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Microbial Quality of *Chhoyla* and *Kachela:* Traditional *Newari* Meat Products

SANTOSH KHANAL[1*], ASMITA KUMAL[1], ROSHAN SHRESTHA[1], SANTOSH SAPKOTA[1], SOYUZ BARAL[1], SUDEEP K. C.[1] AND DEV RAJ JOSHI[2]

[1] Department of Microbiology, National College, Tribhuvan University, Kathmandu, Nepal
[2] Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Chhoyla and Kachela are indigenous and popular meat dishes in the Newar community that are widely consumed in Kathmandu valley. The present study was carried out to investigate the microbial quality of Chhoyla and Kachela from different parts of the valley. The mean total aerobic plate count of Chhoyla and Kachela ranged up to 6.38 and 6.54 log$_{10}$ cfu/g respectively. The mean total coliform count of Chhoyla and Kachela ranged up to 5.25 and 5.82 log$_{10}$ cfu/g respectively. Coliform (93%), Staphylococcus aureus (75%), Salmonella Typhi (12%), Salmonella Paratyphi A (5%) and Shigella spp (3%) were isolated from Chhoyla. Likewise, bacteria isolated from Kachela were coliforms (97%), Staphylococcus aureus (86.66%), Salmonella Typhi (15%), Salmonella Paratyphi A (8%) and Shigella spp (7%). High microbial load and the potent human pathogens isolated from these meat products indicate potential risk of food borne diseases.

Key words: *Chhoyla, Kachela*, meat products, *Total coliforms, Salmonella, Shigella, Staphylococcus aureus.*

Introduction

Newar are the indigenous inhabitants of Kathmandu Valley and are endowed with an exotic cooking; all traditional and a wide variety of cuisines (Shakya, 1993). Meat, meat products and meat varieties are very popular in the Newar community. Buffalo meat locally called buff, is the most important and unavoidable part of Newari culinary (Tuladhar, 2011).

Chhoyla is a smoked meat, which is marinated with oil, salt, chilies and other different varieties of spices. For its preparation, buff meat is taken, washed and smoked in a grill pan under medium heat for 10-15 minutes. The meat is cut into small pieces and roasted flakes of chilies along with ginger paste, chili powder, cumin, coriander, turmeric powder and salt are added and mixed well. Finally, heated oil containing fenugreek seeds are poured over meat and mixed well. It is then garnished using coriander or green garlic leaves (Shrestha, 2012). *Kachela* is a raw and minced buff meat, which is mixed with oil, ginger, salt, turmeric powder and marinated with various local spices such as cumin powder, chili powder, ginger garlic paste along with fried mustard oil containing fenugreek seeds (Shrestha, 2012).

Many pathogenic and toxigenic microorganisms are capable of growing in meat products, which include *Salmonella* spp., *Clostridium botulinum*, *C. perfringens*, *S. aureus*, *Shigella* spp., and *Vibrio parahaemolyticus*. Several newly recognized meat pathogens have become a matter of concern. These include *Listeria* spp., *E. coli* and *Yersinia enterocolitica* (Pearson, 1997).

The contamination in *Chhoyla* and *Kachela* is inevitable. Some of the sources of contamination may be due to the contact with unhygienic surfaces, personal contact and airborne organisms (Zheng *et al*., 2009). Besides, the instruments used in dressings, the contact with steel, knives, scabbards etc. may also act as prominent sources of contamination (Kirkpatrick, 2002). Similarly, water used for the purpose of cleaning the containers and equipment may also contribute to the microbial contamination (Bell, 1997). Contamination occurs when meat comes in contact with dirty hands. In addition to that, storage temperature plays a vital role in multiplication of the organism (Frazier & Westhoff, 1988).

Consumption of *Chhoyla* and *Kachela* may lead to various health issues, ranging from mild to severe as they are not so well cooked. So, the study of microbial quality of these products is of great concern in the field of public health (Shakya, 1993). Therefore, the study was undertaken to determine the load of aerobic mesophilic bacteria and coliforms in *Chhoyla* and *Kachela* along with identification of coliforms and detection of potential human enteric pathogens.

