtered house of Pokhara Valley, six chicken meat samples were taken in the present work. Different microbiological parameters such as total coliforms, fecal coliforms, total plate count, *Salmonella, Shigella,* and *Staphylococcus aureus* were examined. A survey with the help of a questionnaire was also done to assess the sanitary condition of slaughtered houses and butchers. The average value for the total plate count of the meat sample was found to be $6.3406x10^6$ cfu/g, only one sample was found to be *Salmonella* positive whereas all the samples were found to be *Shigella* negative. Only two samples were found to be *Staphylococcus aureus* positive. Likewise, two samples were found to contain total coliforms and there were no any formation of fecal coliform. Based on the results obtained, the risk of contamination depends on how the meat is processed, handled and packed. From the survey, it was clear that in some slaughtered houses, chicken meat is slaughtered unhygienically and unscientifically in an unmanaged way. There is a need for strict compulsion of the meat inspection act and education about sanitation for all the employees working in it.

**KEYWORDS**

Chicken meat, slaughtered house, total plate count, total coliform, *Salmonella, S. aureus*

## 1. INTRODUCTION

Meat is a perishable food item because it provides an ideal environment for the growth of numerous pathogenic and nonpathogenic bacteria (Bantawa, 2018). The microbiological quality of meat is important from a public health point of view. With the lack of advanced equipment and technology used for processing meat, meat quality is degraded. Mishandling of carcasses during evisceration, processing, canning, or packaging of the product and fluctuation in storage temperature leads to the spoilage of meat by various harmful bacteria (Neupane and Kaphle, 2019).

Likewise, major bacterial pathogens found in meat include *Cl. botulinum, Cl. perfringens, B. cereus, Salmonella, Shigella, E. coli,* and *S. aureus* (Pal et al., ,2018). The sanitation of the slaughtered man and the slaughterhouse is meant to be of greater importance for both the consumers and the slaughter man but its inspection has not been done properly regarding its hygienic condition and meat quality. Similarly, very few microbial assessments in meat have been done in Pokhara till the current time (Baral, 2021).

To assess the hygienic status of the slaughter man and slaughterhouse along with the microbial contamination of meat and as well as equipment used during slaughtering around the valley, this study was performed. The main aim of this study is to help in minimizing the contamination of pathogenic microorganisms causing foodborne illness and maintain the cleanliness of the slaughterhouse. And also, to determine the occurrence and levels of pathogenic and non-pathogenic microorganisms present in chicken meat around the valley.

## 2. MATERIALS AND METHODS

### 2.1 Study Area and Sample Collection

The sample of chicken meat was collected from different slaughterhouses located in the different wards of Pokhara, Nepal. Samples were collected on two different days in the morning time. The sample size of 250 g from each place was collected in sterile polythene plastic bags without touching by bare hands which was analyzed within 2 h after collecting it. The collected samples were placed in the ice box for further analysis.

### 2.2 Isolation of Bacteria and Calculation of Cfu/Ml

25 g from each sample were mixed in 225 ml of sterile buffered peptone water. Separately serial dilutions were made for all those samples to isolate bacteria. Approximately, 1 ml of solution from appropriate dilutions (10-3 and 10-5) of each sample were used in plate count agar, and calculation was done for total plate count. Plates were incubated at 37°C for 24 h and a number of colonies were observed (Adhikari, 2019).

Likewise, serially diluted samples on loopful streaking were done on VRBA plates and incubated at 37°C for 48 h for observing total coliform and incubated at 44.5°C for 24 h for observing fecal coliform (Ruban and Fairoze, 2011; Chaudhrya et al.,2011; Ibrahim et al., 2014). After

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incubation, a number of distinct colonies on each sample's plate were enumerated and colony-forming units (cfu/ml) was calculated. Similarly, the loopful sample was streaked on mannitol salt agar and then the inoculated plates were incubated at 37°C for 18-24 h. The presence and absence of *S. aureus* organisms were determined (Devkota et al., 2019). *Salmonella* and *Shigella* were detected according to with some modifications (Maharjan et al., 2019). After incubating the enriched sterilized peptone water at 37°C for 24 h, loopful streaking was done on *Salmonella-Shigella* agar and again incubated at 37°C for 24 h for observing the isolated colonies.

### 2.3 Identification and Characterization of Bacteria

For the identification and characterization of bacteria, pure cultures of bacteria were made separately from mixed bacteria with the help of the streak plate method. The streaked plate was incubated at 37°C for 24 h. For gram staining, similar colonies were used after 24 h. Biochemical tests were carried out for identifying pure isolates which includes gram staining, catalase test, oxidase test, and coagulase test.

