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Research Article

Bacteriological screening of environmental sources of contamination in an abattoir and the meat shops in Mumbai, India

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Abstract

Bacteriological screening of environmental sources of contamination was carried out in Deonar abattoir and traditional meat shops in Mumbai. A total of 54 swab samples from different environmental contaminants were collected and analyzed from the abattoir, while 81 swab samples were analyzed from three meat shops. These samples were processed for total viable count (TVC) and differential counts. The average TVC for all environmental contamination points in the abattoir was 5.80 ± 0.17, whereas in the shops it was 6.05 ± 0.25 log CFU/sq.cm indicating higher microbial load in traditional meat shops. In the abattoir, the maximum numbers of isolates were found on the floor and minimum numbers in water. *S. epidermidis*, *S. aureus*, *Micrococcus* spp. and faecal coliforms were found to be the predominant organisms. In the shops, the maximum numbers of isolates were found on floors and minimum numbers on plastic bags. *Micrococcus* spp., faecal coliforms and *S. epidermidis* dominated the differential flora. The prevalence of *Salmonella* spp. was 1.85% in the abattoir, while in the retail meat shops the prevalence was as high as 14.8%. In addition, wooden logs used in the shops also showed substantial contamination. The bacteria isolated from these sources were of public health importance and thus, these points need proper cleaning and sanitization to avoid cross contamination of the meat. A thorough clean up procedure not only prevents contamination but also creates a clean environment and encourages cleanliness amongst workers. The results

obtained represent an index of the sanitary quality of meat production in an Asian developing country.

Keywords: Swabs, bacteria, total viable counts, differential counts, abattoir, meat shops

Introduction

The safety aspect of meat production is not highly regarded in India and other Asian developing countries. The practices of hygiene and sanitation prevailing in the Indian meat production system encourage access to microbial contamination [1]. The major proven cause of rapidly rising, diet-related morbidity and mortality is food-borne diseases arising from microbial contamination as consumers have very little knowledge about the microbiological hazards in meat [2]. This phenomenon is more concerning, because it is avoidable, as it arises from neglect of simple hygienic precautions. The majority of the population in India consume meat from traditional meat shops, where individual butchers slaughter a few sheep/goats for sale of meat in small quantities throughout the day, but there are a few large modern abattoirs mostly for export purposes. The risk of contamination exists from the point of entry of animals to the slaughter slab until the time of consumption of meat. The abattoir and meat shop environments play important roles in the spreading of microbial contamination. The modern product based emphasis on hazard analysis and critical control point (HACCP) approach has lowered the priority for equipment and non-product contact areas, which are the potential sources of microbial contamination. The inspection of floors, walls, ceilings, drains, overhead lines, platforms, equipment, people, water and transport vehicles should be done routinely. This study was aimed to analyze bacteriological contamination from environmental sources in a modern Indian abattoir and traditional meat shops.

Materials and Methods

Collection of swab samples

A total of 54 samples were taken at the abattoir in Deonar, Mumbai on six occasions from water used for washing carcasses, hand swabs of butchers, knives, evisceration platform, hooks, abattoir floor, abattoir wall, transportation van floor and transportation van wall. A total of 81 samples were collected at three meat shops on nine occasions from water used for washing carcasses, hand swabs of butchers, knives, wooden logs used for chopping meat, hooks, shop floor, shop wall, weighing balance and plastic bags. The surfaces were sampled by sterile cotton wool swabs (3 cm long and 1 cm in diameter) on wooden sticks. Each cotton wool swab was moistened with 0.1% peptone water prior to its use. The swabs were rubbed on sites continuously for 30 seconds and transferred to a sterile screw-capped test tube containing 10 ml of sterile maintenance medium (0.85% NaCl and 0.1% peptone). Ten ml of washing water was also collected in sterile test tube. The screw-capped test tubes were brought to the laboratory in a thermos flask containing ice and processed immediately.

