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Microbiological Quality of Meat Collected from Municipal Slaughter Houses and Retail Meat Shops from Hyderabad Karnataka Region, India

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Abstract

In the present research work, 300 meat samples (50 beef, 50 carabeef, 50 chevon, 50 mutton, 50 pork and 50 chicken) collected from the municipal slaughter houses and the retail meat shops from Hyderabad Karnataka region of Karnataka state, India, were analyzed for the microbiological quality; standard plate count and isolation and confirmation of *Staphylococcus, Salmonella, E. Coli, Listeria* and *Clostridium* by selective plating, microscopic examination and biochemical characterization. As per Food Safety and Standards (FSS) regulations 2011, of the samples analyzed, 89 (29.66%) (21 beef, 26 carabeef, 9 chevon, 7 mutton, 14 pork and 7 chicken) samples exceeded the limit of 10,000 CFU/gram of total viable count. Twenty (6.66%) samples (8 beef, 9 carabeef and 3 pork) exceeded the limit for *Staphylococcus (*100/gram maximum), 15 (5%) samples (9 pork, 4 chicken and 2 mutton) exceeded the limit for *Salmonella (*absent in 25 gram) and 22 (7.33%) samples (11 pork, 4 chicken, 4 beef and 3 carabeef) exceeded the limit for *E. Coli (*100/gram maximum). None of the samples were positive for *Listeria* and *Clostridium* spp. The finding in this study specifies the probable contamination during farming and on-floor slaughtering and accentuates the requirement of the upgrading the municipal slaughter houses and training of retail outlet sellers.

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1. Introduction

The Microbiological quality of meat and meat products is very important with regards to public health significance. There are several reports on outbreaks of food borne illnesses because of consumption of meat (Lunden *et al*., 2003; Prakash *et al.*, 2005; Bhandare *et al.,* 2007). The meat is potentially subjected to contamination from a variety of sources within and outside animal during the slaughter of animal and during its sale. In living animals, those surfaces in contact with the environment harbor a variety of microorganisms. The contaminating organisms are derived mainly from the hide of the animal and the faeces. The place of slaughter, the environment of the slaughter house (Cooper, 1999; Sofos et al., 1999), the floor of the retail outlet, the air in the outlet and the vehicle used for the transport of the meat from the slaughter house to the retail outlet act as the external sources for the contamination of the meat (Sudhakar *et al.,* 2009).

Food habits of Indian society have substantially changed due to rapid urbanization and westernization, resulting in people converting from vegetarian to non vegetarian diet. Hence the demand for meat is increasing. Simultaneously the consumers have become more alert regarding the quality, freshness and health aspects of meat (Selvan *et al.*, 2007).The absence of organized slaughter house facility and the existence of small retail outlets have been the two biggest hurdles for hygienic production of meat.

Hence the present research work was undertaken to check the microbiological quality of the meat collected from the municipal slaughter houses and the retail meat shops from Hyderabad Karnataka region with the following objectives:

- To collect the meat samples from municipal slaughter houses and retail meat outlets of different locations in Hyderabad-Karnataka region of the Karnataka state, India.
- To check the microbiological quality of the meat samples by estimation of standard plate count and isolation and confirmation of the food borne infection causing bacteria - *Staphylococcus, Salmonella, E. Coli, Listeria* and *Clostridium*

2. Materials and Methods

2.1. Sample Collection

Three hundred (300) meat samples were collected from different municipal slaughter houses and the retail meat outlets of various towns and districts of Hyderabad Karnataka region, Karnataka state, India. The samples consisted of 50 beef, 50 carabeef, 50 mutton, 50 chevon, 50 pork and 50 chicken meat samples. 250 g of each meat sample was collected in a sterile polythene bags, packed in a box embedded with ice packs and transported to the lab. The samples were processed within 24 hours after bringing to the laboratory.

2.2. Sample Preparations

Ten grams of meat sample was taken and homogenized into 90 ml of normal saline using a meat grinder under sterile conditions. Ten fold dilutions of the homogenates up to 10^{-5} in normal saline were made using sterile pipettes (Fawole and Oso, 2001).

3. Microbialogical Analysis

3.1. Media

All the media used were purchased from Himedia®

3.2. Total Viable Count

The total viable count (TVC) was determined by standard pour plate method (Scott Sutton, 2011). Dilutions of 10^{-5} were prepared. Dilutions of each sample were inoculated in duplicate in to the standard plate count agar medium just before solidification of the agar. On solidification of agar, the plates were incubated at 37^{o}C for 24 hours. After 24 hours of incubation the colonies were counted using colony counter. The result was calculated using following formula:

$$N = \frac{\sum c}{(n1 + 0.1 \times n2)\, d}$$

Where

- $\sum c$: Sum of colonies counted on all the dishes retained.
- n1 : Number of dishes retained in the first dilution.
- n2 : Number of dishes retained in the second dilution.
- d : Dilution factor corresponding to the first dilution.