Gram staining was performed according to the procedure of for the catalase test, 3% H2O2 was poured into isolated colonies placed in the microscopic slide (Thapa, 2021). Organism showing bubbles formation in the dark background was known to be catalase-positive and vice versa (Reiner, 2010). The coagulase test was performed by the procedure of where this test differentiates between *S. aureus* which produces the enzyme coagulase being coagulase-positive from *S. epidermis* and coagulase-negative from *S. saprophyticus* and citrate utilization test by the procedure of) where citrate agar is used to test an organism's ability to utilize citrate as a source of energy (Aryal, 2018; Aryal, 2019).

## 3. Results

An assessment survey was carried out among six different slaughterhouses of different wards of Pokhara through communication. Hygienic status was cleared in terms of their cleaning, storage, sanitation, and selling condition, controlling flies and dust, education status, etc. as shown in Table 1. The microbial load of total plate count in all chicken meat samples was found to be higher than the prescribed standard of Oregon State Microbiological Standard (5 X log 6/g). The bacterial counts were found to be high due to the poor hygienic condition of the slaughtered house, handlers, and slaughtering equipment. The presence of *E. coli* indicated the possible contamination of meat by intestinal matter. The total coliform count was also higher than that of the prescribed standards of Europe and the United States (5 X log 3/g). Two meat samples were found to contain *S. aureus* and one meat sample was found to contain *Salmonella*.

## 4. Discussion

### 4.1 Assessment Survey

A total of six slaughterhouses out of 12 were interviewed which were located in different wards of Pokhara Valley. Here 50% of the workers were illiterate and 50% of them were untrained by the slaughterhouse and meat inspection act. The assessment revealed that 33.33% of the workers used only water for cleaning the materials, 50% of them used soap or detergent and 16.67% used only clothes for cleaning. Likewise, 50% of workers scraped the chopping block for cleaning while 50% uses water for cleaning it. Only about 33.33% use aprons and covered their hair but the remaining ones neither use aprons nor cover their hair. Similarly, 50% of the workers control the flies in slaughtering areas chemically while 50% of them control it manually.

### 4.2 Total Plate Count

This study recorded the total plate count from the chicken meat sample obtained during the morning time. The data obtained are shown in Table 2. Table 2 shows the values for the total plate count of meat samples. The average value was found to be 6340 X 10^3 cfu/g with a maximum value of 18084 X 10^3 cfu/g and a minimum value of 2 X 10^3 cfu/g. The mean bacterial count (log cfu/g) of raw chicken meat was found to be 5.7035. All the samples obtained a higher total plate count than the many microbiological standards as Oregon State Microbiological Standard and inspected German Quality Meat. This result is also higher than the ISO guideline which has set 10^5-10^7 total viable count in 0.01g of raw meat. This finding is however less than the findings of [1] from Dharan and that from Chitwan (Bhandari, 2013).

The mean TVC from poultry meat has been found to vary in the Nepalese context from 8.31 ± 0.23 in Bhaktapur to 12.2 ± 0.5 in Rampur (Neupane and Kaphle, 2019). The total viable count also varies among different meat types also as has shown higher TVC in pork and least in buffalo meat (Bantawa et al., 2018). Our finding is somewhat similar to the findings of (Maharjan et al., 2019). This variation is due to the difference in time, season of research and sample size. These findings indicate the contamination of products due to unsatisfactory sanitation during handling and processing.

### 4.3 Total and Fecal Coliform

Meat is generally checked for the presence of indicator organisms such as *E. coli* and coliforms to indicate the possible contamination with viscera (Jimenez et al., 2003). According to our study, 33.33% of samples were found to be contaminated with total coliform bacteria whereas there was no growth of fecal coliforms in the given sample shown in Table 3.

Our finding is less than that of [1] who showed 8.13±0.13 cfu/g in meat samples of the Dharan. Similarly, has shown 8.4±0.5 in VDCs in Chitwan (Bhandari et al., 2013). Fewer coliform counts were reported (1.03 log cfu/g) by ( Joshi, 2003). However, our finding is more than that of (2.19 log cfu/g); (2.7 log cfu/g), and (2.6 log cfu/g) in raw market meat(Maharjan et al., 2019; Álvarez-Astorga et al., 2002; Northcutt et al., 2003). Similarly, shows different total coliform in three different locations of dining halls as log 5.77, 3.26, and 2.95 respectively (Hossain et al., 2015). Our coliform counts of 3.69 log cfu/g and 4.36 log cfu/g was much more similar to the studies of (4.97 log cfu/g) (Ku et al., 2011). No fecal coliforms were detected in our finding which is in alignment with that of (Maharjan et al., 2019).