Processing of samples

Test tubes containing swabs were vortexed for 30 seconds for uniform distribution of micro-

organisms. Tenfold serial dilutions up to 10^{-6} of all the samples were prepared using sterile normal saline solution (NSS) and the samples were processed for total viable count and differential counts. All the media used were dehydrated and purchased from Himedia laboratories, Mumbai, India.

Total viable count

The total viable count (TVC) was determined by standard pour plate method. Dilutions of 10^{-4} and 10^{-5} were used. Dilutions of each sample were inoculated in duplicate in to the medium. On solidification of agar, the plates were incubated at 37° C for 24 hours. The counts were expressed as log CFU/cm^2.

Differential counts

Differential counts for various pathogenic and spoilage organisms were enumerated using differential media. The spread or pour plate method was employed with 0.1 ml inoculum from 10^{-2} and 10^{-3} dilutions decided on the basis of the results of the pilot study.

***Staphylococcus* spp.**

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

***Bacillus* spp.**

Egg yolk agar and blood agar were used for isolation and enumeration of *Bacillus* spp. The plates were incubated aerobically at 37^{0}C for 24-48 hours. *B. cereus* isolation agar (Himedia code M833) was used for isolation and identification of *B. cereus* in suspected cases.

Enterococcus counts

Enterococcus counts were estimated by use of Slanetz and Bartley medium. Plates were incubated aerobically at 44^{0}C for 24-48 hours.

***Clostridium* spp.**

The double layer technique with a layer of sodium polymyxin sulphadiazine (SPS) agar at the bottom of a tube, an inoculum in between and then a layer at the top was employed to create anaerobic conditions for *Clostridium* species isolation. The tubes were incubated at 44°C for 24 hours.

Enterobacteriaceae counts

Various members of the family *Enterobacteriaceae* were isolated and enumerated using MacConkey agar with crystal violet. Eosin methylene blue agar (37^{0}C for 24-48 hours) was employed for isolation and identification of *E. coli*.

Faecal coliforms

The pour plate technique was used for enumerating the faecal coliforms. Molten violet red bile agar (at 45-50^{0}C) was poured on 0.1 ml inoculum and the plates were incubated aerobically at 44^{0}C for 24-48 hours.

***Isolation of Salmonella* spp.**

Swab samples were homogenised and pre-enriched in 225ml buffered peptone water at 37^0C for 24-48 hours. One ml of culture was transferred to 10 ml of selenite cystine broth (selective enrichment medium) and incubated at 44^0C for 18 hours. Selective plating was done on brilliant green sulpha agar and bismuth sulphide agar and incubated aerobically at 43^oC for 24 hours.

Characterisation and identification of isolates

Two to three characteristic colonies of each bacterium were further subjected to purification, identification and characterisation. Microscopic examination was carried out on smears stained by modified Gram's staining as described by Beveridge [3]. Characterisation and identification of organisms was done according to the methods described by Barrow and Feltham [4] and Cheesbrough [5].

Statistical analysis

All counts were converted to $\log_{10}$ CFU cm^{-2} for analysis. The differences between slaughtering operations for total viable counts and differential counts on the carcass sites were evaluated by using analysis of variance for one-way classification according to Snedecor and Cochran [6].

Results and Discussion

The average TVC for all environmental contamination points in the abattoir was 5.80 ± 0.17, whereas in the shops it was 6.05 ± 0.25 log CFU/cm^2 (Tables 1 and 2; Figure 1). Simard and Auclair [7] reported TVC of working surface in various plants between 10^6 to 10^8 CFU/cm^2. In the abattoir, the highest TVC was observed on the floor (7.19 ± 0.18 log CFU/cm^2) and the lowest in water (3.90 ± 0.07 log CFU/ml), while at retail meat shops the highest TVC was noted on the shop floor (7.45 ± 0.46 log CFU/cm^2) and the lowest on plastic bags (3.08 ± 0.24 log CFU/cm^2) (Tables 1 and 2). Similarly, Tarwate *et al.* [8] reported TVC between 2.07 ± 0.06 log CFU/ml for water to 6.70 ± 0.15 log CFU/cm^2 for slaughterhouse floor in an organised slaughterhouse. Narasimha Rao and Ramesh [9] also found the count of 2.5 x 10^6 for the floor.

