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Research Article

ENUMERATION OF TOTAL VIABLE AND TOTAL COLIFORM COUNT IN CHICKEN MEAT AND MEAT PRODUCTS USING TEMPO® SYSTEM

MUKESH KUMARI*, ASHOK KUMAR and RENU GUPTA
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar- 125004, India

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ABSTRACT

Bacteriological counts of meat sample indicate the quality of food available for human consumption. Traditionally, the bacterial load is calculated by most probable number (MPN) count and this research highlights the use of an automated instrument (TEMPO®) for estimation of bacterial count in raw and ready-to-eat chicken meat available in the market. A total of 75 chicken meat samples comprising of raw meat (n = 50) and ready-to-eat meat (n = 25) were analysed to evaluate hygienic status by quantifying bacteria. The quantification included total viable count (TVC) and coliform count (CC) with use of TEMPO® system. Of all the examined samples, only 4% (2/50) raw meat samples showed a total viable count within the permissible limit as per FSSAI standards ($\leq \log 10^6$ CFU/g). However, 56% (14/25) ready-to-eat meat samples had total viable count within limit ($\leq 10^4$ CFU/g). As per International Commission on Microbiological Specifications of food (ICMSF) specifications for the total coliform count, 44% (11/25) ready-to-eat meat samples were acceptable (≤100 CFU/g) and all the raw meat samples had coliform count much more than the permissible limit (>2000 CFU/g). The study indicated very high bacterial contamination in the raw meat whereas the ready-to-eat meat samples were somehow in acceptable range though the higher contamination in some ready-to-eat meat samples can lead to spoilage of food with longer period of storage.

Key words: Raw meat, Ready-to-eat meat, TEMPO® system, Total coliform count, Total viable count

Globally, health-conscious consumers are opting leaner and easy digestible chicken meat. In India, poultry meat is an important, low-cost source of animal protein which encourages the consumption of poultry products by a large number of consumers and therefore a safe and quality product without the presence of pathogenic microorganisms is in demand. Illness due to food borne diseases is perhaps the most widespread health problem and an important cause of reduced economic productivity (FAO/WHO, 1984). According to report from Center for Diseases Control and Prevention (CDC), in the United States approximately 48 million people get ill annually due to foodborne diseases despite US having the safest food supplies in the world (CDC, 2011). The data regarding developing countries including India are lacking because of lack of organized food-borne disease surveillance system.

The TEMPO® system (bioMerieux) was developed to improve laboratory efficiency and to replace traditional methods. It is based on an established microbiological method, called Most Probable Number (MPN) method and it is a fast, accurate method with more reliability than the traditional process. Due to many advantages, particularly ease of use, the popularity of ready to use system for the enumeration of hygiene indicator microorganisms is increasing (Ferrati *et al*., 2005). The microbiological quality of chicken meat and meat products was assessed by enumerating total viable and total coliform count using TEMPO® system in the present study.

MATERIALS AND METHODS

Sampling

A total of 50 raw meat and 25 ready-to-eat chicken meat samples were collected from local market of Hisar. At least 25 g of raw meat was collected in sterile sample container with all aseptic precautions. Ready-to-eat chicken meat products including 5 each of chicken meat sausage, chicken finger and chicken nuggets, 3 each of chicken meat loaf and patties and 4 chicken meat keema samples were also collected. All the samples were transported to the laboratory under cold conditions at the earliest. All the ready-to-eat chicken meat products were brought to laboratory without opening the packet. All the samples were processed in the Food Safety Laboratory of Department of Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana.

Test procedure for total viable count

To prepare samples for TEMPO® system, the sample was diluted 1/10 (primary dilution), aseptically by adding 10g of sample to 90ml of sterile peptone water (primary diluent) and then homogenized in the TEMPO bag for 5 min. In case of coarse hard meat, the meat was first chopped into smaller bits using a sterile pair of scissors before homogenization with peptone water. Because of a high level of microbiological contamination of the raw meat, the dilution of the sample 1/400 was applied by mixing 0.1 ml of sample mixture to 3.9 ml of reconstituted media. One aerobic count (AC) card for each vial of inoculated medium was removed from its protective covering, without touching the tip of the transfer tube. The vial containing the inoculated medium was put in the filling rack. The card was inserted in the slot opposite to the vial, placing the transfer tube of the card inside the vial. After giving the sample an identification number in the TEMPO® software, the card was allowed to be filled up by the TEMPO® filler, which transfers the inoculated medium

*Corresponding author : aryamukesh1991@gmail.com

Table 1

Evaluation of microbial quality of chicken meat by analysis of total viable count and coliform count

