

Isolation and Identification of Gram Negative Bacteria from Chicken Meat collected from Retail Meat Shops of Kathmandu Valley

B. Koirala^{1,*}, R. Bhattarai¹, R. Maharjan¹, S. Maharjan¹ and S. Shrestha¹

¹Department of Microbiology, Tri Chandra College, Kathmandu

*Corresponding author: Barsha Koirala, varshakoirala@gmail.com

ABSTRACT

Various health related issues may arise due to the microbial contamination in meat that we consume. Due to lack of proper sanitation, tools and other resources, meat usually gets contaminated with the microorganisms that are capable of causing serious illness to humans. A study was conducted to isolate and identify Gram negative bacteria in chicken meat samples collected during rainy season from different sites of Kathmandu valley. Altogether 40 samples were collected and assessed. The Total Plate Count (TPC) and Coliform Count was determined and found to be highest from Asan area i.e. 9.2 ± 0.04 log cfu/gm and 7.7 ± 0.03 log cfu/gm respectively. The further isolation was done to compare the various biochemical tests of each isolates. Altogether 40 samples were collected and assessed. Altogether ten genera of Gram negative bacteria were isolated. The isolated gram negative microorganisms were *Proteus spp* (32%,22/68), *Pseudomonas* (15%,10/68) *Citrobacter spp.* (12%,8/68), *E. coli* (13%,9/68), *Serratia marcescens* (10%,7/68), *Salmonella spp.* (7%,5/68), *Enterobacter spp.* (4%,3/68), *Morganella morganii* (3%,2/68), *Klebsiella* (2%,1/68), *Shigella* (2%,1/68). *Proteus spp* was found to be predominant. From this research, it was concluded that meat is prone to contamination. Therefore, they need to be cleaned, and cooked properly before consumption.

Keywords: Bacteria, Biochemical tests, Isolation, Meat

INTRODUCTION

Meat and meat products are important source of selenium, phosphorus, niacin, vitamins(B₆ and B₁₂), choline, riboflavin, zinc and iron along with vitamin k (Schurgers and Vermeer, 2000; Lawrie, 2006). The most common sources of meat are domesticated animal species such as cattle, pigs and poultry and to a lesser extent buffaloes, sheep and goats (Bertol, 2004). Meat is an ideal culture medium for many organisms because it has high moisture content, source of nitrogenous food, various minerals and growth factors. It contains fermentable carbohydrates which act as a harbor for most microorganisms. The inner flesh of meat is usually sterile or few numbers of microorganisms can survive there (Aruno *et. al.*, 2007).

Chicken meat refers to whole carcasses or part of carcasses or boned out meat of *Gallus gallus*. The consumption of white meat is increasing day by day because of its popularity, low price, easy access with fast digestion, low religious taboo, tasty, and low calorie food. Therefore, the high quality of poultry meat should be maintain as they are consumed more. Majority of the spoilage microorganisms comes from external sources during unhygienic bleeding, handling, processing,

exterior part of chicken, tools and equipment, water, the area around the slaughtering, mode of transportation, hands and aprons of the meat handlers also play great role for the contamination (Sheridan, 1998; Bertol, 2004). Bacteria of many genera are found in meat, among which some of the more important are *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Micrococcus*, *Streptococcus*, *Sarcina*, *Proteus*, *Flavobacterium*, *Escherichia coli*, *Campylobacter*, *Salmonella*, *Shigella*, *Enterobacter*, and *Morganella etc.* Those bacteria including Coliforms can cause various food related infections and diseases in human (Williams *et. al.*, 2010).

As chicken meat is a diet rich in nutrition, it is also a better place for organisms and various organisms reside and grows. Also, bacteria may enter in meat at the time of slaughtering, washing or during storage. Mainly in the rainy season, there is high chance of contamination of meat because of the low sanitation, water pollution, soil pollution and environmental pollution. Meat is eaten by lots of people so the maintenance of quality in the meat is in the priority of us. So we must be careful regarding its quality. Therefore, the study will help to know the quality of meat from different parts of Kathmandu Valley.

