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Comparative Microbial Load Assessment of Meat, Contact Surfaces and Water Samples in Retail Chevon meat Shops and Abattoirs of Kolkata, W.B, India

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A B S T R A C T

A cross sectional study to quantitatively assess the microbial load viz. total plate count (TPC), fecal coliform count (FCC) and total Staphylococcus count (TSC) was conducted in meat (n=60), water (n=60), and meat in-contact surface swab samples (n=228) collected from floor, cutting knife, hands of workers, cutting wood during processing of chevon in 8 retail meat shops and 2 abattoirs of Kolkata, West Bengal. The TPC, FCC and TSC were 6.27±0.48, 4.16±0.32 and 3.85±0.42 log cfu/cm^2 for floor, 3.46±0.11, 1.36±0.17 and 2.34±0.28 log cfu/cm^2 for hands of the workers and 3.12±0.18, 2.14±0.09 and 2.11±0.36 log cfu/cm^2 for cutting knife used in the abattoir. On the other hand, the TPC, FCC and TSC values were 7.86±0.53, 5.69 ± 0.21 and 4.76±0.40 log cfu/cm^2; 3.85±0.09, 1.98±0.16 and 3.88±0.31 log cfu/cm^2; and 4.31±0.22, 3.04±0.13 and 3.55±0.32 log cfu/cm^2 for floor, hands of workers and cutting knife, respectively in retail meat shops. Besides, water samples from abattoir had mean values for TPC, FCC and SPC as 6.18±0.71, 3.88±0.28 and 4.58±0.25 log cfu/ml, respectively against 9.28±0.68, 8.54±0.32 and 5.39±0.3 log cfu/ml, respectively from retail shops. The meat samples of retail shop had comparatively higher mean values of TPC (6.22 ±0.48 vs 5.14±0.12), FCC (4.22 ±0.28 vs 3.04±0.05) and TSC3.33 ±0.11vs2.74±0.11) log cfu/cm^2 than meat samples from abattoir. Overall, the findings indicate higher level of contamination of the floor, water and equipment used in retail meat shops and reiterates the need for application of scientific interventions and capacity building of workers in order to improve the hygienic conditions of meat during production and processing, thereby ensuring its quality and safety for consumers.

Introduction

Meat, being one of the important sources of animal proteins, needs to be produced hygienically, be pathogen free and retain its natural state and nutritive value so as to be acceptable by the consumers (Govindarajan, 1990; Bhandare *et al.*, 2007). Even though retailing unprocessed raw meat in the street or in public places is common in India as well as in some parts of Asian countries (WHO, 2009), the quality aspects have remained unchanged over many years. This is because meat sector is still one of the poorly organized

sectors in many developing countries and contamination of meat occurs due to the use of poor quality of water while washing, handling carcasses and using equipments during slaughtering/processing meat (Das and Biswas, 2003; Parvin *et al.,* 2017).

Most of the shops lack modern waste disposal facilities resulting in microbial contamination of processed meat (Deriba and Mogessie, 2001). Besides, most of the workers are unaware of the basic importance of personal cleanliness as a result of which the processed products are vulnerable to gross contamination by flies, insects, rodents, dust and other dirt. Meat being a highly perishable food needs to be processed hygienically, but unhygienic processing conditions often make the environment conducive for growth of many hazardous microorganisms (Das *et al.,* 2004; Rao *et al.,* 2009), thereby reducing its keeping quality.

All these factors bring changes in chemical composition and biological characteristics leading to spoilage of meat due to contamination with microorganisms of public health importance and may spread infections in the community (Gorman *et al.,* 2002; Olaoye *et al.,* 2010).

Over the years, consumers' have become more quality conscious and their awareness to get safe and wholesome meat is growing day by day (Selvan *et al.,* 2007). On the other hand, the safety and quality of the meat sold to consumers is becoming a matter of increasing concern in developing countries.

It is against this background that this study was undertaken to assess the level of microbial load in meat and its possible sources of contamination through water, floor, hands of workers and equipment used, while processing meat in retail shops and abattoirs present in and around Kolkata, West Bengal.

Materials and Methods

Study area and sample collection

The study was carried out selecting meat shops both from retail (n=8) and municipality abattoirs (n=2) located in and around Kolkata, West Bengal. A total of 348 (288 from retail and 60 from abattoirs) samples were collected aseptically from the hands of workers, floor, cutting platform (wood), surface of cutting instruments (Knife) along with water and meat samples from retail shops and organized abattoir maintained by Kolkata municipality and is presented in Table 1. At least two visits were made to each retail shop and abattoir, and samples were collected in triplicate.

Swab samples (n=228) from the hands of workers, floor, cutting platform (wood) and surface of cutting instruments (knife), used for various operations during handling and processing, were collected using sterile cotton wool swabs (3cm long and 1cm diameter) held by wooden stick and moistened with 0.1% peptone water. Briefly, an area of 25cm^2 (5cm X 5cm) was marked on the surface of the cutting wood, hand, floor and cutting instruments with a sterile frame. The swabs were rubbed on the sites for around 30 sec and then transferred to individual screw-capped test tube containing sterile maintenance medium (0.85% NaCl and 0.1% peptone). Likewise, water (10 ml, n=60) and meat samples (10g, n=60) from both retail shops and abattoirs were collected in sterile screw capped tubes. Care was taken so that both the swab and water samples were processed within 24 hours of collection.