Amongst the environmental contaminants in the abattoir, the maximum numbers of isolates were found on the floor and minimum numbers of isolates were found in water. *S. epidermidis, S. aureus, Micrococcus* spp. and faecal coliforms were found to be the predominant organisms. In the case of environmental sources of contamination in the shops, the maximum numbers of isolates were found on floors and minimum number on plastic bags. *Micrococcus* spp., faecal coliforms, and *S. epidermidis* dominated the differential flora.

In the abattoir, the highest percentage prevalence was recorded for *Micrococcus* spp. (66.7%) and the lowest for *Salmonella* spp. (1.85%). At the shops, pooled maximum prevalence was seen in the case of *Micrococcus* spp. (63.0%) and minimum in *B. cereus* (12.3%). As indicated earlier, the prevalence of *Salmonella* spp. was 1.85% in the abattoir, while in the shops the prevalence was as high as 14.8% (Tables 1 and 2). Borse *et al.* [10] recovered *Salmonellae* from two samples on the slaughterhouse floor and one each from platform and knife samples. Smeltzer *et al.* [11] found *Salmonella* on knives, steels etc. and other equipment that accidentally or indirectly

contaminate the carcasses. However, Gupta *et al.* [12] found the prevalence of *Salmonella* spp. as 13.3% for slaughterhouse and 10% for retail shops.

Analysis of variance between environmental contaminants in the abattoir revealed highly significant differences ($P<0.01$) for all the organisms (Table 1). Pooled averages of all the organisms in the shops also revealed highly significant ($P<0.01$) differences, except for *B. subtilis,* faecal coliform and *E. Coli,* which showed non-significant variations amongst the sources of environmental contamination (Table 2).

Floors, platforms and walls on most occasions are contaminated due to microorganisms brought in by animals along with hides and faeces and also through blood droppings and rupture of viscera [13]. Animals have their own normal micro-flora and tend to harbour various types of organisms found in their environment which may have been reflected in the occurrence of a wide range of bacteria in the present study. Carcasses themselves contaminate the walls and platforms through contact and the movement of personnel adds to the bacterial load on floors and platforms. The situation is further aggravated by the ridged surfaces on platforms, uneven surfaces, cracks and crevices on the floors and walls where meat particles and moisture accumulate resulting in the growth and multiplication of bacteria. The muddy floors without any concrete work in the traditional meat shops further accentuated the situation. Free access to crows, dogs, cats, rodents and flies in the traditional meat shops might have worsened the situation leading to cross contamination. Butchers and other workers in the abattoir investigated were untrained and thus they neglected the maintenance of hygienic standards which invariably contributed to the microbial contamination and also elevated the bacterial load. Prevalence of bacteria on workers' hands due to contact with carcasses and other body parts during operations is much higher.

It was also observed that the abattoir workers pushed knives into their pant belts directly touching the backside. Besides, knives were not washed intermittently between the operations in the abattoir, although there was provision of a hot water bath for knives. The bacterial flora obtained from knives, hooks and hands in the present study can be attributed to the direct contact with carcasses, viscera and the organs. This is also evident from the prevalence of common types of bacteria observed on the environmental contaminants (Tables 1 and 2). The results can be substantiated with the observations of Sammarco *et al.* [14], who studied the slaughterhouse environment, work surfaces, equipment and workers hands.

In the abattoir and the shops, maximum total and differential load, along with high prevalence of organisms, was noticed on the abattoir as well as shop floors. Besides, wooden logs in the shops showed substantial contamination. Blood droppings, meat/fat particles, crushed bone and gut contents falling/sprinkling continuously on the floor, their ineffective removal or their spread from one point to another due to continuous movement of personnel in the slaughter area may have resulted in higher counts on the floor. Wooden logs are used for cutting the carcass into bits and chopping or mincing of meat in the shops. They are not washed after use nor can they be sterilized. Wood being absorptive in nature may deposit blood/drip, which serves as an ideal medium for growth and multiplication of food borne microorganisms. All of these factors could also have resulted in a higher load on the wooden logs used in the meat shops.

