

Bacterial Assessment of Buffalo Meat in Kathmandu Valley

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ABSTRACT

Meat is highly nutritious and mostly consumed food. Usually, meat gets contaminated because of improper handling, open transportation practice, unhygienic cutting tools, and can cause various food-borne illnesses. This study was focused to determine bacterial load and occurrences of potential pathogenic bacteria in the raw buffalo meat sold in Kathmandu valley. Altogether 40 raw buffalo meat samples were collected and assessed. In addition, antibiotic susceptibility of gram-negative bacterial isolates was tested. The total plate count (TPC), and coliform count (CC) in the samples were found comparatively highest from the Asan area of Kathmandu with 9.6 ± 0.02 log CFU/gm and 8.9 ± 0.00 log CFU/gm respectively. Altogether 10 genera of gram-negative bacteria were identified based on the various biochemical tests. The isolated gram-negative bacteria included *Proteus* spp. (39%, 21/54), *Pseudomonas* (19%, 10/54) *Citrobacter* spp. (9%, 5/54), *E. coli* (7%, 4/54), *Serratia marcescens* (5%, 3/54), *Salmonella* spp. (9%, 5/54), *Enterobacter* spp. (2%, 1/54), *Morganella morganii* (2%, 1/54), *Klebsiella* (2%, 1/54), *Yersinia enterocolitica* (6%, 3/54). The antibiotic susceptibility tests for the isolates was carried against six different antibiotics including-Ofloxacin (OF), Ciprofloxacin (CIP), Nalidixic Acid (NA), Nitrofurantoin (NIT 300), Amoxycillin (AMX) and Chloramphenicol (C). Out of total 54 isolates, 36 were found to be multidrug-resistant. The results of this study clearly revealed buffalo meat sold in Kathmandu valley was contaminated with potential bacterial pathogens which may cause various food-borne illnesses. The occurrence of multi-drug resistant bacteria in the meat samples is alarming threat to public health. Adequate measures to protect bacterial contamination in buffalo meat is highly recommended.

Keywords: Buff meat, gram-negative bacteria, AST

1. INTRODUCTION

Since ancient times, people domesticate animals like chicken, sheep, pig, buffalo, goat, and consume them as meat (Lawrie *et al.* 2006). Meat is nutritionally rich food composed of water, protein and amino acids, minerals, fats, fatty acids, vitamins, bioactive components, and carbohydrates (Charlton *et al.* 2008). Because of these nutritional varieties in meat, it is one of the better mediums for microbial contamination. Enterobacteriaceae are a large family of gram-negative bacteria that includes *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella*, *Shigella*, *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter*. They are also called enterobacteria or enteric bacteria, as various members of this family are the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on

a variety of different animals and plants (Williams *et al.* 2010). Food-borne infection is caused by the ingestion of food contaminated with microorganisms and the reaction of the body to their presence or their metabolites. Examples include Salmonellosis, Cholera. Likewise, Food intoxication is caused by ingesting food containing preformed toxins. Examples include–Staphylococcal Intoxication (Enterotoxin), botulism (neurotoxin) (Gwida *et al.* 2014; Frazier & Westhoff 1988). The nature and level of microbial contamination in meat have a necessary consequence on public health, storage life, and the type of spoilage of meat. The rearing, slaughtering, and processing condition of meat are essential factors for determining the microbial contamination in meat (Brown & Parker 1982).