The total viable counts were expressed as CFU/g.

3.3. Isolation and Enumeration of Specific Bacteria

The specific bacteria; *Staphylococcus, Salmonella, E. Coli, Listeria* and *Clostridium,* which cause food poisoning, were isolated by using selective media. The specific bacteria were identified and confirmed by the colony characters on the selective media, microscopic examination (Beveridge, 2001) and biochemical characterization. The samples which were positive for the aforesaid bacteria were diluted up to 10^{-3} and inoculated on to the selective media and the specific counts were enumerated. The spread or pour plate method was employed for the inoculation with 0.1 ml inoculum.

3.4. Staphylococcus spp.

Baird Parker agar was used for isolation and enumeration of *Staphylococcus* spp. The plates were incubated aerobically at 37^{0}C for 24-48 hours.

3.5. Salmonella spp.

Each of the samples (25 g) were homogenised and pre-enriched in 225 ml buffered peptone water at 37^{0}C for 24-48 hours. One ml of culture was transferred to 10 ml of selenite cystine broth (selective enrichment medium) and incubated at 44^{0}C for 18 hours. Selective plating was done on Brilliant Green Agar incubated aerobically at 43^{o}C for 24 hours.

3.6. E. coli

The samples were enriched in MacConkey broth and incubated at 44^0C for 24 hours. Then selective plating was done on MacConkey agar and incubated at 44^0C for 24 hours. The E. Coli was further confirmed by inoculating the colonies from MacConkey agar on to Eosin Methylene Blue (EMB) agar.

3.7. Listeria spp.

The samples were enriched in two steps using Fraser Broths I and II and incubated at 30 °C for 24 hours. The selective plating was done on Polymixin B Acriflavin Lithium Chloride Ceftazidime Ascolin and Mannitol (PALCAM) agar and incubated at 30 °C for 48 hours.

3.8. Clostridium spp.

The samples were enriched in Robertson Cooked Meat Medium incubated at 44 °C for 24 hours. The selective plating was done on Sulphate Polymixin Sulphadiazine Agar and incubated at 37 °C for 24 hours in anaerobic conditions using anaerobic jar.

3.9. Statistical Analysis

In the present study mean as a measure of central tendency and the standard error as a measure of random error were employed for the statistical analysis (Snedecor and Cochran, 1994). The two sample test with *P* value of 0.05 was used to know the significant variation between the two groups.

4. Results

4.1. Total Viable Count

Of the 300 meat samples analyzed, 89 (29.66%) samples exceeded the limit of 10,000 CFU/gram of total viable count, the limit set by Food Safety and Standards (FSS) Regulations, India, 2011. The 89 samples consisted of 21 (42%) beef, 26 (52%) carabeef, 9 (18%) chevon, 7 (14%) mutton, 14 (28%) pork and 7 (14%) chicken) (Table 1).

Isolation and enumeration of specific bacteria: The bacteria responsible for food poisoning; Staphylococcus, Salmonella, E. Coli, Listeria and Clostridium, were isolated and enumerated by using selective media. The bacteria were identified and confirmed by the colony characters on the selective media, microscopic examination (Beveridge, 2001) and biochemical characterization.

The result is presented in the Table 1.

Of the 300 meat samples analysed 15 (5%) samples were positive for Salmonella, 30 (10%) samples were positive for E. Coli and 25 (8.33%) samples were positive for Staphylococcus. None of the samples were positive for Listeria and Clostridium spp. None of the chevon samples was positive for any of the above stated food poisoning causing bacteria.

As per FSS regulations 2011, 15 (5%) samples consisting of 9 pork, 4 chicken and 2 mutton exceeded the limit for *Salmonella (*absent in 25 gram), 22 (7.33%) samples consisting of 11 pork, 4 chicken, 4 beef and 3 carabeef exceeded the limit for *E. Coli (*100/gram maximum) and 20 (6.66%) samples consisting of 8 beef, 9 carabeef and 3 pork exceeded the limit for *Staphylococcus (*100/gram maximum).

Table 1. Microbiological quality of the meat samples

Sample/ parameter		Beef	Carabeef	Mutton	Chevon	Pork	Chicken	Total
No. of Samples analyzed		50	50	50	50	50	50	300
Salmonella	*	--	--	2 (4%)	--	9(18%)	4 (8%)	15(5%)
	**	--	--	2 (4%)	--	9(18%)	4 (8%)	15(5%)
E. coli	*	8(16%)	6 (12%)	--	--	11(22%)	5(10%)	30(10%)
	**	4(8%)	3 (6%)	--	--	11(22%)	4(8%)	22(7.3%)
Staphylococcus	*	11(22%)	11(22%)	--	--	3(6%)	--	25(8.3%)
	**	8(16%)	9(18%)	--	--	3(6%)	--	20(6.66%)
Listeria	*	--	--	--	--	--	--	--
	**	--	--	--	--	--	--	--
Clostridium	*	--	--	--	--	--	--	--
	**	--	--	--	--	--	--	--
Total viable count exceeding the limit		21(42%)	26(52%)	9(18%)	7(14%)	14(28%)	7(14%)	89(29.6%)

