

Assessment of bacterial load in broiler chicken meat from the retail meat shops in Chitwan, Nepal

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ABSTRACT

INTRODUCTION: Keeping quality of meat and meat related food hazard relates to microbes present in the meat during processing or storage. The poultry slaughtered and dressed under Chitwan conditions carrying high initial contamination would be present in meat as inherent contamination in the finished products.

MATERIALS AND METHODS: This cross-sectional study included 26 fresh broiler meat samples from registered retail shops. The samples were subjected to bacteriological analysis such as total viable count (TVC), total enterobacteriaceae count (TEC), total coliform count (TCC) and total staphylococcal count (TSC). Also, the samples were analyzed for the detection of *Salmonella* spp. and identified by different biochemical tests.

RESULTS: The mean counts in $\log_{10} \pm SE$ colony forming units per gram (cfu/g) for 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 TVC; 8.5 ± 0.2 , 9.2 ± 0.3 and 10.2 ± 0.4 TEC; 6.5 ± 0.3 , 7.6 ± 0.3 and 8.4 ± 0.5 TCC; 6.5 ± 0.2 , 6.8 ± 0.3 and 7.7 ± 0.4 TSC respectively. No samples were found to be within the permissible limits given by different agencies. Prevalence of *Salmonella* spp. in retail broiler meat in Chitwan was found 46.2%.

CONCLUSION: The retail broiler meat samples from the locations contain high count of bacteria suggesting deplorable state of hygienic and sanitary practices. The presence of *Salmonella* and *Staphylococcus aureus* organisms over the permissible limits are of special concern because these account for potential food borne intoxication. So, the need for microbial assessment of fresh meats can be emphasized.

KEY WORDS: Bacterial load, Broiler meat, Chitwan, *Salmonella* spp., *Staphylococcus aureus*, Permissible limits

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INTRODUCTION

Meat, a good source of animal protein along with sensory attributes, appeals to the consumers very easily. Meat is the most perishable of all important foods since it contains sufficient nutrient needed to support the growth of microorganisms¹. Annual production of chicken meat in Nepal is 16662 metric ton and annual production of chicken meat in Chitwan district is 1422 metric ton. So, Chitwan has 8.5 percent share of chicken meat production annually in Nepal². Chicken meat can be contaminated at several points throughout the processing operations. Moreover, retail cuts could result in greater microbial load owing to large amount of exposed surface area, more readily available water, nutrient and greater oxygen penetration which leads to spoilage of meat³. Meat borne zoonotic diseases such as Salmonellosis, Campylobacteriosis, *E. coli* enteritis and food poisoning by *Clostridium*, *Staphylococcus*, etc. are the major problems encountered by the consumers eating contaminated meat. Only little is known about microbial aspects, shelf life and food safety of commercial retail chicken meat in Chitwan. The poultry slaughtered and dressed under Chitwan conditions carrying high initial contamination would be exhibited to the point the consumers are offered as retail meat. So, retail meat would harbour all the bacteria that are already present in meat as inherent contamination through infection and that are introduced during handling, improper dressing, cleaning, insanitary condition and retailing. To increase meat quality, assurance in accordance with microbial load assessment is deemed necessary⁴. Hence, this study was conducted to assess the microbiological situation of fresh chicken meat which can be the reflection of hygienic condition of meat consumed and the possible hazards of public health. The study also compares the bacterial load to the acceptance criteria given by different international agencies since no such criteria for raw chicken meat has been set in Nepal.

MATERIALS AND METHODS

Study Area

This was a cross sectional study carried out in 6 months period from July to December 2011. The research was focused on retail broiler meat shops of Ratnanagar Municipality, Bharatpur Municipality and Institute of Agriculture and Animal Science, IAAS vicinity (Mangalpur, Sharadanagar, Gitanagar and Parbatipur VDCs) of Chitwan, Nepal.

Experiments were carried out at Veterinary Microbiology Laboratory of IAAS, Rampur. A total of 26 samples including 13 samples out of 17 registered retail meat shops in Bharatpur Municipality, 9 samples out of 13 registered retail meat shops of Ratnanagar Municipality, and 4 samples out of 4 registered retail meat shops of VDCs around IAAS vicinity (1 from Mangalpur VDC, 1 from Sharadanagar VDC, 1 from Gitanagar VDC and 1 from Parbatipur VDC) were collected by simple random sampling method.

