

Prevalence of Coliform in Frozen Poultry Meat Marketed at Kathmandu Valley

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Abstract

Microbiological analysis of poultry meat was carried in Kathmandu valley during September to November 2018 with an objective of finding out Total Coliform Count (TCC) in the frozen meat samples collected from different meat shops of the valley. Out of 50 samples 11 (22%) samples were found to be positive for coliform. The mean value of TCC in the frozen meat were found to be 6.82 log CFU/g. The prevalence of Coliform indicates the unhygienic handling of meat and risk to the consumers.

Keywords: Frozen Poultry Meat; Total Coliform Count; ISO Standard

Introduction

Nepal is predominantly an agricultural country where 65.5% people depend on agriculture for their livelihood. Among them 65.6% are male and 72.8% are female [3]. The total population of animals contributing to the meat production is comprised of 5.1 million buffaloes, 0.8 million sheep, 10 million goats, 1.2 million pigs, 60.8 million poultry and 0.3 million ducks [3]. Nepal is mainly based on small holder subsistence production system except for some recently developed units of commercial poultry and pigs. Meat and meat products originating from all domestic farm animals except cattle are consumed in Nepal. Animal slaughter is a common practice not only for consumption but also religious sacrifice and other traditional ceremonies. Among the farm livestock reared by farmers goats, pigs and broilers poultry are reared specifically as meat animals whereas buffaloes, sheep, rural poultry and layer birds are reared for other outputs also contribute to meat supply as an additional output [10].

Meat and meat products contribute significantly to high incidence of food borne diseases and zoonotic diseases. Microbes in meat and meat products are the main cause of diseases in humans [12]. Meat serves as an excellent medium for the growth of microorganisms. The microorganisms responsible for lowering the sanitary quality of meat are mainly derived from external environment. The pathogenic bacteria like *Escherichia coli*, *Clostridium* spp., *Salmonella* spp., *Staphylococcus* spp., *Campylobacter* spp., etc. not only spoiled meat but also cause food poisoning and other illness to consumers. Measures should be adopted in such a way that these organisms should not be present in meat and meat products [1].

In Nepal lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses in meat which is aggravated by its slaughtering places, and which are frequently polluted with street dust, garbage, human excreta, animal blood, intestinal contents and dirty effluents [9]. The condition of animal handling, meat production and marketing are primitive. Pre-slaughter handling is cruel, antemortem and postmortem inspections are not practiced. The slaughtering condition is far from satisfactory. Meat market is filthy and totally unhygienic. Adulteration of meat is a common practice. Thus, the consumers are deprived of clean and wholesome meat [14].

Fresh meat butcher shops are the major retail outlets for meat in Nepal. However, the trending of purchasing from cold stores which trade in frozen meat and meat products has been rising in these days. Main contamination of meat is through unclean water used while washing, transporting and slaughtering. Due to the insufficient supply of clean water in Kathmandu, Butchers frequently use untreated ground water for meat cleaning and processing. Besides, these unsatisfactory slaughtering techniques, environmental pollution, unhygienic way of transportation of slaughtered meat, slaughtering of unhealthy and parasite infected animals are other equally important sources of contamination [12].

Coliforms are gram negative, asporogenous rods that ferment lactose within 48 hours and produce dark colonies with metallic sheen on endo type agar. Coliforms are represented by four genera of family Enterobacteriaceae namely *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella*. Coliforms are capable of growth in the presence of bile salts which inhibit the growth of gram-positive bacteria [7].

E. coli infection or Colibacillosis is the most common, the most troublesome and economically the most important bacterial disease of poultry. It occurs throughout the year and is difficult to deal with. Bacteria of the species *Escherichia coli* normally live in the digestive tract of poultry and most strains do not produce disease [8]. Certain strains can cause disease in poultry. These diseases include Colisep-ticaemia (blood infection) and yolk sac infection in young chickens; egg peritonitis (-inflammation of the peritoneum) and Coligranuloma in adult chickens [8].

E. coli is also involved in number of other conditions. These include synovitis (inflammation of the synovial membrane), arthritis (inflammation of the joint), osteomyelitis (inflammation of the bone marrow), salpingitis (inflammation of the oviduct), panophthalmitis (inflammation of the eye) and localized abscesses. All these conditions are collectively referred to as Colibacillosis. *E. coli* produces several toxins in human body. These include enterotoxins, cytotoxins, hemolysins and haemagglutinins and several disease producing virulence factors such as adhesins [8].

