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Microbial quality of poultry meat in an ISO 22000:2005 certified poultry processing plant of Kathmandu valley

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Abstract

Poultry meat can be contaminated by different types of microorganisms during processing in processing plant. The microbiological quality of chicken carcasses and along with processing steps and environmental condition was analyzed in this study in an ISO 22000:2005 certified poultry processing plant of Kathmandu. Standard plate count method was applied for the enumeration and detection of total mesophilic bacteria, total coliform, total faecal coliform, *Staphylococcus* load along with selected pathogens like *Salmonella* spp., *S. aureus*, *Escherichia coli*, *Clostridium perfringens*, and *Listeria* spp. in chicken meat at four processing step (evisceration, final washing, frozen and market). It was observed that the level of microbial load decreased with subsequent processing phases in poultry processing plant where high level of bacteria were reduced during final washing and frozen phase. After processing poultry meat in an ISO 22000:2005 certified meat processing plant, total aerobic mesophilic count, total coliform count, total faecal coliform count, total *Staphylococcus* count were decreased from 6.92 to 4.45 log CFU/g, 3.49 to 2.19 log CFU/g, 2.41 to nil log CFU/g, and 3..43 to 1.99 log CFU/g respectively. Pathogenic bacteria like *Salmonella* spp., *C. perfringens*, and *Listeria* spp. were absent in chicken meat at the fourth processing step. Prevalence of *E. coli* was reduced from 37.4% to 10.2%, whereas *S. aureus* was decreased from 18.57% to 17.1%. It was concluded that the final washing and freezing steps were the Critical Control Point (CCP) to control microbial hazards in poultry processing phase.

Keywords: Poultry processing plant, ISO 22000:2005, HACCP, CCP, Pathogen

Introduction

Chickens are domestic fowl having red fleshy wattles and combs on their heads raised for meat which are low acid food, rich in nutrients, phosphorous, other minerals, and B-complex vitamins (Gamble, 2015). The consumption of highly nutritious and safe poultry meat has increased worldwide. Globally, in 2013 the average consumption of chicken meat was 15 kg per person annually (Gonzalez-Ortiz et al., 2013). According to the Central Bureau of Statistics (CBS) and 'Nepal Commercial Poultry Survey 2014-15', per capita chicken consumption for Nepali stands about 4.1 kg. According to the statistical information on Nepalese agriculture issued by Ministry of Agriculture Development (MoAD), Nepal, the total poultry meat production of the country was 55,041 metric ton (MT) in the fiscal year 2015/2016. Government of Nepal had formulated and endorsed the Animal Slaughterhouse and Meat Inspection Act 1999 and regulation 2001 to ensure the quality of meat products. However, there is no meat and meat products standard, while more than 100 food standards have been issued by the government.

The HACCP concept was pioneered in the 1960s, through a joint collaboration between the US National Aeronautics and Space Administration (NASA), Pillsbury Company, and the US Food and Drug Administration (FDA) (Finucane & Holup, 2005). In the year 2000, a number of safety standards had developed by many industries which created problems in the implementation of third party audits and certification of food industries. This led to the development of

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International Organization for Standardization (ISO) standards and ISO 22000 food safety management system for the implementation of a HACCP system (Weinroth et al., 2018).

Hazard Analysis and Critical Control Point (CCP) is worldwide recognized scientific system for the identification and to control of physical, chemical and biological hazards in food processing plant and reduce hazard from farm to consumption for safety assurance of food (Panisello et al., 2000). The microbial quality of poultry meat depends on the time of feed withdrawal before slaughter, transportation, contamination from live birds, efficiency of processing method, temperature, sanitary and hygienic condition in the plant (Mead, 2004). The number of contaminating bacteria on poultry carcass may decrease or increase in different processing steps of the plant (Althaus et al., 2017).