Collection of Samples

Pooled sampling was done by taking samples from neck, thigh, and breast region by excision weighing a total of 25 g. The samples were stored in sterile sealed polythene bag on crushed ice until microbiological analyses. Samples were collected at time between 9 to 10 am. The samples were transported within few hours and analyzed immediately at the Veterinary Microbiology Laboratory of IAAS, Rampur.

Microbiological Analysis of samples

Two main assessments were performed for bacteriological analysis after collection of samples. Firstly to evaluate sanitary quality of meat, 225 ml of 0.1% buffered peptone water was transferred into the plastic bag containing 25 g of meat sample and a homogenized suspension was made. Thus 1:10 dilution of the sample was obtained and later different serial dilutions ranging from 10^{-2} - 10^{-13} was prepared according to the recommendation of International Organization for Standardization, 1995⁵. All these samples were subjected to bacteriological analysis such as determination of total viable count (TVC), total enterobacteriaceae (TEC), total coliform count (TCC) and total staphylococcal count (TSC). A 100 μ l (0.1ml) from serial dilution was used on prepared duplicate plates and spreading was performed for the determination of counts. For this 100 μ l of each tenfold dilution (10^{-2} - 10^{-13}) was transferred on duplicate agar plates using separate sterilized micro- pipette for each dilution. So, duplicate agar plates prepared using the Nutrient agar (NA), Violet red bile glucose agar (VRBG), MacConkey agar with crystal violet and Baird-Parker agar (BPA) as supplied by HIMEDIA[®] Pvt Ltd., India were used for determination of TVC, TEC, TCC and TSC respectively. The diluted samples were spread as quickly as possible on the surface of the plate with sterile glass spreader. One sterile spreader was used for each plate. The plates were incubated at 37°C for 48

hours before counting. The plates were interpreted as <30 too few to count, 30 – 300 counted and >300 too many to count. The average number of colonies in particular dilution in particular agar plate was multiplied by the dilution to obtain the total count. The results of the total bacterial count were expressed as the number of colony forming units cfu/per g of meat samples. Bacterial counts were then transformed and represented as \log_{10} cfu/g of the sample.

Secondly, 25 g samples were analyzed for the presence or absence of *Salmonella* spp. For selective isolation of *Salmonella*, enriched (in buffered peptone water) sample was streaked into Brilliant Green Agar (BGA), MacConkey agar and Xylose Lysine Tergitol 4 (XLT4) agar plates. The inoculated plates were incubated for 48 hrs at 37°C. The bacterial isolates obtained from the samples were purified by re-streaking them on the same selective media (BGA, MacConkey, XLT4) used for their isolation and were characterized by different biochemical tests as described by Chakraborty⁶ and Quinn et al.⁷ The biochemical tests such as Triple Sugar Iron (TSI), Oxidase, Catalase, Methyl Red (MR), Voges Proskauer (VP), Urease, Motility, Ornithine decarboxylase, Indole etc. were used for identification of *Salmonella* spp.

Ethical Approval

This study was carried out as a part of partial fulfillment of degree of Bachelor of Veterinary Science and Animal Husbandry (B. V. Sc & A.H) and approved by Institute of agriculture and animal science (IAAS) internship committee. Consents of the shopkeepers were taken before moving to the collection of the samples.

Statistical Analysis

The recorded data were tabulated using Microsoft Excel 2007. Significant differences were analyzed by

one-way ANOVA using MSAT-C version 1.3(1994). For mean comparison, LSD at $P < 0.05$ was performed. Correlation analysis was done for between the agars. Descriptive statistics was used to describe the result of prevalence analysis. Prevalence was estimated as the number of samples detected positive to *Salmonella* spp. isolation from the total sample analyzed.