Meat is a shelter for many organisms because of its moisture content, nutritious values, and various growth factors. The microorganism can contaminate meat from external sources like unhygienic bleeding, improper handling and processing, exterior parts of animals- like skin, hairs, soil, water, feed and manure, natural surface flora. Considering poor hygiene and other factors, we hypothesize that raw Buffalo meat might be contaminated with potential pathogenic gram-negative bacteria. Since the Buffalo meat consumption is higher in Kathmandu, the maintenance of its quality should be the priority. This study is carried out to understand the occurrence of microbial potential pathogens which may indicate possible incidence of food-borne illnesses. The study also describes the antibiotic susceptibility test pattern of the contaminants along with frequency of multiple antibiotic resistant bacteria. The study provides information on bacteriological quality of buffalo meat from different parts of Kathmandu Valley.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Total 40 raw meat samples (flesh) of Buffalo, each weighing 25 grams were collected from local markets of Kathmandu valley (Asan, Gongabu, Kalanki, Kirtipur, Godawari, Patan, Bhaktapur city & Thimi) in the month of August. The samples were collected in sterile Zip lock polyethylene bags in the morning hours and transported to the laboratory for analysis and processed immediately. The microbiological assessment of the meat samples was conducted at Research Laboratory for Biochemistry and Biotechnology (RLABB), Kathmandu.

2.2 Laboratory Analysis

Total bacterial count and coliform count was determined by heterotrophic plate count and pour plate technique using plate count agar (PCA) and violet red bile salt agar

(VRBA) media respectively (Feng *et al.* 2002, Prathab and Lalitha 2012). Selected gram-negative bacteria were isolated on respective media and identified by standard microbiological techniques, and their antibiotic susceptibility Test (AST) was done by Kirby-Bauer disc diffusion method.

2.2.1 Heterotrophic Plate Count and Coliform Count

Total 225 ml of sterilized buffer peptone water (BPW) and 25 gm of raw meat sample were mixed at 80 rpm for 30 minutes and diluted to the required dilutions (USFDA 2012). For the pour plate, 1ml of meat homogenate from appropriate dilutions was transferred to sterile Petri dishes, on which molten media were poured (PCA & VRBA for heterotrophic and coliform count, respectively) and then mixed gently. The inoculated plates were incubated at 37°C for 24 hours. The isolated colonies on the plates were counted.

2.2.2 Isolation and Identification of Bacteria

Since gram negative-bacteria were targeted in this study, isolated selected colonies from VRBA media were purified to recover coliform bacteria. However, raw meat homogenate was enriched for 24 hours at 37°C in Selenite Broth (1:10) and alkaline peptone water (1:10) to isolate *Salmonella*, *Shigella* and *Vibrio* species (Shrestha *et al.* 2017). Further isolation of bacteria was done on Xylose Lysine Deoxycholate (XLD) agar and Thiosulfate-citrate-bile salts-sucrose (TCBS) agar incubating at 37°C for 24 hours (Da Silva *et al.* 2013). Typical colonies grown on the media were purified on Nutrient Agar (NA) and lactose fermenting bacteria were distinguished on MacConkey Agar (MA) (Frazier & Westhoff 1988; Sharma *et al.* 2015). The isolates were then identified using microbiological techniques as colony morphology, Gram's staining, and biochemical properties. Only gram-negative cultures were selected for further identification using catalase, oxidase, indole production, methyl red, Voges-Proskauer, motility, citrate utilization, triple sugar iron agar, urea hydrolysis and oxidation-fermentation tests (Happy *et al.* 2018; Shrestha 2009).

2.2.3 Antibiotic Susceptibility Test

Antibiotic susceptibility testing was performed by the Kirby Bauer disc diffusion method on Mueller-Hinton agar (MHA) as recommended by the Clinical Laboratory Standard Institute (CLSI 2020) guidelines. In this technique, a test organism inoculum in nutrient broth following 4 hours incubation at 37 °C was adjusted to the turbidity equivalent to 0.5 McFarland scale and carpet cultured on MHA. The antibiotic discs were laid over the

carpet culture on the media. After overnight incubation, the clear zone size around the disc was measured, and the result was interpreted according to the standard zone size interpretative chart (Frazier & Westhoff 1988) as CLSI (2020) guidelines. Antibiotics used in this study were Ciprofloxacin, Ofloxacin, Nalidixic Acid, Nitrofurantoin, Amoxicillin, and Chloramphenicol (Gautam *et al.* 2018) which were also purchased from Hi-media laboratory Pvt. Ltd. India.