Joshi., *et al.* [13] conducted a microbiological analysis of poultry meat. 15 meat samples from different meat shops of Kathmandu valley were taken for coliform count. Among the samples only 20% were within the ISO standard. The average coliform count was found to be 11.48 log cfu/g. Bhandari [2] conducted a qualitative and quantitative bacteriological study in goat meat of Kathmandu valley and out of 101 samples 66.33% were found to be positive for coliform while in a study conducted by Mukhopadhyay., *et al.* [11] on, fresh chicken samples collected from retail outlets in and around Pondicherry were analyzed for coliform count and the mean coliform count was found to be 5.85 log₁₀ cfu/g.

In a study conducted by Dams in Jordan [5] in imported frozen broiler meat, a total of 100 consecutive lots of imported frozen broilers received at the laboratories of Jordan Food and Drug Administration. The total coliform count varied between < 10 to > 10⁵ cfu/g.

In a study conducted by Hassani., *et al.* [6] at government hospital Kalyobia governorate Egypt, a total of 100 samples were taken from chicken breast and thigh (50 of each), randomly to evaluate average coliform count in frozen meat, the average coliform count varied from 2.07 × 10³ ± 0.60 × 10³ cfu/g for breast and 2.61 × 10³ ± 0.60 × 10³ cfu/g for thigh.

Objectives of the Study:

- To count the total coliforms in the frozen poultry meat
- To assess the microbial quality of meat marketed at Kathmandu valley.

Materials and Methods

Sample size: A total of about 50 meat samples were collected from different meat shops of Kathmandu valley.

Study site: The frozen meat samples were collected from different retail meat shops of Tokha, Phutung and Gongabu areas of Kathmandu valley randomly.

Study period: The study was conducted from September 2018 to November 2018.

Laboratory processing: About 25 gram of meat sample from the frozen poultry meat were collected in zip lock bags which were kept in ice box and transported to the Central Veterinary Laboratory Tripureshwor. The culture of bacteria was done by Spread plate method as described by Loeffler and Gaffky.

Procedure of spread plate method

The sample was diluted to 10 folds. 0.1 ml suspension was poured in the desired agar plate and spread using shape loop. It was incubated at 37°C for 24 to 72 hours according to the nature of bacteria. Count the bacterial colonies:

$$\text{No. of viable cells per ml} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Volume of incorporate sample}}$$

Regarding microbial standard of meat: Till date there is not any universally accepted standard for the microbial quality of meat but most of the organizations like EU, WHO and ISO have established their own standards for qualitative assessment of the meat from microbial point of view. Likewise, Nepalese National Microbial Standards of meat are given in the table 1.

Meat Sample	Microorganism	Limits		Analytical Reference Method
		m	M	
Chilled/Frozen meat	Coliform	$\leq 5 \times 10^2$	$\leq 5 \times 10^3$	ISO 16649-1 and 2
Fresh meat	Coliform	$\leq 10^2$	$\leq 10^3$	ISO 21528-2

Table 1: Recommended microbial standards for meat and meat products [4].

The interpretation of the obtained values was compared and analyzed as follow:

- Satisfactory, if all the values observed are $\leq m$ (minimum)
- Acceptable if maximum values are between m and M (Maximum)
- Unsatisfactory, if one or more of the values are $> M$.

Results and Discussion

Altogether 50 poultry meat samples were collected from different meat shops of Kathmandu valley. Out of 50 samples 22% were found to be positive for TCC and 78% were found negative. The mean value of TCC was found to be 6.82 log CfU/g. The result so obtained were compared to the ISO standard where it was found even higher than the maximum limit. The graphical representation of the same is given in the figure 1 and 2.