MATERIALS AND METHODS

Sampling site and sample size

A total of 40 raw chicken meat samples were collected (purposive sampling method) from different local markets of Kathmandu valley that consists of Kathmandu, Bhaktapur and Lalitpur districts. The research was conducted on Research Laboratory for Biochemistry and Biotechnology (RLABB), Sitapaila, Kathmandu. Meat samples were collected in clean polyethene bags in the morning hours as the public were offered for meat sale at different retail meat shops located in Kathmandu valley. The samples collected were then transported to RLABB maintaining aseptic conditions, and then processed immediately. The laboratory analysis steps included all the works related with the enumeration, isolation, and identification of the isolates.

Total Plate Count (TPC) and Coliform Count (CC)

25 gm of raw meat sample was aseptically transferred into blender and blended with 225 ml of sterilized buffer peptone water (BPW) at 15000 to 20000 rpm for 2 minutes and was successively diluted to the required dilutions. Pour plating was done on two different agar mediums i.e. Plate Count Agar (PCA) for Total Plate Count and Violet Red Bile Agar (VRBA) for Coliform Count. For pour plate, 1ml of meat homogenate sample from appropriate dilutions were transferred to sterile petridish to which molten, cooled medium (PCA and VRBA) about 18-20 ml was poured and mixed gently. The petridish were then incubated at 37°C for 24 hours. Total count of organisms was performed from plate count agar (PCA) and coliform count was performed using violet red bile agar (VRBA) media, both of which were incubated at 37°C for 24 hours (Sharma and Chattopadhyay *et.al.*, 2015).

10 ml of meat homogenate sample from buffered peptone water (BPW) was enriched into 2 sterile conical flasks, each containing 90 ml of alkaline peptone water (APW) and selenite F broth (SFB). The tubes were then incubated at 37°C for 24 hours (Sharma and Chattopadhyay *et. al.*, 2015).

Isolation and identification of bacteria

A loopfull of sample from APW and SFB was streaked on Thiosulfate-citrate-bile salts- sucrose (TCBS) and Xylose Lysine Deoxycholate (XLD) agars respectively with the help of sterile inoculating loop. The petri dishes were incubated at 37°C for 24 hours. The isolated colonies from TCBS and XLD were then again inoculated on Nutrient Broth (NB) for about 4 hrs and then to Nutrient Agar (NA) and MacConkey Agar (MA) plates and further incubated at 37°C for 24 hours (Frazier and Westhoff, 1988). The bacteria were then identified using microbiological techniques as described in Bergey's manual comprising of colony morphology, Gram's staining and biochemical properties. Gram's staining was performed for the presumptive identification of bacteria according to the standard technique. The only gram negative cultures were used for the further identification. Catalase test, Oxidase test, Indole production test, Methyl red (MR) test, Voges-Proskauer (VP) test, Motility test, Citrate utilization test, Triple sugar iron (TSI) Agar test, Urea Hydrolysis test, Oxidation-Fermentation test were performed for the final identification of gram negative bacteria (Happy *et.al.*, 2018 ; Shrestha, 2009).

Data Analysis

The results were carried out in triplicates and expressed as mean±S.D. using MS Excel 13. The one way Anova was performed and the p- value was observed to be statistically significant ($p < 0.05$).

RESULTS

In the present study, altogether 40 meat samples (chicken) were collected from different places of Kathmandu valley for isolation and identification of gram negative microorganisms.

Table 1: Total Count and Coliform Count in meat

Name of districts and local area		Total samples collected	Chicken meat microbial load (log cfu/gm)	
			TPC	CC
Kathmandu	1.Asan	15	9.2±0.04	7.7±0.03
	3.New buspark		7.5±0.08	4.3±0.16
	4. Kalanki area		6.8±0.00	4.9±0.00
Bhaktapur	1. Bhaktapur Durbar square	10	8.6±0.02	6.1±0.08
	2. Old thimi area		8.4±0.08	6.2±0.00
Lalitpur	1.Patan durbar square	15	8.5±0.01	6.3±0.03
	2. Godawari area		6.1±0.03	4.3±0.09
	3. Kirtipur		8.1±0.00	3.03±0.02

All the values were expressed as mean ± standard deviation and found to be statistically significant ($p < 0.05$)

The Total Plate Count (TPC) for chicken was lowest count from Godawari area (6.1±0.03 log cfu/gm) and highest from Asan area (9.2±0.04 log cfu/gm). Likewise, the Coliform Count (CC)

was lowest count from Kirtipur (3.03 ± 0.02 log cfu/gm) and highest was from Asan (7.7 ± 0.03 log cfu/gm).