Materials and Methods

Study site and study period

The research work was conducted in the microbiology laboratory, Department of Microbiology, National College, Kathmandu from February to August 2016.

Sample collection and transportation

A total of 120 samples (60 *Chhoyla* and 60 *Kachela*), each of 250 g, were collected from 24 different locations of Kathmandu, Bhaktapur and Lalitpur in sterile plastic bags. Considering minor cultural and food variation in Newar community (Shakya, 1993) of three different districts of Kathmandu valley, the random samples were collected from these places. The samples were transferred to the laboratory maintaining the cold chain and processed within 2 hours of collection. All the samples were analyzed in duplicates.

Sample processing and total count of bacteria

The food homogenate of samples was prepared and serially diluted up to 10^{-6}. The homogenate from the tubes of dilution of 10^{-2}, 10^{-4} and 10^{-6} were subjected to pour plating

*Corresponding author: santoshkhanal007@gmail.com

using Plate Count Agar (PCA) for total mesophilic count (FDA, 1998) and Violet Red Bile Agar (VRBA) for total coliform count respectively. The coliforms isolated from VRBA were further identified by various biochemical tests (Kayisoglu *et al.*, 2003).

Isolation and identification of *S. aureus*

For the isolation of *S. aureus*, 0.1 ml of homogenate was taken and spread on pre-poured Mannitol Salt Agar (MSA). The plate was then incubated at 37°C for 24 hours. After incubation, typical golden yellow colonies of Staphylococci were taken and sub-cultured on nutrient agar. The isolated pure culture was identified by Gram staining and biochemical tests; Catalase, Oxidase, Oxidative/ Fermentative (O/F) and Coagulase test (Cheesbrough, 2006).

Isolation and identification of *Salmonella* and *Shigella*

For the isolation of *Salmonella* spp. and *Shigella* spp., samples mixed with buffered peptone water were pre-enriched by incubating at 37°C for 24 hours. Then, 5 ml of the mixture was dispensed in 45ml sterile Selenite F broth in an aseptic condition and incubated at 37°C for 48 hours. After enrichment, a loopful of enriched mixture was aseptically streaked in Xylose Lysine Deoxycholate (XLD) Agar and incubated at 37°C for 24 hours. After incubation, typical *Salmonella-Shigella* like colonies were taken and sub-cultured on nutrient agar. The isolates were then identified by Gram staining and biochemical tests; Catalase, Oxidase, O/F, SIM, MR, VP, Citrate, TSIA and Urease according to Bergey's Manual of Determinative Bacteriology, (9th edition, 1994) for further analysis.

Data analysis

The data collected were explored using statistical software SPSS version 20. One-way ANOVA was used and a *p*-value less than or equal to 0.05 was considered to be statistically significant.

Results and Discussions

In *Chhoyla* samples, the highest total aerobic mesophilic bacterial count was from the sample of Kathmandu with mean 6.38 $\log_{10}$ cfu/g and standard deviation 0.54 $\log_{10}$ cfu/g. The lowest mesophilic bacterial count was from the sample of Lalitpur with mean 5.38 $\log_{10}$ cfu/g and standard deviation 0.77 $\log_{10}$ cfu/gm, but the data was not significant statistically (p=0.129). Similarly, the highest coliform count was from Kathmandu with mean 5.25 $\log_{10}$ cfu/g and standard deviation 1.04 $\log_{10}$ cfu/g and the lowest coliform count was from Bhaktapur with mean 4.21 $\log_{10}$ cfu/g and standard deviation 1.33 $\log_{10}$ cfu/g, which was statistically significant (p=0.02) (Table 1).