In chicken, presence of total mesophilic count is an indicator of hygienic level, total coliform count and total faecal coliform count are indicator of faecal and environment contamination whereas total Staphylococci count and *S. aureus* count are indicator of poor hygienic, handling and temperature control condition (González-Fandos & Dominguez, 2006; Rindhe et al., 2008) . Presence of food poisoning bacteria in meat is an important public health issue (Mbata, 2005).

HACCP concept was forthput for food processing industry to produce safe food. In Nepal small- and medium-sized food processing industries play an important role in industrial development and food supply. However, they have been suffering from a raw materials of low grades, lack of proper skilled manpower and the problems associated with these things can be reduced by the HACCP concept. HACCP enhanced food safety, cosumer confidence, market access, product consistency while it reduced risk of food borne illenes, production cost, trade risk, public health cost and timely response to the problems (Code, 2012; Satin, 2005). This study was designed with an aim to study the microbial quality of poultry meat at different points of an ISO certified processing plant of Kathmandu valley.

Materials and methods

Live birds are transported in the poultry processing plant and upon verification by Veterinarians are then processed in the processing phase at 1200 birds/ hours. After humane slaughtering and bleeding for 5 mins transferred in scalding tank for 1.5 min at 62 °C which is followed by defeathering, head removal, vent cutting, evisceration, removal of internal organs, lung vacuuming, in-out washing, hock cutting, water chilling (35 mins at 0–5 °C), final washing, vacuum packaging, blast freezing (− 30 °C for 24 h), cold storage (− 18 °C for storage) and market supply as frozen meat as shown in flow chart (Additional file 1).

The study was conducted in an ISO 22000:2005 certified poultry processing plant of Kathmandu valley from July 2016 to December 2016. In July, field observation, case study and preparation were done. Specifically, three times whole chicken samples were aseptically collected from each processing phase (evisceration, final washing, frozen and market) in each month of the study period (August, September, October, November, December). Similarly, altogether 10 water samples (Tank water and Pipeline water), 30 air samples (lairage, bleeding, evisceration, spin chilling, grading, packaging) and 25 equipment samples (packaging material, table, floor, machine, and bucket) were collected and processed in same 5 months. The samples were immediately transferred to microbiology laboratory in sterile plastic bags placed in an insulated ice container and processed on the same day according to the standard laboratory procedure (Food & D. Administration, 2012).

From whole chicken, 25 gram (gm) of chicken meat samples were cut with its skin by sterilized scissor, and placed in 225 ml of 0.1% sterilized buffer peptone water (Food & D. Administration, 2012). The homogenate sample was serially diluted in 9 ml of 0.1% sterilized buffer peptone water to achieve a 10-fold dilution. The required dilutions were pour plated on plate count agar, violet red bile agar, M-Endo agar, while spread plated on sterilized Mannitol salt agar. (Maturin & Peeler, 2001; Bennett et al., 2001; Feng et al., 2002). All inoculated plates were then incubated at 37 °C for 24–48 h while M-endo was incubated at 44.5 °C for 24–48 h. Culture suspected *S. aureus* strain was confirmed by result of Gram staining along with biochemical tests; Catalase, Oxidase, and Coagulase while *E. coli* was confirmed based on the result of series of biochemical tests; Methyl Red (MR), Voges Proskauer (VP), Oxidase, Catalase, Urea Hydrolysis, Triple Sugar Iron agar (TSI), Citrate utilization, Indole and Sulfide Motility test as recommend by Bailey and Scott's Diagnostic Microbiology (2007).

For the detection of *Salmonella* spp., 25 g of chicken meat was enriched in 225 ml of sterile buffered peptone water and incubated at 37 °C for 24 h. Five ml pre-enriched sample was inoculated to 45 ml Selenite-F broth and incubated at 37 °C for 24 h. A loopful of enriched sample was streaked on *Salmonella-Shigella* (SS) agar and incubated at 37 °C for 24 h (Feng et al., 2002). After culture, isolated colonies were subcultured on MacConkey agar and Nutrient agar for isolation of pure colonies at 37 °C for 24 h. For the confirmation of isolated pure colonies, different biochemical tests like Methyl Red (MR), Voges Proskauer (VP), Oxidase, Catalase, Urea Hydrolysis, Triple Sugar Iron agar (TSI),

Citrate utilization, Indole and Sulfide Motility test were performed.