Table 2: Biochemical properties of isolated organisms

Ox	Cit	Mot	Ind	Urea	Slope	Butt	H ₂ S	Gas	Species
-	-	+	+	-	Y	Y	-	+	<i>E. coli</i>
-	-	-	+	-	R	Y	-	-	<i>Shigella</i> spp.
-	-	+	-	-	R	Y	+	-	<i>S. Typhi</i>
-	+	+	-	+	R	Y	+	+	<i>Citrobacter</i> spp.
-	+	-	-	+	Y	Y	-	+	<i>Klebsiella</i> spp.
-	+	+	-	-	Y	Y	-	+	<i>Enterobacter</i> spp.
-	+	+	+	+	R	Y	+	+	<i>Proteus</i> spp
-	-	+	+	+	R	Y	-	+	<i>Morganella morganii</i>
+	+	+	-	+	R	R	-	-	<i>Pseudomonas aeruginosa</i>
-	+	+	-	+	R	Y	-	-	<i>Serratia marcescens</i>

Key: Ox=oxidase test, Cit=Citrate test, Mot=Motility, Ind=Indole test, H₂S=Hydrogen sulphide, R=Red-Pink (alkaline reaction), Y=Yellow (acid reaction),

This research focuses on isolation and identification of gram negative bacteria. Therefore, the isolates with gram staining test negative were used for further identification. Total 68 gram negative bacterial isolates were obtained. Of these, predominant Gram negative microorganism was found to be *Proteus* spp (32%), *Pseudomonas* (15%) *Citrobacter* spp. (12%), *E. coli* (13%), *Serratia marcescens* (10%), *Salmonella* spp. (7%), *Enterobacter* spp. (4%), *Morganella morganii* (3%), *Klebsiella* (2%), *Shigella* (2%) was isolated (Figure 1).

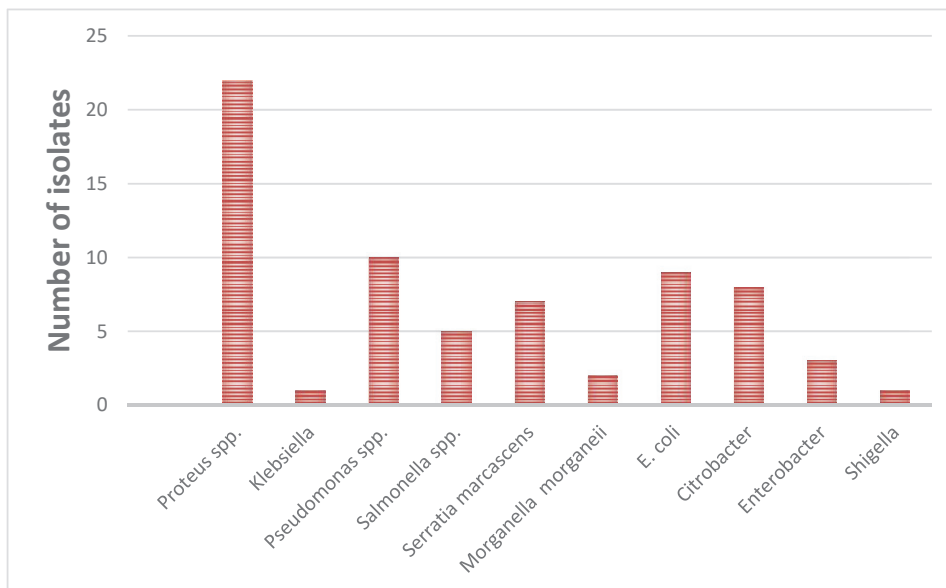


Figure 1: A bar diagram representing gram negative bacteria isolated from chicken meat

DISCUSSION

The TPC and CC was highest in the meat samples of Asan area i.e. 9.2 ± 0.04 log cfu/gm and 7.7 ± 0.03 log cfu/gm respectively. This shows that the hygiene practices were worst in Asan area compared to other areas. Therefore, the meat vendors need to be careful regarding the sanitation and hygiene. The slaughterhouse, the knives used for cutting chicken, the water, the feed for chicken, etc. should be clean and good.