2.2.4 Quality Control

The performance of laboratory equipment including incubator, refrigerator and autoclave was assessed periodically during the study period. Control was used for every media prepared to check the contamination during the media preparation (King & Brown 2001). Purity plate technique was applied to avoid any misleads due to contaminants. Performance of each media and antibiotic disc was maintained with parallel experiments using standard American type culture collection strains as per the recommendations.

2.2.5 Data Analysis

The results were carried out in triplicates and expressed as mean±S.D. The one-way ANOVA was performed and the *p*-value less than 0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Buffalo meat in Nepal is almost consumed without proper washing because of some religious taboos. Buffaloes are slaughtered at the slaughterhouse and transported to the different local vendors. The slaughterhouses of Nepal do not follow the hygienic process for meat processing. Meat is then transported using a rickshaw or small vehicles without proper covering responsible for the bacterial contamination compared to the Isolation obtained by the investigators from other parts of the world where hygienic practices are maintained during the meat processing (Adhikari *et al.* 2012). The main objective of this study was to understand the microbial quality of raw buffalo meat sold in Kathmandu Valley of Nepal.

In this study, the total plate count (TPC) and coliform count (CC) of 40 raw buff meat samples from Kathmandu valley were assessed and the results are presented in Table 1. As shown in the table 1, the average total plate count for raw buffalo meat was found to be somewhat similar to the findings of Ayten *et al.* (2014) and Baskaya *et al.* (2004)

who reported 6.36 log CFU/gm and 6.14 log CFU/gm, respectively. However, the results in this study were lower than the finding of the Adhikari *et al.* (2012) who reported 7.55 log CFU/gm, and higher than Kandeepan *et al.* (2011), Siddiqui and Harsojo, and Sari (2015) whose findings were 3.52, 3.58, and 5.87 log CFU/gm, respectively.

Various researchers from different parts of the world have enumerated coliform bacteria in meat samples and have reported in range of 4.15 - 5.09 log CFU/gm (Harsojo & Sari 2015; Ayten *et al.* 2014; Baskaya *et al.* 2004; Daly *et al.* 1976). In contrast to these reports, we found higher values of the average coliform count in raw buffalo meat samples (Table 1). These differences in total count might be because of the seasonal differences of the sample collection, practices of hygiene in the slaughterhouse, meat from the different body parts of buffalo and so on.

Table 1: Total Plate Count (TPC) and Coliform Count (CC) in buff meat

Name of districts and local area		Chicken meat microbial load (log cfu/gm)	
		Total Plate Count (TPC)	Coliform Count (CC)
Kathmandu	1.Asan	9.6±0.02	8.9±0.00
	2. Gongabu	8.2±0.09	7.2±0.15
	3. Kalanki	7.5±0.00	3.9±0.00
	4. Kirtipur	4.1±0.10	3.5±0.02
Bhaktapur	1.Bhaktapur city	8.2±0.00	8.1±0.09
	2. Thimi	7.3±0.01	7.2±0.43
Lalitpur	1.Patan	5.2±0.05	4.5±0.03
	2. Godawari	4.8±0.24	3.9±0.21

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

The presence of coliforms in meat products is the indication of improper techniques that are being used during preparation, handling, processing, and temperature of holding. The contamination may be from water too. Therefore, it is necessary to use good water quality to avoid contamination (Vanderlinde *et al.* 1998). Coli forms are present in the gut of warm-blooded animals. They are an indicator for fecal pollution and unsanitary condition of meat and meat products, and their presence indicates enteropathogenic and toxigenic microorganisms that constitute a public health hazard and economic loss (Morshidy & Roushdy 1983).

In this study, we isolated and identified ten different genera of gram-negative bacteria from raw buffalo meat samples. The identification was based on biochemical properties of the isolates (Table 2).