For the isolation and identification of *Clostridium perfringens*, 25 g of chicken sample was transferred in 225 ml of sterile buffered peptone water and an aliquot of 1 ml from food homogenate was transferred into 9 ml of 0.1% peptone water then heated at 75 °C for 20 min. Further dilution was made up to 10^{-6} by transferring the 1 ml aliquot into a tube containing 9 ml sterile buffered peptone water (Solomon & Lilly Jr, 2001). About 6–7 ml of Tryptose Sulfite Cycloserine Agar (TSC) without egg yolk was poured into petri plates and spread evenly on bottom. After solidification of agar, 1 ml of each dilution sample was transferred into the center of duplicate agar plates. Then, additional 15 ml of TSC agar without egg yolk was poured into plates and mixed well. After solidification, 5–6 ml of TSC agar was poured to make overlapping (dual layer pour plating). The plates were placed in upright position in anaerobic jar and incubated at 37 °C for 48 h (Velugoti et al., 2007).

Twenty five gram of the chicken sample was enriched in 225 ml of Listeria Enrichment broth at 25 °C for 6 days (Food & D. Administration, 2012). After incubation, on 3rd and 6th day 1 ml of aliquot was pour plated on Listeria identification agar base (PALCAM) and incubated at 25 °C for 48 h (Food & D. Administration, 2012). After incubation period, grey-green with black center and black halo colonies were subcultured on nutrient agar for isolation of pure culture at 37 °C for 24 h. Identification of *Listeria* spp. was done by microscopy, catalase test, oxidase test, biochemical tests, hanging drop motility test, and sugar fermentation test with inverted Durham's tube (Food & D. Administration, 2012).

Water samples from main tank and pipeline water were collected and microbiologically analyzed for total mesophilic count, total coliform count and total faecal coliform count (Cunniff, 1996). Diluted samples were poured on plate count agar, violet red bile agar and incubated at 37 °C for 24 h where M-Endo agar was incubated at 44.5 °C for 24 h. After incubation, from the countable plates number of colonies between 25 and 250 colonies were counted and expressed in CFU/ml (Cunniff, 1996).

Samples from equipments (packaging material, table, floor, machine, and bucket) used in abattoir were collected by swabbing method. Sterilized swabbing bud was dipped in normal saline (0.85%) and drained excess by pressing in side of test tube. With swabbing bud, marked swabbing area (10 cm^{2}) was swabbed in vertical and horizontal position then transferred in test-tube containing 10 ml sterile buffered peptone water (Jørgensen et al., 2002). Then serial dilution was performed as required. Pour plating was done in Plate Count Agar (PCA) then incubated at 37 °C for 24 h and observed for the significant growth of colonies and enumerated accordingly.

Sterilized and air dried solidified Plate Count Agar (PCA) and Potato Dextsrose Agar (PDA) of about 90 mm diameter (approximate internal area 64 cm^{2}) plates were taken and exposed for 15 min in lairage, bleeding, evisceration, spin chilling, grading, and packaging section of poultry processing plant. Then PCA was incubated at 37 °C for 24 h and PDA plates was incubated at 30 °C for 3–5 days then observed for the significant growth of colonies and enumerated accordingly (Prathab & Lalitha, 2012).

Data anlysis

All data were analyzed using SPSS version 21.0. One-way anova was calculated where p value of < 0.05 was considered statistically significant at 95% of confidence level and tukey pairwise comparison was done between processing phase at 95% confidence. Graphs were plotted using Minitab 18 software.