In *Kachela* samples, the highest total aerobic mesophilic bacterial count was from the sample of Kathmandu with mean $\log_{10}$ cfu/g value of 6.54 and standard deviation of 0.55 $\log_{10}$ cfu/g and the lowest total aerobic mesophilic bacterial count was from Bhaktapur with mean $\log_{10}$ cfu/g value of 5.86 and standard deviation of 0.58 $\log_{10}$ cfu/g. Similarly, the highest coliform count was from the sample of Kathmandu with mean $\log_{10}$ cfu/g value of 5.28 and standard deviation of 0.73 $\log_{10}$ cfu/g and the lowest coliform count was from the sample of Bhaktapur with mean $\log_{10}$ cfu/g value of 4.28 and standard deviation of 0.66 $\log_{10}$ cfu/g. But, the total bacterial count (p=0.381) and coliform count (p=0.718) were not significant statistically (Table 2).

Table 1
Total mesophilic and coliform count in *Chhoyla* samples

Sampling site	Total mesophilic count ($\log_{10}$ cfu/g)		Total coliform count ($\log_{10}$ cfu/g)	
	Mean±SD	*p*-value	Mean±SD	*p*-value
Kathmandu	6.38±0.54		5.25±1.04	
Bhaktapur	5.57±0.85	0.129	4.21±1.13	0.02
Lalitpur	5.38±0.77		4.25±0.99	

SD= standard deviation; p<0.05 was considered a significant difference (one-way ANOVA).

Table 2
Total mesophilic and coliform count in *Kachela* samples

Sampling site	Total mesophilic count ($\log_{10}$ cfu/g)		Total coliform count ($\log_{10}$ cfu/g)	
	Mean±SD	*p*-value	Mean±SD	*p*-value
Kathmandu	6.54±0.55		5.28±0.73	
Bhaktapur	5.86±0.58	0.381	4.28±0.66	0.718
Lalitpur	6.02±0.74		4.67±0.83	

SD= standard deviation; p<0.05 was considered a significant difference (one-way ANOVA).

Among 60 *Chhoyla* samples processed from Kathmandu (n=20), Bhaktapur (n=20) and Lalitpur (n=20), 75% had *S. aureus* and the remaining 25% were coagulase negative Staphylococci (CoNS). *Salmonella* Typhi was detected in 11.7% of the samples and *Salmonella* Paratyphi A was detected in 5% of the samples. Two samples also contained *Shigella* spp. and 93.3% of the samples were found to be contaminated with coliforms (Table 3).

Table 3
Occurrence of organisms in *Chhoyla* samples

Organisms	Frequency	%
Staphylococcus aureus	45	75.0
Coagulase negative Staphylococci	15	25.0
Salmonella Typhi	07	11.7
Salmonella Paratyphi A	03	05.0
Shigella spp.	02	03.3
Coliforms	56	93.3

In 56 *Chhoyla* samples that were found to be contaminated with coliforms, 46.4% contained *Escherichia coli*. Similarly, *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. were found in 25%, 9% and 19.6% of the samples respectively (Table 4). Among 60 *Kachela* samples, most of them were contaminated with coliforms (96.7%) followed by *S. aureus* (86.7%). *Salmonella* was isolated from 23.3% of the samples and *Shigella* spp. from 6.7% of samples. (Table 5).

Table 4
Occurrence of different genera of coliforms in *Chhoyla* samples

Organism	Frequency	%
Escherichia coli	26	46.4
Enterobacter spp.	14	25.0
Citrobacter spp.	05	09.0
Klebsiella spp.	11	19.6

Among 58 *Kachela* samples that were found to be contaminated with coliforms, 51.7% contained *Escherichia coli*. Similarly, *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. were found in 27.6%, 6.9% and 13.8% of the samples respectively (Table 6).