RESULTS

Total viable count (TVC)

The result presented in Table 1 reveals that the mean value of the TVC of broiler meat of Bharatpur Municipality, Ratnanagar Municipality and that of VDCs around IAAS Rampur Campus did not differ significantly with mean counts of \log_{10} cgu/gram being 11.1 ± 0.3 , 11.5 ± 0.3 and 12.2 ± 0.5 respectively.

Total Enterobacteriaceae count (TEC)

The analysis of variance revealed a highly significant difference ($p = 0.0093$) for TEC. The TEC for Bharatpur Municipality was found to be 8.5 ± 0.2 and that of Ratnanagar Municipality and VDCs around IAAS as 9.2 ± 0.3 and 10.2 ± 0.4 respectively. However, mean counts for both the municipalities didn't exhibit significant differences as shown by LSD.

Total Coliform Count (TCC)

Highly significant difference ($p = 0.0017$) was also noted for the TCC. Significant difference in TCC was found in the municipalities with mean counts being 6.5 ± 0.3 and 7.6 ± 0.3 . The highest coliform load was found in the VDCs around IAAS vicinity having mean count of 8.4 ± 0.5 .

Total Staphylococcal count (TSC)

Significant difference at ($p = 0.043$) was found in case of TSC of the retail broiler meat shops. The

Table 1. Mean total counts of retail broiler meat in \log_{10} CFU per gram \pm Standard error (SE)

Sample n=26)	Mean TVC	Mean TEC	Mean TCC	Mean TSC
Bharatpur Municipality (n=13)	11.1 ± 0.3^a	8.5 ± 0.2^b	6.5 ± 0.3^b	6.448 ± 0.22^b
Ratnanagar Municipality (n=9)	11.5 ± 0.3^a	9.2 ± 0.3^b	7.6 ± 0.3^a	6.8 ± 0.3^b
VDCs around IAAS (n=4)	12.2 ± 0.5^a	10.2 ± 0.4^a	8.4 ± 0.5^a	7.7 ± 0.4^a
F-value	1.800 ^{ns}	5.769 ^{**}	8.545 ^{**}	3.673 [*]

ns= non significant

Single asterisk (*) = statistically significant at $P < 0.05$

Double asterisk (**) = statistically significant at $P < 0.01$

Means in the column with different superscript letters (a and b) are significantly different ($P < 0.05$).

Table 2. Summarized results of bacteriological examination of samples in comparison with the standards

Samples	Bharatpur Municipality(n=13)	Ratnanagar Municipality(n=9)	VDCs around IAAS (n=4)
Type of analysis			
TVC permissible limit by ICMSF,1986	log ₁₀ 7(for raw chicken, fresh or frozen)	log ₁₀ 7	log ₁₀ 7
No. of samples within the limit	0	0	0
No. of samples exceeding the limit	13	9	4
TCC permissible limit by Directive 88/657/EEC,BIS 1969	log ₁₀ 2	log ₁₀ 2	log ₁₀ 2
No. of samples within the limit	0	0	0
No. of samples exceeding the limit	13	9	4
TSC permissible limit by Directive 88/657/EEC	log ₁₀ 2	log ₁₀ 2	log ₁₀ 2
No. of samples within the limit	0	0	0
No. of samples exceeding the limit	13	9	4
<i>Salmonella</i> permissible limit by Directive 88/657/EEC,BIS 1969	Absent in 25 grams	Absent in 25 grams	Absent in 25 grams
No. of samples within the limit(absent)	8	6	0
No. of samples exceeding the limit (present)	5	3	4

Since, no such standards have been recommended for the permissible limit of raw poultry meat in Nepal, the European Economic Committee (EEC) provision for raw meat⁸, Bureau of Indian Standards (BIS)⁹ and International Commission on Microbiological Specifications for Foods(ICMSF)¹⁰ has been used for comparison.

Table 3. Prevalence of *Salmonella* spp. in Chitwan

Sn	Sites	Total samples	Positive for <i>Salmonella</i>	Prevalence of <i>Salmonella</i> (%)
1	Bharatpur Municipality	13	5	38.5
2	Ratnanagar Municipality	9	3	33.3
3	VDCs Around IAAS	4	4	100
Total		26	12	46.2

LSD analysis found no significant differences in count between the two municipalities having mean count of 6.5±0.2 and 6.8±0.3. But the count of both municipalities differed significantly to that of VDCs around IAAS which had highest mean count of 7.7±0.4.