Results

Table 1 represents the mean log count of total mesophilic bacterial count, total coliform count, total faecal coliform count and total Staphylococci count enumerated from 60 chicken samples at four different phases of processing line. The results show that higher bacterial load were found in evisceration followed by final washing and frozen and least in market meat. Graph 1 represents interval plot of mean log count vs processing phase (95% CI for the mean). Bacterial mean log count in evisceration, final washing, frozen and market lies between 3.4–4.8, 2.8–4.2, 1.8–3.2 and 1.6–3.0 respectively. Table 2 shows pairwise comparisons between processing phase using the Tukey Method and 95% Confidence tukey. This result shows that there is significant difference in bacterial load in first, second and third phases. However, there is no significant difference in the load between last two phases. Table 3 shows one way Anova which represents p-value (0.001) < 0.05, so there is a significant difference in mean bacterial in different phases of processing plant.

Graph 2 shows mean of mean log count of bacterial load in four different phases which illustrates that all bacterial load decreases with processing phases. After washing process, high load of bacteria was found to be decreased in total *Staphylococcus* count (0.91 log CFU/g). Total mesophilic count, total coliform count and total faecal coliform count were found to be highly decreased after freezing process by 1.27 log CFU/g, 0.82 log CFU/g and 1.84 log CFU/g respectively. However, on moving from frozen to market bacterial load remain constant. Graph 3 represents mean of mean log count of bacterial count with processing phase and month. Bacterial load significantly decreases with processing phase

Table 1 Mean log count of bacterial load of chicken meat in poultry chain

Sampling time	Biological hazards	Mean log $_{10}$ cfu/gm at different phases in poultry chain			
		Evisceration	Final washing	Frozen	Market
August	TMC	7.84	5.96	4.84	4.66
	TCC	3.4	3.3	2.2	2
	TFCC	2.24	2.2	1	0
	TSC	3.25	2.4	2.2	1.9
September	TMC	7.65	6.04	4.64	4.60
	TCC	3.29	3.28	2.5	2.3
	TFCC	2.25	2.2	0	0
	TSC	3.04	2.38	2.1	2.08
October	TMC	6.29	5.6	4.04	4.0
	TCC	3.74	3	2	2
	TFCC	2.69	2.3	0	0
	TSC	3.47	2.25	2.04	2
November	TMC	6.43	5.89	4.79	4.49
	TCC	3.14	2.4	2.35	2.2
	TFCC	2.2	2	1	0
	TSC	3.81	3.3	2.52	1.95
December	TMC	6.41	5.87	4.69	4.51
	TCC	3.86	3.73	2.53	2.43
	TFCC	2.69	2.5	0	0
	TSC	3.56	2.29	2.2	2.04

Note
TMC Total Mesophilic Bacterial Count, *TCC* Total Coliform Count
TFCC Total Faecal Coliform Count, *TSC* Total Staphylococi Count

whereas environment temperature donot have much effect over it.

The result from Table 4 showed that pathogenic microorganism were analyzed at four different phases of processing plant. During the study the result did not show the presence of *Salmonella* spp., *Clostridium perfringens* and *Listeria* spp. According to this study, prevalence of *E. coli* and *S. aureus* in evisceration stage was found to be 37.4% and 18.57% which was eventually decreased to 10.2% and 17.1% in market stage respectively.

The Table 5 result showed that 10 water samples of tank water (untreated water) and pipeline water (treated water- treated by abbaitor water treatment plant and chlorine dosing) were analyzed for total mesophilic bacterial count, total coliform count and total faecal coliform count which were then compared with drinking water standard. Tank water was found out of the standard value however, treated water was within the standard value in terms of total mesophilic count (2.69 log CFU/ml) and absence of total coliform count and total faecal coliform count.