Table 5
Occurrence of organisms in *Kachela* samples

Organism	Frequency	%
Staphylococcus aureus	52	86.7
Coagulase negative Staphylococci	08	13.3
Salmonella Typhi	09	15.0
Salmonella Paratyphi A	05	08.3
Shigella spp.	04	06.7
Coliforms	58	96.7

In this study, a total of 60 *Chhoyla* samples from three different locations were analyzed by quantitative and qualitative methods. Mean total aerobic mesophilic bacterial count ranged from 5.38 to 6.38 $\log_{10}$ cfu/g and mean total coliform count ranged from 4.25 to 5.25 $\log_{10}$ cfu/g in *Chhoyla*, Kathmandu showing the highest number of bacterial contaminations.

Table 6
Occurrence of different genera of coliforms isolated in *Kachela* samples

Organism	Frequency	%
Escherichia coli	30	51.7
Enterobacter spp.	16	27.6
Citrobacter spp.	04	06.9
Klebsiella spp.	08	13.8

Kachela samples had similar results as that of *Chhoyla*; mean total aerobic mesophilic bacterial count ranged from 5.86 to 6.54 $\log_{10}$ cfu/g and mean total coliform count ranged from 4.28 to 5.28 $\log_{10}$ cfu/g. The samples from Kathmandu district displayed the highest number of bacterial pathogens. The results obtained are similar to that of Al-Mutairi (2011) who reported mean aerobic plate count of Kofta (also a meat product) to be 6.45 $\log_{10}$ cfu/g.

Kachela being a raw meat product, the total bacterial count was found to be slightly higher than that of *Chhoyla*. Slight heat and smoke are produced during preparation of *Chhoyla.* Since smoke contains different antimicrobial compounds like phenols and different acids, it extends the shelf life of product by preventing damage caused by spoilage causing and pathogenic bacteria (Girard, 1992). This might be the reason for comparatively low bacterial count in *Chhoyla*.

Coliforms are the indicators of post processing contamination of meat. Coliforms form a group of Enterobacteriaceae family and are primary environmental saprophytes, often found in the intestinal tract of man and lower animals. Because of their association with the intestinal tract, they easily become introduced into food during slaughter of animals or subsequent processing and food handling. These enteric pathogens may grow in foods and cause food infections after ingestion by attaching in intestinal walls, causing symptoms of nausea, vomiting, pain, diarrhea and headache (ANFZA, 2001).

In both of the *Chhoyla* and *Kachela* samples, the coliform load was extensively high. The results obtained in the current study were relative to that of Hussein (1996) who reported the mean value of coliform count of 5.26 $\log_{10}$ cfu/g for Kofta. Similarly, the current results were found to be higher than that recorded by Hassanin *et. al.* (2015) who recorded the mean coliform count value of Kofta to be 3.27 $\log_{10}$ cfu/g and El-Rayes (2008) who presented the mean value of coliform count of Kofta to be 3.45 $\log_{10}$ cfu/g. The difference in coliform count across three districts was statistically significant with higher number of contaminations in the samples from Kathmandu. Overpopulation, lack of inadequate quality water supply (DoHS, 2019) and inefficacy in the management of sewage systems (Prasai, 2007) could be the possible reasons for higher occurrence of coliforms in the meat samples.

The coliform load was higher in *Kachela* samples than that of *Chhoyla* samples. This might be due to the fact that no heat treatment is used during preparation of *Kachela* but slight heat treatment is done during preparation of *Chhoyla*. Also, *Kachela* being finely minced is exposed to higher microbial load than *Chhoyla* due to the large surface area (Salihu, 2010).

According to Microbial Guidelines of Food Product (2014), the bacteriological range for ready to eat meat products must be less than 6 $\log_{10}$ values. The $\log_{10}$ value more than 7 is considered to be unacceptable. The $\log_{10}$ value in-between 6-7 is considered to be in borderline. The mean bacteriological load obtained from the present study in *Chhoyla* was in the acceptable range. Among 60 samples of *Chhoyla*, microbial loads of 48 samples were within acceptable range and that of 10 samples were borderline. Microbial load in 2 of the samples were in unacceptable range. Similarly, among 60 samples of *Kachela*, microbial loads of 50 were within acceptable range and that of 8 were borderline. Microbial load in 2 of the samples were in unacceptable range.