* No. of samples positive
* No. of samples exceeding limit

5. Discussion

In this study, 29.66% meat samples were found to be exceeding the limit of total viable count set by FSS regulations 2011 which is significantly very high. 42% beef samples and 52% carabeef samples exceeded the limit. The percentage of other species meat samples that exceeded the limit for total viable count were 28% pork, 18% mutton and 14% each of chevon and chicken. 16% beef and 18% carabeef samples exceeded the limit for Staphylococcus. During the collection of the samples it was observed that on-floor slaughtering was followed to slaughter cattle and buffaloes, where in the ubiquitous Staphylococcus might have contaminated the meat and may also be responsible for high total viable count (Bradeeba and Sivakumaar, 2013).

22 % and 18% of pork samples exceeded the limit for *E. Coli* and *Salmonella,* respectively. Among other species, 8% beef, 6% carabeef and 8% chicken exceeded the limit for *E. Coli.* 4% mutton and 8 % chicken exceeded the limit for *Salmonella.* The reason for more number of pork samples exceeding the limit for total viable count, *Salmonella* and *E. Coli* could be the unorganized farming of pigs.

None of the chevon samples was positive for any of the above stated food poisoning causing bacteria. And even the percent of chevon samples exceeding the total viable count limit was less (14%) compared to other species meat. None of the samples were positive for *Listeria* and *Clostridium* spp. This could be because the meat is consumed fresh and is not processed nor stored at lower temperatures (Lunden *et al.,* 2003).

The findings in this study are in tandem with the findings of other research workers (Prakash *et al.,* 2005; Bhandare *et al.,* 2007; Sudhakar *et al.,* 2009 and Sengupta *et al.,* 2011). However the results are in contrast to the findings of Selvan *et al.,* 2007, where in mutton samples were of poor quality and pork samples were better compared to other species meat. The result obtained in this study signifies the importance of upgradation of the municipal slaughter houses and retail outlets and also the training of the personnel involved in the meat production and marketing chain.

References

[1] Lunden, J.M., T.J. Autio, A.M. Sjoberg and Korkeala, H.J. Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *Journal of Food Protection* 2003; 66: 2062-2069.

[2] Prakash, B., Krishnappa, G., Muniyappa L. and Kumar, B.S. Epidemiological characterization of avian Salmonella enterica serovar infections in India. *International Journal of Poultry Science* 2005; 4 (6): 388-395.

[3] Bhandare, S. G., A. T. Sherikar, A. M. Paturkar, V. S. Waskar, and R. J. Zende. A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food. Contr* 2007; 18:854-868.

[4] Cooper, K. The Plant Environment Counts: Protect your Product through Environmental

[5] Sampling. *Meat and Poultry* 1999; 5: 5.

[6] Sofos JN, Kochevar SL, Bellinger GR, Buege DR, Hancock DD, Ingham SC, Morgan JB, Reagan JO, Smith GC. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *J Food Prot* 1999; 62(2):140-5.

[7] Sudhakar G. Bhandare*, A. M. Paturkar, V. S. Waskar and R.J. Zende. Bacteriological screening of environmental sources of contaminationin an abattoir and the meat shops in Mumbai, India. *As. J. Food Ag-Ind* 2009; 2(3): 277-287.

[8] Selvan, P., Narendra,R., Babu,S., Sureshkumar., Venkataramanujamv. Microbial Quality of Retail Meat Products Available in Chennai city. *American journal of Food Technology* 2007; vol.2 (1): p.55-59.

[9] Fawole, M.O. and Oso, B.A. Laboratory manual of Microbiology: Revised edition spectrum books Ltd, Ibadan 2001; p.127.

[10] Scott Sutton. Accuracy of Plate Counts. *Journal of Validation Technology* 2011; 42-46.

[11] Beveridge, T.J. "Use of the gram stain in microbiology". *Biotechnic and Histochemistr* 2001; 76 (3): 111–8.

[12] Snedecor, GW and Cochran, WG (1994). Statistical methods. 8th Edn. Iowa State University. Press, Ames, Iowa.

[13] Bradeeba, K. and P.K. Sivakumaar. Assessment of microbiological quality of beef, mutton and pork and its environment in retail shops in Chidambaram, Tamil Nadu. *International Journal of Plant, Animal and Environmental Sciences* 2013; 3(1): 91-97.

[14] Sengupta R., R. Das, S. Ganguly and S. K. Mukhopadhayay. Survey on microbial quality of chicken meat in Kolkata, India. *International Journal of Research in Pure and Applied Microbiology* 2011; 1 (3): 32-33.