Table 2 Tukey pairwise comparisons: processing phase grouping information using the Tukey method and 95% confidence

Processing Phase	N	Mean	Grouping		
Evisceration	20	4.0625	A		
Final Washing	20	3.4445		B	
Frozen	20	2.3820			C
Market	20	2.1580			C

Means that do not share a letter are significantly different

Altogether 30 air samples from processing area of poultry chain was assessed by exposing the plates in different area as shown in Table 6. The total bacterial count were more than total yeast and mold count from all sampling sites. The microbial load was found to be high in lairage section followed by bleeding, evisceration, spin chilling, grading, and least in packaging section. Table 7 represents the microbial load of different sites where 25 swab samples were taken from different equipments used in poultry processing plant (packaging material, table, floor, machine and bucket). Total mesophilic bacterial count was found high in floor and bucket with least contamination in packaging materials.

Table 3 One-way Anova table

Null hypothesis	All means are equal				
Alternative hypothesis	Not all means are equal				
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	*P*-Value
Processing Phase	3	48.34	16.112	6.08	0.001
Error	76	201.37	2.650		
Total	79	249.71			

Conclusion: Since *p*-value (0.001) < 0.05, we reject our null hypothesis

Discussion

As the result of the highest count was observed in evisceration and final washing stage,which was decreased during in final washing after evisceration, the number of total mesophilic bacterial count by more than 1 log cycle which is compatible with a report reported by Goksoy et al. (2004) (James et al., 2000). Nevertheless, the rate of decrease in total coliform count, total faecal coliform count and total Staphylococci count were less compatible which may be due to the strong attachment of these microorganism in chicken and less effective of washing process applied in the plant (Althaus et al., 2017).

A significant changes in bacterial count occurred at freezing steps which reduced the number of total bacterial count by 1 log cycle. Cooling and freezing of poultry meat has great influence on poultry meat microflora as cold condition has reduced microorganisms (James et al., 2006). During freezing of poultry the destruction of microorganisms occurs but this destruction is never absolute and is only interested in a limited number of microorganisms, which may be higher or lower depending on type of microorganisms (Mbata, 2005).

The total aerobic plate count 4.45 log CFU/g in market meat of this study is consistent with previous studies conducted by Chaudhey et al. (2011) (5.07 log CFU/g) (Chaudhrya et al., 2011). Sengupta et al. (2012), Omorodion and Odu (2014), and Bhandari et al. (2013) have reported higher counts of total aerobic bacteria 6.39 log CFU/g, 5.96 log CFU/g and 7.24 log CFU/g respectively in market chicken meat (Ibrahim et al., 2015; Omorodion & Odu, 2014; Bhandari et al., 2013). On the other hand, lower counts were reported by Rindhe et al. (2008) (3.67 log CFU/g) (Rindhe et al., 2008), and Al-jasser et al. (2012) (4.03 log CFU/g) (Al-Jasser, 2012). Total viable count in raw poultry indicates hygienic conditions of processing plants under which the food are processed and high load increases the risk of microbial spoilage (Cohen et al., 2007; Javadi & Safarmashaei, 2011). Studies have shown the correlation of food spoilage with the total bacterial count on the surface part of carcass: off-odor and sour are the noticeable evident when the bacterial count on carcass reached about 10^7 CFU/g and visible slime formation can be observed when the count reached approximately 10^8 CFU/g threshold (Wabeck, 2002).

In this study, total coliform count 2.19 log CFU/g of market meat was similar with results reported by Capita et al. (2002) (2.7 log CFU/g) and Northcutt et al. (2003) (2.6 log CFU/g) (Capita et al., 2002; Northcutt et al., 2003). In contrast, less coliform counts were reported by Joshi et al. (1.03 log CFU/g) and Selvan et al. (2007) (1.13 log CFU/g) (Joshi & Joshi, 2010; Selvan et al., 2007) while higher coliform counts were found in studies conducted by Kumar et al. (2012) (4.97 log CFU/g), Sengupta et al. (2012) (32.2 log CFU/g), and Bhandari et al. (2013) (6.5 log CFU/g) (Ibrahim et al., 2015; Bhandari et al., 2013; Selvan et al., 2007). In this study, total faecal coliform count was absent in market meat. *E. coli* was used as an indicator organism of sanitary quality and potential faecal contamination of meat which can originate even from workers or environment of the processing plant (Wabeck, 2002). *E. coli* count in poultry products can be minimized by controlling cross contamination, maintaining sanitary practices, and temperature of carcass. It is necessary to ensure hygienic food production for the safeguard of public health (Althaus et al., 2017).