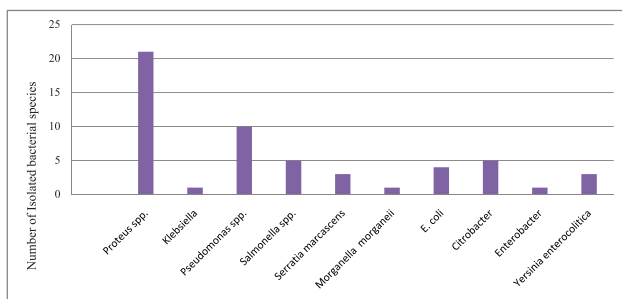
Table 2: Results for Biochemical tests for isolates

Ox	Cit	Mot	Ind	Urea	TSIA				Bacterial Species
					Slope	Butt	H ₂ S	Gas	
-	-	+	+	-	Y	Y	-	+	<i>Escherichia coli</i>
-	-	+	-	-	R	Y	+	-	<i>Salmonella Typhi</i>
-	+	+	-	+	R	Y	+	+	<i>Citrobacter spp.</i>
-	+	-	-	+	Y	Y	-	+	<i>Klebsiella spp.</i>
-	+	+	-	-	Y	Y	-	+	<i>Enterobacter spp.</i>
-	+	+	+	+	R	Y	+	+	<i>Proteus spp.</i>
-	-	+	+	+	R	Y	-	+	<i>Morganella morganii</i>
+	+	+	-	+	R	R	-	-	<i>Pseudomonas spp.</i>
-	+	+	-	+	R	Y	-	-	<i>Serratia marcescens</i>
-	-	+	+	+	R	Y	-	-	<i>Yersinia enterocolitica</i>

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

Besides coliform genera, the presence of *Salmonella Typhi*, *Yersinia enterocolitica* and other potential pathogens in the raw buffalo meat samples was found in this study. *Salmonella* is found anywhere in nature, including the digestive tracts of different animals, poultry products, milk products, and seafood. The nature and level of microbial contamination in meat have a necessary consequence on public health, storage life and the type of spoilage of meat. The rearing, slaughtering, and processing condition of the meat determines its microbiology. The extent to which the contamination occurs, and the flora's composition reflect the hygiene standard (Brown & Parker 1982).

The most frequent bacteria contaminating meat samples was *Proteus* species, followed by *Pseudomonas*, *Salmonella* and *Citrobacter* species (Figure 1).

**Fig. 1.** Total isolates of the organism from buff meat

The equal sample size was taken for the analysis from three different districts namely Kathmandu, Bhaktapur, and Lalitpur. However, the highest microbial count and micro-

organisms were isolated from the Kathmandu district (Table 3). This implies that compared to Bhaktapur and Lalitpur, meat vendors in Kathmandu are less conscious regarding sanitation and hygiene.

Table 3: Distribution of isolated organism in the districts (Kathmandu, Bhaktapur & Lalitpur) of Kathmandu valley

Name of bacteria	Distribution of isolated organisms in different districts		
	Kathmandu	Bhaktapur	Lalitpur
<i>Proteus spp.</i>	12	4	5
<i>Klebsiella</i>	1	-	-
<i>Pseudomonas spp.</i>	4	4	2
<i>Salmonella spp.</i>	1	2	2
<i>Serratia marcescens</i>	1	1	1
<i>Morganella morganii</i>	1	-	-
<i>E. coli</i>	2	1	1
<i>Citrobacter</i>	2	1	2
<i>Enterobacter</i>	1	-	-
<i>Yersinia enterocolitica</i>	1	-	2
Total	26	13	15

All the organisms isolated from both meat samples were subjected to antibiotic susceptibility test by using the Kirby-Bauer disc diffusion method using different antibiotic discs- Ofloxacin (OF), Ciprofloxacin (CIP), Nalidixic acid (NA), Nitrofurantoin (NIT 300), Amoxycillin (AMX) and Chloramphenicol (C). The isolates were differentiated as susceptible or resistant by measuring the inhibition zone concerning zone size interpretative chart (Table 4).