Preparation of sample

Before processing, the tubes containing swabs were vortexed for 30s to maintain uniformity in distribution of microorganisms. Meat samples were homogenized, using a sterile

warring blender, in sterile jar containing 90 ml sterile normal saline. A serial dilution of sample for inoculation was prepared according to ICMSF (2002). Accordingly, ten-fold serial dilutions (up to 10^{-6}) of all the samples were prepared using sterile maintenance medium (0.85% NaCl and 0.1% peptone). Using pour plate method, 0.1 ml of each ten-dilution was transferred on a solidified prepared plate count agar for TPC, whereas MacConkey agar and Mannitol Salt agar were used for FCC and TSC, respectively. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. The whole procedure was carried out inside the laminar flow and all the media used were dehydrated and purchased from Himedia Laboratories, India. The inoculated plates were incubated aerobically at 37°C for 24 hrs. After incubation, plates with countable colonies (30-300 numbers) were counted using the illuminated colony counter. The total count so obtained was multiplied with the average number of colonies in a particular dilution with the dilution factor ISO (2009) and expressed as the number of organisms or colony forming unit (CFU) of each sample and calculated into its log value. The microbiological data were expressed in log cfu/cm^2, log cfu/ml and log cfu/g in case of swab, water and meat samples, respectively.

Statistical analysis

The means and standard deviations were determined using Graph pad Prism. Student's 't' test for paired samples was used to determine the levels of statistical significance at 95% confidence interval. Microsoft Excel 2010 was used for the Student's 't' test computation.

Results and Discussion

In total, 348 samples [swab samples (n=228) from hands of workers, floor, cutting platform (wood), surface of cutting instruments (knife); water and meat samples (n=120)] were processed to quantitatively assess the microbial load viz. TPC, FCC and TSC during processing of chevonin retail meat shops and abattoirs. Mean values of TPC, FCC and TSC of swab samples collected from abattoir were 6.27±0.48, 4.16±0.32 and 3.85±0.42 log cfu/cm^{2}for floor; 3.46±0.11, 1.36±0.17 and 2.34±0.28 log cfu/cm^2 for hands of the workers and 3.12±0.18, 2.14±0.09 and 2.11±0.36 log cfu/cm^2 for cutting knife used, respectively. On the other hand, the TPC, FCC and TSC values of retail meat shop swab samples were 7.86±0.53, 5.69 ± 0.21 and 4.76±0.40 log cfu/cm2 for floor; 3.85±0.09, 1.98±0.16 and 3.88±0.31 log cfu/cm^2 for hands of workers and 4.31±0.22, 3.04±0.13 and 3.55±0.32 log cfu/cm^2 for cutting knife, respectively. Our results are in tandem with reports by Bhandare *et al.*, (2009) who observed significantly higher level of contamination of meat contact surfaces (hand, floor and knife) for total viable count (TVC) in an abattoir and the meat shops in Mumbai, India. Similarly, Tarwate *et al.*, (1993) also reported higher microbial load in meat contact surfaces in traditional meat shop environments compared to the abattoir.

According to Timm *et al.*, (2013), contact surfaces with contamination level exceeding 4.0 log cfu/cm^2 is sufficient to initiate biofilm favoring microbial growth. Among all the meat contact surfaces; floors of retail meat shops had higher mean TPC as compared to others.

Most of the retail shops, from where the samples were taken, practice the slaughter on the floor. These retail meat shops either do not use sanitizer or detergent or adopt proper cleaning method. So, higher microbial loads on retail meat shop floors could be due to minimum hygienic practices that are followed compared to abattoirs (Parvin *et al.*, 2017).

Table.1 Total number of samples from retail meat shops and abattoirs for assessment of bacterial load

Sl. No.	Site of sample collection	Number of samples						Total
		Floor (swab)	Worker's Hand (swab)	Cutting Knife (swab)	Cutting wood (swab)	Water	Meat	
1.	Retail meat shops (n=6)	48	48	48	48	48	48	288
2.	Abattoirs (n=2)	12	12	12	0	12	12	60

Table.2 Comparison of mean bacterial count of meat contact surfaces (log cfu/cm^2) and water (log cfu/ml) samples of abattoirs and retail meat shops

Sample	Total Plate Count		Faecal Coliform Count		Total Staphylococcus Count	
	Abattoirs	Retail meat shops	Abattoirs	Retail meat shops	Abattoirs	Retail meat shops
Floor (swab)	6.27±0.48	7.86±0.53*	4.16±0.32	5.69±0.21*	3.85±0.42	4.76±0.40
Hand (swab)	3.46±0.11	3.85±0.09	1.36±0.17	1.98±0.16	2.34±0.28	3.88±0.31
Knife (swab)	3.12±0.18	4.31±0.22*	2.14±0.09	3.04±0.13	2.11±0.36	3.55±0.32*
Cutting wood (swab)	----	7.56±0.54	----	4.86±0.47	-----	5.72±0.62
Water	6.18±0.71	9.28±0.68**	3.88±0.28	8.54±0.32**	4.58±0.25	5.39±0.30