The count of total Staphylococci 1.99 log CFU/g in market meat was lower than the studies carried by Sengupta et al. (2012) (3.7 log CFU/g), Joshi et al. (2010) (4.07 log CFU/g) (Joshi & Joshi, 2010; Sengupta et al., 2012). Presence of Staphylococci in meat reflects insanitary condition, cross contamination between processing phase, and surrounding environment, processing temperature and personal contact. *Staphylococcus aureus* is a commensal organism of human skin and also a common pathogen, which causes minor to severe infections including food poisoning (Carroll et al., 2015). In a study conducted by

Table 4 Occurrence of pathogenic microorganism at 4 processing phases of the processing phase

Processing stage	*E. coli*	*S. aureus*	*Salmonella* spp.	*Clostridium perfringens*	*Listeria* spp.
Evisceration	37.4%	18.57%	–	–	–
Final washing	22.8%	19.4%	–	–	–
Frozen	11.4%	17.1%	–	–	–
Market	10.2%	17.1%	–	–	–

Table 5 Parameters of water samples from the poultry processing plant

Water sources	Mean bacterial count (log CFU/ml water)		
	Total mesophilic bacterial count	Total coliform count	Total faecal coliform count
Tank water (untreated water)	3.2	1.2	–
Pipeline water (treated water)	2.5	–	–
Drinking water standard	2.69	–	–

Hotee et al. (2011) at the Central Health Laboratory in Mauritius have isolated *S. aureus* as the second common pathogen from analyzed food samples (Heetun et al., 2015). The presence of 17.1% *S. aureus* in market meat of this study is in agreement with the study conducted Kozacins et al. (2012) where prevalence was 17.9% (Kozačinski et al., 2012). In contrast, Joshi and Joshi (2010) had reported higher prevalence of *S. aureus* (100%) in meat (Joshi & Joshi, 2010). Generally chicken meat becomes contaminated with *S. aureus* when an infected person does coughing, sneezing, talking or breathing inside the plant (Wabeck, 2002). The microbial load were found in initial processing step, comprising the receiving-killing and defeathering areas, whereas count towards the evisceration, air chilling, packaging and dispatch area decreased (James et al., 2000; Hinton Jr et al., 2004).

Another important pathogen of meat contamination is *Salmonella* which habitats in the intestinal tract of animals and shed along with feces of the animals that makes its presence in surrounding environment (Wabeck, 2002). In this study, *Salmonella* spp. was not detected in market chicken meat. As we applied plating culture technique for the isolation of *Salmonella* spp. so we can't claim that *Salmonella* spp. were completely absent. There could be viable but non-culturable cells of the strain. Similar findings have been reported by Vaidya et al. (2005), and Lindblad et al. (2006) (Vaidya et al., 2005; Lindblad et al., 2006). In contrast, in another study carried by Joshi and Joshi (2010) had reported *Salmonella* spp. in all the examined chicken carcasses (100%) (Joshi & Joshi, 2010). On the other hand, lower percentage of *Salmonella* spp. was reported by Cohen et al. (2007) (1.6%); Abdellah et al. (2008) (2.08%); Colmegna et al. (2009) (1.1%) (Cohen et al., 2007; Abdellah et al., 2008; Colmegna et al., 2009). Presence of *Salmonella* spp. in market meat of chicken suggests poor hygienic status of meat processing plant during slaughtering, cross contamination between machines, scalding tanks, defeathering machines, and workers. During the slaughtering and manual evisceration process of intestinal contents may spill and contaminate the muscle and organs of the chicken which is an important source of *Salmonella* spp. contamination in meat and water chilling tanks (Colmegna et al., 2009).