Contamination of ready-to-eat foods with *S. aureus* is largely as a result of human contact. *S. aureus* are habituated in the warm, damp and congenital atmosphere of the nose, throat, in the pores and hair follicle of the skin and on the surfaces of skin (Baron, 1996). *S. aureus* can be transferred

to meat during processing, packaging, preparation and service by touching, breathing, coughing or sneezing. *S. aureus* can also colonize itself on food processing equipment and so food can become contaminated during preparation. Its presence in the samples might be due to contamination from the working environment of the meat preparation and the sale point. Levels of ≥10^4 cfu/g are considered potentially hazardous as foods with this level of contamination may result in food borne illness if consumed (Bolder 2007). In this study, 80.83% of samples were contaminated with *S. aureus*. Incidence rate of *S. aureus* was very high in comparison to the study carried out by Shaltout *et al.* (2017) in which *S. aureus* was detected in 32% of beef Kofta.

Salmonella spp., the causative agent of typhoid and paratyphoid, causes mortality and morbidity in a large fraction of people in underdeveloped and developing countries like Nepal and other South Asian countries. The route of transmission is feco-oral; any food contaminated with feces may transmit the organism to a new host (Joshi, 2005). The infective dose of *Salmonella* depends on several factors such as age and immunological status of the consumer. For a healthy person, an infectious dose of 10^3-10^6 bacteria has been indicated. However, infections caused by much lower numbers have been reported, especially in connection with intakes of fatty products (Karmi, 2013).

Ready-to-eat foods should be free from *Salmonella* species and other potential pathogens. Most of the food borne illnesses is due to the consumption of contaminated foods. Therefore, possible source of contamination should be avoided. Raw materials used for manufacturing of meat products should also be carefully selected and tested for absence of *Salmonella* spp. (Farhang *et al.*, 2012). Furthermore, hygienic food preparation and safer handling practices should be strictly followed. Maintenance of personal hygiene and periodic screening of infections among food handlers are also recommended.

A variety of spices are used during the preparation of *Chhoyla* and *Kachela*. These ingredients show antimicrobial activities against a lot of microbes including pathogenic organisms. The antimicrobial properties of spices are mostly due to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (Ebana *et al.*, 1991). Besides the antimicrobial activities of different ingredients used in *Chhoyla* and *Kachela*, they harbored a large number of contaminants and some pathogens as well. This may be due to the fact that the ingredients might not be in enough concentration that is required to suppress the growth and development of pathogens. Similarly, the organisms might have already developed resistance against these compounds (Ebana, 1991).

Large numbers of bacteria in *Chhoyla* and *Kachela* might be due to lack of unmanaged and unfurnished slaughterhouses and inadequate water supply. Also, the raw meat used for preparation of these products is not transported properly maintaining the cold chain. As meat products were contaminated with potential human pathogens like *Salmonella* and *S. aureus*, it also indicates poor personal hygiene practices among workers and food handlers. Therefore, food handlers should be informed about the importance of personal hygiene practice. Food poisoning / illnesses are entirely preventable by practicing good sanitation and food handling techniques. Similarly, further thermal processing of meat products can be of great importance. Therefore, strict regulation and implementation of food hygiene and safety practices will ensure the decline in food borne illnesses.

Conclusions

There was considerable contamination of *Chhoyla* and *Kachela* samples by mesophilic bacteria, coliforms and human enteric pathogens and Kathmandu district had the highest rate of microbial load. Since coliforms are fecal indicator bacteria, consumption of contaminated food with fecal coliform is not recommended. It is recommended to follow Good Hygiene Practice (GHP) during preparation and processing of meat and meat products. In addition, regular quality monitoring is also encouraged for regulatory authority to ensure microbial safety of those products in the market.

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