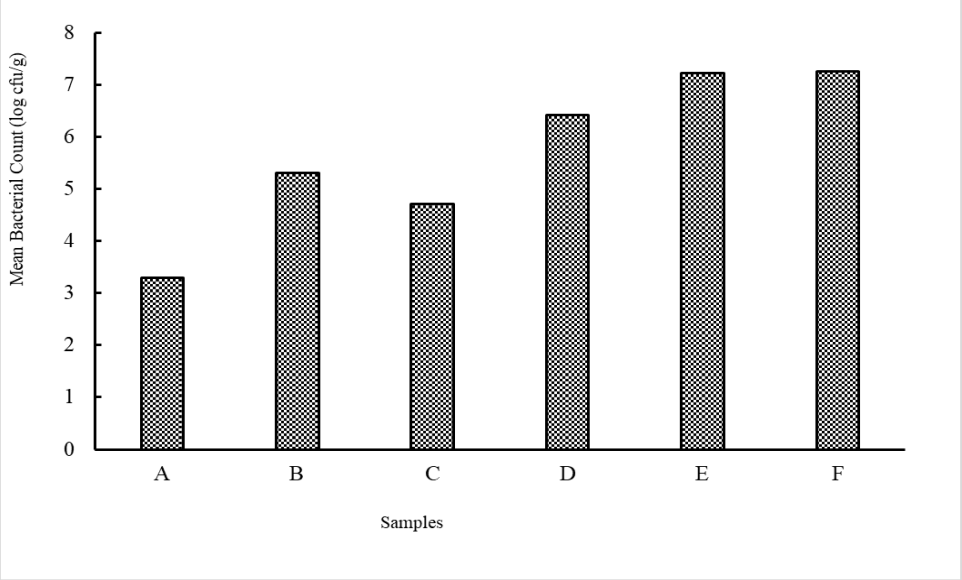


**Figure 1:** Mean Bacterial Count of Chicken Meat

**Table 1:** Detail output of the Survey
Total no of respondents = 6

| S. N. | Questions raised to employees | Options | Frequency | Percentage (%) |
|---|---|---|---|---|
| 1 | Meat storage location | Cemented place | 6 | 100% |
| | | Wooden table | 0 | 0% |
| | | Tin plate | 0 | 0% |
| | | Carpet | 0 | 0% |
| 2 | Provision of meat wire fence | Yes | 5 | 83.33% |
| | | No | 1 | 16.67% |
| 3 | Hide of the animal | Selling on the meat shops | 2 | 33.33% |
| | | Do not handle | 1 | 16.67% |
| | | Selling with hide | 3 | 50% |
| 4 | Selling the feet and shanks of the slaughtered animal | Selling alongside of meat | 0 | 0% |
| | | Selling far from meat | 6 | 100% |
| | | Do not handle | 0 | 0% |
| 5 | Using apron during procedure | Yes | 2 | 33.33% |
| | | No | 4 | 66.67% |
| 6 | Selling the viscera | Remain attached with meat | 1 | 16.67% |
| | | Outside the slaughter house | 5 | 83.33% |
| | | Do not handle | 0 | 0% |
| 7 | Controlling flies in your slaughtering area | Chemically | 3 | 50% |
| | | Manually | 3 | 50% |
| | | Do nothing | 0 | 0% |
| 8 | Cleaning the slaughtered house on a week | Seven times | 0 | 0% |
| | | Two - four times | 4 | 66.67% |
| | | One time | 2 | 33.33% |
| | | Zero | 0 | 0% |
| 9 | Frequently used materials while cleaning | Water | 2 | 33.33% |
| | | Soap/ Detergent powder | 3 | 50% |
| | | Cloth | 1 | 16.67% |
| 10 | Handling leftovers | Refrigeration | 3 | 50% |
| | | Selling next day | 1 | 16.67% |
| | | Left as it is | 1 | 16.67% |
| | | Dispose off | 1 | 16.67% |
| 11 | Cleaning the chopping block | Scrapping | 3 | 50% |
| | | By water | 3 | 50% |
| | | Do not clean | 0 | 0% |
| 12 | Cleaning of equipment used for processing | Yes | 4 | 66.67% |
| | | No | 2 | 33.33% |
| 13 | Slaughtering animal examined before killing | Yes | 6 | 100% |
| | | No | 0 | 0% |
| 14 | Sanitary condition in the slaughter house | Well cleaned | 2 | 33.33% |
| | | Dirty | 2 | 33.33% |
| | | Satisfactory | 2 | 33.33% |
| 15 | Cold stores in the slaughtered house | Yes | 6 | 100% |
| | | No | 0 | 0% |
| 16 | Soaps and wash basins in the slaughtered area | Yes | 6 | 100% |
| | | No | 0 | 0% |
| 17 | Toilet near the slaughtering area | Yes | 4 | 66.67% |
| | | No | 2 | 33.33% |
| 18 | Source from where water is used | Tap water/ tank | 3 | 50% |
| | | River | 3 | 50% |
| 19 | Transportation of meat from slaughtering house | Motorcycle | 2 | 33.33% |
| | | Bicycle | 1 | 16.67% |
| | | Zeep | 2 | 33.33% |
| | | Carrying on foot | 1 | 16.67% |
| 20 | Separate knives used for meat and intestines | Yes | 5 | 83.33% |
| | | No | 1 | 16.67% |
| 21 | Opinion in "Meat is a source of disease for human being" | Yes | 4 | 66.67% |
| | | No | 1 | 16.67% |
| | | No idea | 1 | 16.66% |
| 22 | Familiar with the slaughterhouse and meat inspection act | Yes | 3 | 50% |
| | | No | 3 | 50% |
| 23 | Rating the meat you are selling | Well | 3 | 50% |
| | | Satisfactory | 2 | 33.33% |
| | | Not good | 1 | 16.67% |