Clostridium perfringens is an obligate anaerobe which is found in the alimentary tract of poultry. *C. perfringens* was not isolated in chicken meat samples of this study which complies with the study carried by Shaltout et al. (2009) in Egypt (Shaltout, 2009). On the other hand, *C. perfringens* were isolated by Chhetri and Karki (2014) (80.8%) in raw poultry meat of Kathmandu; Nowell et al. (2010) (66%) in Canada; Cohel et al. (2007) (7.2%) in Casablanca (Morocco) and Thangamani and Subramanin (2012) (3.81%) in Tamilnadu, India (Cohen et al., 2007; Nowell et al., 2010; Thangamani & Subramanian, 2012; Chhetri & Karki, 2014). If the raw meat originally contains *C. perfringens,* it is almost impossible to make final product free from this contamination since heat treatment only destroys vegetative cells of this bacterium activating spores for further germination (Van Immerseel et al., 2004).

Listeria spp. do spread either by inhalation or direct contact. Listeriosis typically occur after consumption of contaminated foods. For humans contaminated sources include raw meat (Reiter et al., 2005). In this study, *Listeria* spp. was not isolated from the chicken meat

Table 6 Mean log count of microorganism isolated from surrounding air of different section of processing plant

Sampling site	Mean count (CFU/15 min)	
	Total mesophilic bacterial count	Total yeast and mold count
Lairage section	*	*
Bleeding section	1.65	1.2
Evisceration section	1.55	1.14
Spin chilling section	1.53	0.95
Grading section	1.45	1.08
Packaging section	1.43	0.93

Mean count of 5 plates
*Higher than the maximum number that can be counted in plate

Table 7 Mean log count of microorganism isolated from different equipment used in processing plant

Sampling area	Mean log CFU per $10cm^2$
	Total mesophilic bacterial count
Packaging material	2.18
Table	4.07
Floor	5.43
Machine	4.36
Bucket	5.37

samples during the slaughter process which was similar with another study reported by Svobodova et al. (2012) (Svobodová et al., 2012). Prevalence of *L. monocytogenes* was reported by Colmegna et al. (2009) (3%) in Milano, Italy; Kozacins et al. (2012) (4.5%) in Croatia; Molla et al. (2004) (1.9%) in Addis Ababa, Ethiopia and Cohen et al. (2007) (0.5%) in Casablanca (Morocco) (Cohen et al., 2007; Kozačinski et al., 2012; Colmegna et al., 2009; Molla et al., 2004). Loura et al. (2004) have reported frequent findings of *L. monocytogenes* on poultry cuts and especially, on the hands of poultry processing plant staffs, poultry processing plant desks and equipment (Gottlieb et al., 2006). *L. monocytogenes* is well documented as a major foodborne pathogen in the different parts of the world which is habitat event in natural environments like mud, soil, water, sewage, gut of poultry and feces (Yeh, 2004).

The tank water is present with high load of total viable count 3.2 log CFU/ml and coliform 1.2 log CFU/ml. After water treatment in water treatment plant and chlorine dosing, processing water (Pipeline water) is nearly free of contamination showing acceptable microbiological water quality with total viable count of 2.5 log CFU/ml, nil coliform and faecal coliform. The total plate count is an indirect indicator or index for pathogens of concern in water, which helps in assessing the efficacy and proper functioning of water treatment and supply process and is related to the acceptability of water (Figueras & Borrego, 2010).