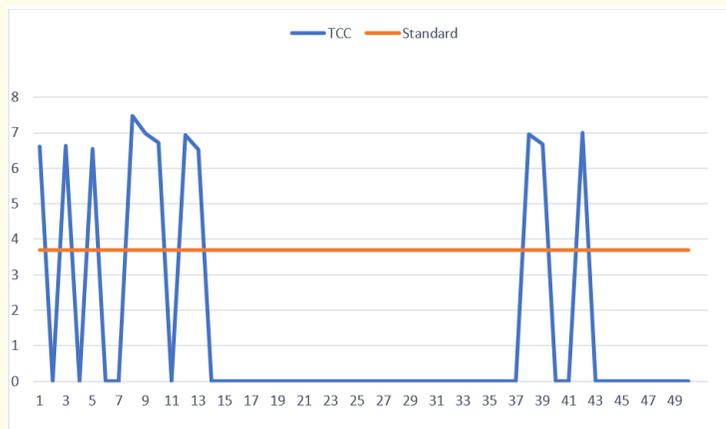


Figure 1: Comparison of TCC with ISO standard.

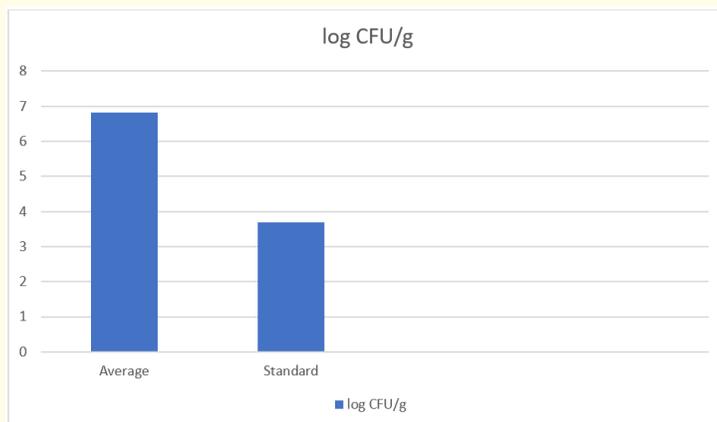


Figure 2: Comparison of average coliform count with the ISO standard.

The total coliform count varied from < 1 to 7.47 log CFU/g. The mean value of Coliform was found to be 6.82 log cfu/g i.e. 8.2×10^6 CFU/g which is more than the standard prescribed by ISO. The finding was found to be higher than the result given by Bhandari [2] i.e. 3.50×10^5 cfu/g and Mukhopadhyay, *et al.* [11] i.e. 5.85×10^2 cfu/g. But this finding was lower than that found by Joshi, *et al.* [13] i.e. 11.48

log cfu/g. This level of coliform contamination revealed that there might be chances of fecal contamination either from animal itself or human sources. Contaminated water might be another risk factor.

Contamination of meat by equipment's begins right from the time of slaughter. During slaughtering, the animal encounters various equipment surfaces. The microflora on the outer surface can easily be transferred to the cut surface by knives. Therefore, the unclean utensils, knives and other equipment's are highly responsible for the poor quality of the meat.

In Nepal lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses in meat as well as its slaughtering places which are frequently polluted with street dust, garbage, human excreta, animal blood, intestinal contents and dirty effluents and which are not protected against dogs, rodents and insects. Meat and meat products under such conditions are generally of poor quality due to bacterial infections. Besides, meat quality is adversely affected by careless handling conditions in the slaughtering places as well as in the meat markets or shops [9]. Such factors also might have influenced in this study also. So microbial safety can only be guaranteed when all overall processing and handling are duly considered.

Conclusions and Suggestions

Out of 50 poultry samples from different meat shops of Kathmandu valley 22% were found positive for Coliform. Based on this study, it can be concluded that the sources of contamination might be due to contaminated water used for dressing and washing of carcass, faeces of poultry, environment or the unhygienic practices of the butcher itself for the materials or equipment's to be used in the slaughter slabs.

Improved hygienic standard must be maintained in the poultry meat production. Meat quality should be increased by improving meat shops sanitation and personal hygiene of the workers. Water used for cleaning and washing of carcass should be treated, proper disposal of sewage and wastage of slaughterhouse and meat shops should be done.

Limitations of the Study

This study has focused only on certain microbiological standards. This study has not isolated and identified all possible microbes in meat. As the sample size of this study was very small the results of this study are expected to be verified with a large number of samples with reference to confirmatory diagnosis by cultural examination and biochemical tests as practiced worldwide although serological tests, PCR and other advanced tests would have been specific for species identification.

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