A survey conducted on broiler chicken meat in different places of Chitwan i.e. Bharatpur, Ratnanagar and Institute of Agriculture and Animal Science (IAAS) vicinity were obtained as 11.1 ± 0.3 , 11.5 ± 0.3 and 12.2 ± 0.5 log cfu/gm for total plate count and 6.5 ± 0.3 , 7.6 ± 0.3 and 8.4 ± 0.5 log cfu/gm for coliform count respectively. Both total plate count and coliform count was higher compared to the result obtained by our study. Likewise, the coliform count from our study was closer to that of 5.12 log cfu /gm (Darshana et al 2014) and 5.85 log cfu /gm (Mukhopadhyay et al (2004). The TPC estimated by our research was much higher than that of TPC reported by Prasai (2000) and Poudel (1999). Prasai (2000) reported as high as 5.39 to 6.5 log cfu /gm and Poudel (1999) reported upto 7.13 to 7.7 cfu/gm. Likewise, 3.88 to 4.71 log cfu /gm and 4.69 to 6.9 log cfu /gm TPC were reported by Karki (1995) and Zobegow (2008) in chicken meat respectively.

The bacterial load may be due to contaminated feed, water used for washing the chicken and the other parameters like slaughtering houses, vehicles used for the transportation and food provided to chicken might be more contaminated and unhygienic. Bone crushers are provided to chicken which is also a reason for the high load of bacteria in chicken. A cross-sectional study of raw meat samples from the local meat market of Kathmandu, *Salmonella* spp was found in 11.4% of meat samples (Joshi *et. al.*, 2006). Likewise, Ahmed MUD *et. al.*, (2013) in his study reported that *E.*

coli was isolated from 9(45%) samples, and *Salmonella* from 7(25%) samples. The *Salmonella* may be present due to inappropriate method of storage of meat, poor handling, transportation etc. (Aftab *et. al.*, 2012; Anihouvi *et.al.* 2013).

Saika and Joshi, (2010) isolated *E.coli* by 98% from raw chicken meat samples and Odwar *et. al.*, (2014) reported 78% of the collected samples were contaminated with *E.coli*. The presence of Coliform and *E.coli* in chicken and meat product is the indication of defective techniques that is being used during preparation, handling, processing and temperature of holding, water. (Vanderlinde *et.al.*, 1998). Coliforms are present in gut of warm blooded animals and are indicator for faecal pollution and unsanitary condition of meat and meat product and their presence is the indication of enteropathogenic and toxigenic microorganisms which constitute public health hazard and economic loss (Morshidy and Roushdy, 1983). In the study conducted by Sharma and Chattopadhyay *et.al.*, (2015) the pathogenic microorganisms isolated were *E. coli*, *Staphylococcus aureus*, *Pseudomonas* spp., *Salmonella* spp., *Klebsiella pneumoniae*, *Enterococcus*, *Citrobacter* spp., *Proteus* spp. Which was similar to this study done.

CONCLUSION

The Total Plate Count and Coliform Count were done along with the isolation and identification of gram negative bacteria. All together 10 genera of Gram negative bacteria (*Salmonella*, *Escherichia*, *Shigella*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Citrobacter*, *Serratia*, *Enterobacter*, *Morganella*) were isolated, and identified. From the result of this study, it has been concluded that the meat of various places of Kathmandu valley was contaminated with health hazardous pathogens and the meat from such places was not of good quality. Meat should be cooked properly and adequately before consumption. Also the regular monitoring of quality of meat and meat market is necessary to prevent the outbreak of food borne illness caused by them as well as to prevent large economic crisis.

ACKNOWLEDGEMENTS

We would like to acknowledge our supervisor, Lecturer Mrs. Shova Shrestha and Ms. Rama Khadka for their continuous guidance, support and supervision during this research work. We are grateful to our Head of Department, teachers and staff members of the Microbiology Department for their support and help.

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