Total Viable Count (CFU/g)			
FSSAI Standards	Acceptable ($\leq 10^6$)	Marginally acceptable ($10^6 - 5 \times 10^6$)	Unacceptable ($> 5 \times 10^6$)
Raw meat (n=50)	2 (4%)	21 (42%)	27 (54%)
FSSAI Standards	Acceptable ($\leq 10^4$)	Marginally acceptable (10^4–10^5)	Unacceptable ($>10^5$)
Ready-to-eat meat (25)	14 (56%)	8 (32%)	3 (12%)
Sausage (5)	1 (20%)	3 (60%)	1 (20%)
Nuggets (5)	5 (100%)	-	-
Fingers (5)	5 (100%)	-	-
Keema (4)	3 (75%)	-	1 (25%)
Meat loaf (3)	-	2 (67%)	1 (33%)
Chicken Patties (3)	-	3 (100%)	-
Total Coliform Count (CFU/g)			
ICMSF Standards	Acceptable (≤ 100)	Marginally acceptable (100 – 2000)	Unacceptable (>2000)
Raw meat (n=50)			50 (100%)
ICMSF Standards	(≤ 100)	(100 – 1000)	(>1000)
Ready-to-eat meat (25)	11 (44%)	7 (28%)	7 (28%)
Sausage (5)	1 (20%)	1 (20%)	3 (60%)
Nuggets (5)	5 (100%)	-	-
Fingers (5)	3 (60%)	2 (40%)	-
Keema (4)	2 (50%)	1 (25%)	1 (33%)
Meat loaf (3)	-	1 (33%)	2 (67%)
Chicken Patties (3)	-	2 (67%)	1 (33%)

from the vial into the card that contains three sets of 16 wells (small, medium and large wells) with a one-log difference in volume for each set of wells (volumes 2.25µl, 22.5µl, and 225µl). The cards were then removed from the filling station and incubated for 40-48 hours at 37°C. At the end of incubation, the cards were read using TEMPO® reader. The machine records the fluorescence in the wells and interprets the results. At the end of the analysis, the cards were removed from the rack and disposed of after autoclaving.

Test procedure for total coliform count

This procedure is same as total viable count except for the dilution and incubation time. To prepare samples for TEMPO® system, the sample was diluted 1/10 (primary dilution), aseptically by adding 10g of sample to 90ml of sterile peptone water (primary diluent) and then homogenized in the TEMPO bag for 5 min. The total coliform culture medium was reconstituted by dispensing 3ml of sterile distilled water (secondary diluent) in each vial. Then 1ml of the homogenized mixture was transferred to the 3ml of reconstituted media. It was mixed properly for approximately 30 seconds using a vortex-type mixer. The 4 ml of inoculated medium obtained corresponds to a 1/40 dilution of the sample (as recommended by the manufacturer as this dilution enables enumeration of bacteria between 10 and 4.9×10^4 CFU/g). The dilution can be increased according to the expected level of contamination i.e. more the contamination of the suspected sample, more the dilution like 1/400, 1/4000 and so on. One total coliform (TC) card for each vial of inoculated medium was used. Incubation was done for 24-27 hours at 37 °C and reading was noted (as explained earlier for total viable count).

RESULTS AND DISCUSSION

In the present study, a total of 75 chicken meat samples comprising of raw meat (n = 50) and ready-to-eat meat (n = 25) were analysed to evaluate hygienic status by quantifying bacteria. Total viable count (TVC) and total coliform count (TC) were assessed using TEMPO® system.

The International Commission on Microbiological Specifications of Foods (ICMSF) guidelines were taken into consideration for coliform count because there are no recommendations on this in FSSAI guidelines in India. As per ICMSF specifications for the total coliform count, 44% (11/25) ready-to-eat meat samples had the coliform count within the acceptable level i.e. ≤100 CFU/g, however, all the raw meat samples had total coliform count way more than the permissible limit (>2000 CFU/g) (Table 1). According to the Food Safety and Standards Authority of India (FSSAI) recommendations, the acceptable contamination level of the raw meat for aerobic mesophilic microorganisms is $\leq 10^6$ CFU/g. Of all the examined samples, only 4% (2/50) raw meat samples showed a total viable count within the permissible limit. However, 56% (14/25) ready-to-eat meat samples were acceptable for the same. All the nugget samples were found to be acceptable in terms of both total viable count and coliform count.