Table 4: Antibiotic susceptibility test of various isolates

Name of organism	No. of isolates	Frequency of resistant isolates (percentage) against different antibiotics					
		AMX	C	CIP	NA	NIT	OF
<i>Proteus</i> spp.	21	18(85.72)	16 (76.20)	11(52.39)	16(76.20)	16 (76.20)	14(66.67)
<i>Klebsiella</i>	1	1(100)	-	-	1(100)	1(100)	1(100)
<i>Pseudomonas</i> spp.	10	1(10)	5(50)	2(20)	2(20)	8(80)	7(70)
<i>Salmonella</i> spp.	5	3(60)	3(60)	-	3(60)	3(60)	5(20)
<i>Serratia marcescens</i>	3	-	1(33.33)	-	1(33.33)	1(33.33)	1(33.33)
<i>Morganella morganii</i>	1	1(100)	-	-		1(100)	-
<i>E. coli</i>	4	2(50)	-	-	3(75)	-	-
<i>Citrobacter</i>	5	3(60)	1(20)	-	-	-	-
<i>Enterobacter</i>	1	-	-	1(100)	1(100)	-	-
<i>Yersinia enterocolitica</i>	3	2(66.66)	3(100)	2(66.66)	2(66.66)	2(66.66)	1(33.33)

The figures in parentheses indicate the percentage

All the organisms isolated from both meat samples were subjected to antibiotic susceptibility test by using the Kirby-Bauer disc diffusion method using different antibiotic discs-Ofloxacin (OF), Ciprofloxacin (CIP), Nalidixicacid (NA), Nitrofurantoin (NIT300), Amoxicillin (AMX) and Chloromphenicol (C). The isolates were differentiated as susceptible or resistant by measuring the inhibition zone concerning zone size interpretative chart (Table 5).

Out of 54 isolates, 36 isolates of Gram-negative bacteria were found to be resistant to the multiple antibiotics (MDR) (Table 6). Total Multiple Drug-resistant bacteria can cause serious health problems directly or by transferring traits (Doyle & Ericson 2006). Those traits can be circulated to the environment through animal wastage. The MDR for the isolated microorganism from the raw buffalo meat might be due to the prolonged use or overdose/misuse of antibiotics in animal farms as growth promoters. The use of antibiotics can prevent it as directed and in a low dose. The animal should be given antibiotics only in the diseased condition, and Antibiotic-free policies for meat supplies needs to be made (Economou & Gousia 2015).

Table 5: Multiple Drug Resistant (MDR) pattern of isolated organisms

Bacteria	MDR		No MDR	
	Number of isolates	%	Number of isolates	%
<i>Proteus</i> spp.	16	76.19	5	23.81
<i>Klebsiella</i>	1	100	-	-
<i>Pseudomonas</i> spp.	7	70	3	30
<i>Salmonella</i> spp.	3	60	2	40
<i>Serratia marcescens</i>	1	100	2	-
<i>Morganella morganii</i>	1	100	-	-

<i>E. coli</i>	2	50	2	50
<i>Citrobacter</i>	2	40	3	60
<i>Enterobacter</i>	1	100	-	-
<i>Yersinia enterocolitica</i>	2	66.67	1	33.33
Total	36		18	

4. CONCLUSION

Altogether, 10 genera of Gram-negative bacteria (*Salmonella*, *Shigella*, *Escherichia coli*, *Proteus*, *Yersinia*, *Klebsiella*, *Pseudomonas*, *Citrobacter*, *Enterobacter*, *Morganella*, were isolated from buffalo meat samples from three different districts of Kathmandu valley. Among 54 isolated organisms, 36 isolates were found to be MDR positive.

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