Air microflora in abattoir changes with rate of movement of people from one place to another, hygienic condition, humidity and room temperature (Haagsma et al., 2012). So, in order to monitor air quality, the plate exposure method was applied in this study for total mesophilic bacterial count and total yeast and mold count which showed that lairage was most contaminated whereas packaging, grading, and spin chilling section showed least contamination.. The presence of fungi in abattoir air may be due to migration from outdoor environment as well as presence in ceilings and walls of production area (Update, E. H. E. D. G, 2006). In poultry processing plants, the reception of birds is the major source of air pollution with pathogenic microorganisms. Along the processing phase of poultry processing plant air microflora tends to decrease reaching the lowest values in the freezing (Whyte et al., 2001). Findings of this stay are in agreement with the data reported by Whyte et al. (2001), they have reported that microbial contamination of the air was widespread and varied considerably between different processing locations of a poultry processing plant (Whyte et al., 2001).

The presence of high load of bacteria on the floor poultry processing plant may be due to cross- contamination of litter as chicken faeces are rich in microorganisms (Svobodová et al., 2012). During this study, the cleanliness of table, equipment, machine, buckets used in the poultry processing were assessed to check the standards of hygiene and efficiency of cleaning procedures. In this study, there was high load of total mesophilic bacterial count indicating lack of good cleaning practices which ultimately played a great role in cross-contamination of poultry meat. Contaminating bacteria on the equipment would soon be found on meat in various parts of the carcasses by increasing their microbial load and reducing their storage quality and safety (Bhaisare et al., 2014). Implementation of HACCP system in the food industry and even in home can maintain food safety by eliminating or reducing food-borne hazards (Wallace, 2014). Many researches have shown that application of HACCP systems in food industry leads to more efficient prevention of food-borne diseases (Scoti & Stevenson, 2006; Pal et al., 2016).

Conclusion

This study shows that microbial load was found to be slowly decreased with further processing steps whereas month/weather have no effect in microbial load in an ISO 22000:2005 certified poultry processing plant in Kathmandu valley. The final washing, and freezing phases are determined as Critical Control Point (CCP) to combat microbial hazard because, besides this, on poultry processing phase, there are no additional reducing operations for reducing the contamination at acceptable level. In the poultry processing plant, microbial contamination of poultry meat occur in every phase of processing so it can be reduced by implementation of good manufacturing practice, proper use of sanitation equipment, proper clean in place system, use of contamination free water, good personnel hygiene, and training of plant workers.

Limitations

Due to short duration of study we couldn't collect and investigate meat samples from other poultry processing plants of the city. So, we can't reveal the exact figure of

microbial load in chicken meat at different points of other processing plant. Further study in poultry meats of different sites of the country is recommended to generalize the result on implementation of HACCP principles in poultry processing plants.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40550-019-0078-5.

Additional file 1: Flow chart and major sampling points (*)
Additional file 2: Graphs

Abbreviations

CBS: Central bureau of statistics; CCP: Critical control point; CFU: Control forming unit; FDA: Food and drug administration; HACCP: Hazard analysis critical control point; ISO: International organization for standardization; MoAD: Ministry of agriculture development; MR: Methyl Red; MSA: Mannitol salt agar; MT: Metric ton; NASA: National aeronautics and space administration; PALCAM: Listeria identification agar base; PCA: Plate count agar; PDA: Potato dextrose agar; SOP: Standard operating procedures; SS: Salmonella-Shigella agar; TQM: Total quality management; TSC: Tryptose sulfite cycloserine agar; TSI: Triple sugar iron agar; VP: Voges proskauer

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Ethics and consent to participate

Not applicable

Authors' contributions

First author: SM is primary author who designed the study methodology, performed laboratory investigations and prepare the manuscript. Second authors: BR and SS edited, proof read, helped in data analysis and revised the complete manuscript for submission. VSC, OPP, and TBK helped in data analysis and proof read of the manuscript. All authors approved the final manuscript before submission to the International Journal of Food Contamination, BMC.

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Availability of data and materials

The datasets used and analysed during this study are available in excel sheets which can be obtained from the corresponding author on reasonable request.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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