In present study, 54% (27/50) of samples had total viable count more than 5×10^6 CFU/g, which indicates high contamination in raw meat. The results pertaining to the total viable count (TVC) of raw chicken meat samples were nearly in agreement with the findings of Bhandari *et al*. (2013) where they reported samples of raw chicken meat had TVC more than $\log 10^7$ CFU/g, which exceeds the permissible limit in Nepal. Higher level of total viable count in this study is in accordance with Ahmad *et al*. (2013) who reported higher mean APC (aerobic plate count) in chicken meat from retail outlets to the extent of 7.22 $\log_{10}$ CFU/cm^2. Our result is also similar to a research conducted in Morocco by Amara *et al*. (1994) where they reported total viable count of raw meat to be 6.56-7.15 $\log_{10}$ CFU/g. Another research conducted in Morocco by Cohen *et al*. (2007) reported an aerobic plate count of 5.9-6.6 $\log_{10}$ CFU/g in hot seasons and 4.5-5.9 $\log_{10}$ CFU/g in cold seasons in raw chicken meat. They also reported a fecal coliform count of 2.9-3.8 $\log_{10}$ CFU/g in hot seasons and 2.6-3.6 $\log_{10}$ CFU/g in cold season. Their results showed 29.2% of the total tested samples were beyond the safety limit in terms of total viable count i.e. 6.7 $\log_{10}$ CFU/g and 22.4% of the samples were beyond the safety limit for fecal coliform i.e. 4 $\log_{10}$ CFU/g according to the Moroccan standard regulations. In Chennai, Selvan *et al.* (2007) reported a little lower total viable count for chicken meat i.e. 4.52 $\log_{10}$ CFU/g and a total coliform count of 1.13 $\log_{10}$ CFU/g. Khalifa and Abd El-Shaheed (2004) examined raw chicken meat and

reported the aerobic plate count with an average of 3.0 x 10^4 CFU/g. Similarly Kim *et al.* (2018) who reported the total viable count and coliform Count in meat processing plants were 3.46 $\log_{10}$ CFU/g and 0.55 $\log_{10}$ CFU/g in chicken meat, respectively in Korea. Mawia *et al*. (2016) reported the mean values of standard plate count (SPC) for poultry meat to be 6.65± 0.06 $\log_{10}$ CFU/g. Singh *et al*. (2014) conducted a study in Agra and reported standard plate count of raw poultry meat to be satisfactory i.e. 6.75 $\log_{10}$ CFU/g and mean total coliform count was found to be 3.82 $\log_{10}$ CFU/g. Álvarez-Astorga *et al*. (2002) reported mean counts ($\log_{10}$ CFU/g) ranged from 5.56 to 7.28, 5.96 to 7.87, 3.49 to 5.42, for mesophiles, psychrotrophs, coliform, respectively and 80% of the samples of hamburgers and sausages were also regarded as being of unacceptable quality of poultry meat.

AL-Dughaym and Altabari (2009) reported the mean total bacterial count ranging from 2.7×10^4 CFU/g for nuggets to 3.3×10^7 CFU/g for burgers, while, for other ready-to-eat products counts were in the range of 10^5-10^6 CFU/g. The higher counts could be due to the unhygienic practices followed during meat handling and processing. Jeffery *et al*. (2003) identified the workers hand and equipment were sources of contamination. Adu-Gyamfi *et al*. (2012) reported 52% and 70% of meat samples had total viable counts and total coliform counts, respectively, within the microbiologically safe limits. They reported mean total viable counts for the supermarkets, local markets and farms were 6.46, 6.91 and 6.57 $\log_{10}$CFU/g, respectively. However, the total coliform counts for the supermarkets, local markets and farms were found to be 3.80, 3.46 and 3.14 $\log_{10}$ CFU/g, respectively. Sengupta *et al*. (2011) reported total aerobic bacterial count ranging from 51-55 x 10^4 to 4-25 x 10^4CFU/g and mean coliform count per gram of poultry meat from semi-urban and urban markets in Kolkata were 3.20 x 10^2 CFU/g and 6.50 x 10^2 CFU/g, respectively. The variation in results may be due to difference in geographical location, time of sample collection, environmental conditions and managerial practices etc. According to our study, it is crucial that sanitary operation in which meat are processed are strictly controlled to prevent microbial contamination of the meat. All the raw chicken meat obtained from local market showed significantly high level of microbial contamination. Whereas, 14/25 (56%) ready-to-eat meat samples showed acceptable results. The reason behind high microbial load in raw chicken meat could be the unhygienic process of slaughter and meat handling whereas the ready-to-eat meat showed low microbial count which could be due to hygienic manufacturing process, less direct human contact and sterile packaging system.

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