*p<0.05; **p<0.01 under Table 2

Table.3 Comparison of mean bacterial load (log cfu/g) in meat samples collected from abattoirs and retail meat shops

Type of microbial load	Abattoirs	Retail meat shops
Total plate count	5.14 ± 0.12	6.22±0.48**
Fecal coliform count	3.04 ± 0.05	4.22±0.28**
Total Staphylococcus count	2.74±0.11	3.33±0.10*

The mean values of TPC, FCC and TSC on the cutting platform (wood) used for mincing of chevon in retail shops were found to be 7.56±0.54, 4.86±0.47 and 5.72±0.62 log cfu/cm^2, respectively which is very high. The presence of bacterial pathogens in meat-contact surface (wood) could be due to their repeated use and presence of blood/drip, which might have served as an ideal medium for growth of micro-organisms thereby contributing for the increase in microbial load, as also observed by Bhandare *et al.*, (2009).

Water is used at various stages during processing of chevon in retail meat shops and abattoirs, as well. Therefore, the role of water used in abattoirs and retail shops assume significance in determining the microbial load of the carcass and cuts of the meat produced. Water samples from abattoir and retail shops had mean values for TPC, FCC and SPC as 6.18±0.71 vs 9.28 ±0.68, 3.88±0.28 vs 8.54±0.32 and 4.58±0.25 vs 5.38 ±0.3 log cfu/ml, respectively (Table 2). In a similar study, Adeyemo *et al.*, (2002) reported mean coliform and total bacterial counts of 4.3 log

cfu/ml and 5.18 log cfu/ml, respectively in water samples of abattoir situated in Ibadan. Tarwate *et al.,* (1993) and Bhandare *et al.,* (2009) reported total aerobic viable counts (TAVC) of 2.07±0.06 and 3.90±0.07 log cfu/ml, respectively for water used in abattoirs. In comparison, the TPC, FCC and TSC of retail shops were 9.28±0.68, 8.54±0.32 and 5.39±0.3 log cfu/ml, respectively.

The high TPC, FCC and TSC in water of retail meat shops in this study indicate repeated use of unclean water during slaughtering process, as these values are above the recommended permissible level of TPC for portable water. From public health point of view, it is very important to have a provision of clean water supply during processing of meat.

The microbial load of meat samples collected from abattoirs and retail shops is presented in Table 3. Mean values of TPC, FCC and TSC of meat samples collected from abattoir and retail meat shops were 5.14±0.12 vs 6.22 ± 0.48, 3.04 ± 0.05 vs 4.22 ± 0.28 and 2.74 ± 0.11 vs 3.33 ± 0.10 log cfu/gm, respectively. The bacterial counts for the meat samples although high were below 10^{-7} where spoilage of meat occur (Warriss, 2001). But retail meat had significantly higher microbial load compared to samples collected from abattoirs. Previously, it has been demonstrated that mincing meat using dirty equipment significantly increases the level of contamination in meat as compared to that in whole carcasses and the process of mincing has the potential to increase microbial load and introduce important pathogens (Fenlon *et al.,* 2008).

From the findings, it is clear that the higher microbial load of meat contact surfaces (equipment and floor) could be the reason for cross contamination of the carcass vis-à-vis meat and higher TPC, FCC and TSC in retail shop meat compared to abattoir samples. This is in agreement with the reports of different researchers that the place of slaughter and its surrounding environment (floors, platforms, walls, knives, axe, saw-blade, hooks and hand swabs) are the critical points in the slaughterhouse and main sources of contamination of meat (Tarwate *et al.,* 1993; Cooper, 1999; Sofos *et al.,* 1999).

This study presents the contamination status of meat during processing, handling and equipment used in retail shops and abattoirs. The higher TPC obtained from the floor, hands, cutting wood and knife in this study is an indication of their repeated use with inadequate cleaning, either before commencement or after completion of work and is suggestive of lack of good management practice (GMP) at the retail shops. This could either be due to the lack of knowledge on personal hygiene or absence of sterilization and sanitation facilities for the equipment used; repeated use of a single knife in spite of contact with dirty or contaminated surfaces. Another reason for high level of counts in meat of retail meat shop may be due to the untrained personnel engaged in slaughtering and processing. The whole process is carried on the floor and the same person performs all operations and uses the same knife. In contrast, the evisceration process in abattoir is carried out on a production line and by different persons for different operations.

In conclusion, the contamination of the meat contact surfaces, equipment and water used in retail shops coupled with poor level of personnel hygiene of workers may be the main reason for higher microbial load in chevon in retail meat shops. To minimize the contamination of meat and to reduce the risk of transmission of meat borne pathogens of public health importance, there is an urgent need to implement simple scientific meat

safety interventions. To ensure the quality of meat, enhancing the knowledge of butchers on personal and environmental hygiene is required through awareness and training programmes, which would go a long way towards providing wholesome and safe meat to the consumers.

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