Source: (Adhikari, 2019)

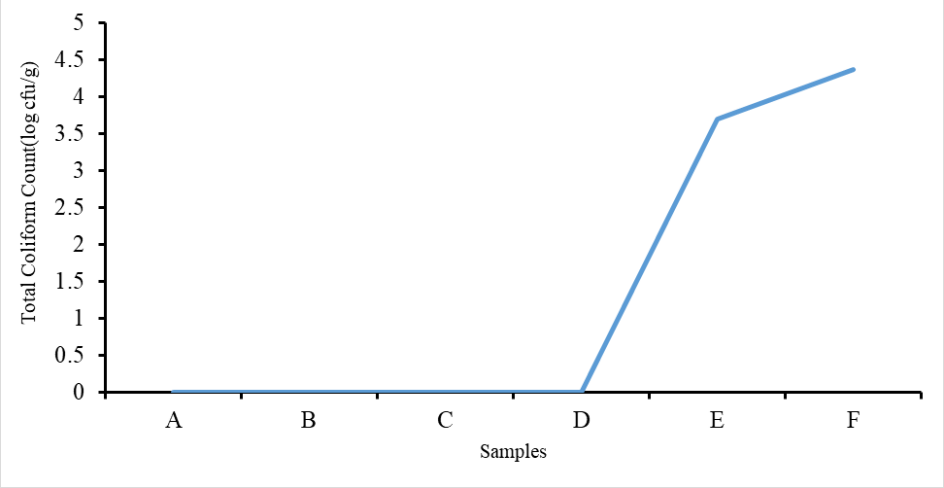


**Figure 2:** Total coliform count of chicken meat

### *4.4 Salmonella Spp.* And *S. Aureus* in Chicken Meat

The principal source of *Salmonella* spp. contaminating broiler carcasses including the hands of workers, clothes, wiping clothes, tools of workers, knives, skin, eviscerating neck, etc. (Hossain et al., 2015). According to our study, 33.33% of samples were found to be contaminated with *S. aureus* whereas 16.67% were found to be contaminated with *Salmonella* spp. These organisms were identified using biochemical tests.

Only one sample was found to contain *Salmonella* spp. i.e. 16.67%. The distribution of *Salmonella* spp has been found to vary in several types of research has shown 345 in poultry meat. Similarly, within the Chitwan region variation has been observed as has shown as 46.2%, has shown 12%, has shown 26.1%, and 47.8% in Chitwan (Bantawa et al., 2018; Bhandari et al., 2013; Dhakal and Manandhar, 2005; Acharya, 2007). This indifference is due to the difference in time and season of research. *Staphylococcus aureus* was detected in 2 (33.33%) of the samples which is less than that of who showed 68% in poultry meat of Dharan (Bantawa et al., 2018; Tiwari, 2002). The presence of *Staphylococcus aureus* in meat samples indicates unsatisfactory handling, cleaning and post-processing contamination from the polluted atmosphere in the slaughtered house. Their presence in raw meat and handlers contains hazards like toxin-mediated virulence and invasiveness to consumers (Kadariya et al., 2014).

## 5. Conclusion

Hygiene quality of chicken meat marketed in Pokhara from slaughtered house was assessed by questionnaire survey on slaughter house and butchers and also from the assessment of microbial load. Randomly chosen six places out of twelve were used to take meat samples. All those chicken meat samples were found to contain higher microbial load of *total plate count* due to poor sanitary condition and slaughtering premises than prescribed standards of Europe and United States. Presence of *total coliforms* indicated that the meat might be contaminated by the visceral content. Two meat samples were found to contain *Salmonella* out of six different samples. This survey indicates the degree of contamination of microbial load depends upon the unhygienic and unscientific method of handling, lack of sanitation and knowledge of microorganisms which needs to be improved.

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