Prevalence of *Salmonella* spp.

A total of 26 retail broiler meat samples of Chitwan district (2 Municipalities and 4 VDCs around IAAS) were processed for isolation of *Salmonella*. Out of the total samples tested, *Salmonella* was detected from 12 samples (5 from Bharatpur Municipality, 3 from Ratnanagar Municipality and 4 from VDCs around IAAS). Thus prevalence of *Salmonella* in Chitwan was found to be 46.2 % which is shown in table 3.

DISCUSSION

The results were higher than those obtained for fresh poultry meat samples in Slaughter Slabs of Kathmandu valley, Nepal.¹¹ Also very high count were found than those obtained in local chicken market with non-sophisticated outlets of Bangalore, India.¹² Similarly, the TVC was also greater than that found in chicken meat samples collected from retailers in Tabriz, Iran which was 5.1±0.1 in terms of log₁₀cfu/gram±SE.¹³ The high TVC in the examined samples reported here indicate that the contamination of the product could be attributed to unsatisfactory sanitation during handling and processing.

For raw chicken meat, the TEC values are very high

and indicate high contamination of both coliform and non-coliforms which form the majority of Gram negative pathogens.¹⁴ The coliform contamination was found highest in the VDCs which may be due to non sophisticated slabs and poor hygiene of the retail shops. The results are very high than those obtained for fresh poultry meat samples in Slaughter Slabs of Kathmandu valley, Nepal ($2 \log_{10}\text{cfu/g}$).¹¹ Also the results are higher than those from Iran which was found to be 4.0 ± 0.1 .¹³

The presented data revealed that, the *Staphylococcus aureus* counts were found at highest number in case of VDCs at an average of $7.7 \pm 0.4 \log_{10}\text{cfu/g}$ where as similar counts were found for both Bharatpur and Ratnanagar municipalities at averages of 6.5 ± 0.2 and 6.8 ± 0.3 respectively. These results are higher than those obtained in local chicken market with non-sophisticated outlets of Bangalore, India which were at an average of $4.2 \pm 0.1 \log_{10}\text{cfu/g}$.¹² The use of hand evisceration technique along with infrequent hand washing could be the cause of such a high occurrence of this bacteria.

Results presented in table 2 declared that, all of the examined samples of raw chicken meat had $\text{TVC} > \log_{10}7 \text{ cfu/g}$ which exceeded the permissible limit recommended by the ICMSF, 1986. Similarly, none of the samples were in agreement with TCC permissible limit by Directive 88/657/EEC, BIS 1969 and TSC permissible limit by Directive 88/657/EEC. For *Salmonella* spp, 8 samples out of 13 in Bharatpur Municipality and 6 samples out of 9 in Ratnanagar Municipality were in agreement with the standard given by Directive 88/657/EEC and BIS 1969. However, none were in agreement with the standard in case of 4 samples of VDCs around IAAS Rampur Campus.

According to this study prevalence of Salmonella in retail broiler meat in Chitwan was found to be 46.2 % which is higher than that obtained by Dhakal and Manandhar (12%)¹⁵; and by Acharya (26.1 %)¹⁶ for Chitwan Valley. However, this prevalence rate is lower than that of Tiwari who isolated 47.8% Salmonella in poultry meat in Chitwan¹⁷. This difference can be related to difference in time and season of research from those researches.

CONCLUSIONS

The marked growth of bacteria concludes that retail broiler meat is not suitable for consumption. The high total viable counts recorded in this study showed the microbial diversity (differences in form

or species) in these places, condition of the market and the hygienic practice employed by meat sellers and butchers. The presence of *Salmonella* and *Staphylococcus aureus* organisms demonstrates a potential health risk since the organisms are pathogenic and gives warning signal for the possible occurrence of food borne intoxication. The need for microbial assessment of fresh meats and other meat products processed and packaged for human consumption is therefore emphasized and recommended to reduce possible hazard.

CONFLICT OF INTEREST: None to declare

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