Table 1. Mean bacterial counts (log CFU/sq.cm. ± S. D.), analysis of variance between environmental contaminants and % prevalence of various organisms in the abattoir.

		Water ^	Hand swabs	Knives	Evisceration platform	Hook	Abattoir floor	Abattoir wall	Transportation van floor	Transportation van wall	Average counts	F values	% prevalence
1	Total Viable Count	3.90 ± 0.07	4.87 ± 0.40	5.52 ± 0.03	6.04 ± 0.15	5.10 ± 0.14	7.19 ± 0.18	6.22 ± 0.11	7.10 ± 0.05	6.24 ± 0.41	5.80 ± 0.17	16.50**	-
2	*S.aureus*	-	3.11 ± 0.08	3.56 ± 0.19	4.08 ± 0.84	3.01 ± 0.06	4.20 ± 0.31	3.63 ± 0.84	3.13 ± 0.70	3.50 ± 0.56	3.53 ± 0.45	8.50**	59.3
3	*S.epidermidis*	-	3.65 ± 0.06	3.58 ± 0.16	5.19 ± 1.10	3.87 ± 0.24	5.68 ± 0.78	5.04 ± 0.30	4.06 ± 0.72	4.94 ± 0.38	4.50 ± 0.47	12.40**	59.3
4	*Micrococcus* spp.	2.08 ± 0.17	3.56 ± 0.81	3.14 ± 0.28	4.88 ± 0.54	4.17 ± 0.08	5.88 ± 0.73	5.26 ± 0.91	5.82 ± 0.34	5.03 ± 0.68	4.42 ± 0.50	13.98**	66.7
5	*Enterococcus faecalis*	-	3.01 ± 0.08	3.10 ± 0.17	4.17 ± 0.38	-	4.50 ± 0.28	3.81 ± 0.73	4.13 ± 0.38	4.05 ± 0.56	3.82 ± 0.37	181.01**	50.0
6	*B.cereus*	-	-	-	3.18 ± 0.06	-	4.27 ± 0.08	3.93 ± 0.21	3.18 ± 0.18	-	3.64 ± 0.13	34.44**	18.5
7	*B.subtilis*	-	3.30 ± 0.00	3.54 ± 0.09	4.22 ± 0.01	4.20 ± 0.04	4.69 ± 0.55	3.80 ± 0.00	4.18 ± 0.05	4.54 ± 0.52	4.06 ± 0.16	9.97**	37.0
8	*Clostridium* spp.	-	3.39 ± 0.55	3.10 ± 0.13	4.11 ± 0.26	-	4.39 ± 0.12	4.00 ± 0.28	3.39 ± 0.12	4.08 ± 0.35	3.78 ± 0.26	39.03**	35.2
9	F.coliforms	-	-	3.80 ± 0.15	4.00 ± 0.30	3.11 ± 0.18	4.68 ± 0.38	3.72 ± 0.43	4.41 ± 0.83	3.81 ± 0.45	3.93 ± 0.39	53.87**	55.6
10	*E.coli*	-	-	3.54 ± 0.09	3.81 ± 0.05	-	4.13 ± 0.12	3.20 ± 0.38	3.55 ± 0.81	3.89 ± 0.14	3.71 ± 0.27	16.68**	33.3
11	*K.aerogenes*	2.00 ± 0.00	3.13 ± 0.04	3.09 ± 0.13	3.53 ± 0.61	-	4.31 ± 0.84	3.78 ± 0.34	3.42 ± 0.22	3.86 ± 0.18	3.39 ± 0.30	29.79**	50.0
12	*Salmonella* spp.	-	-	-	-	-	Present(1)	-	-	-	-	-	1.85

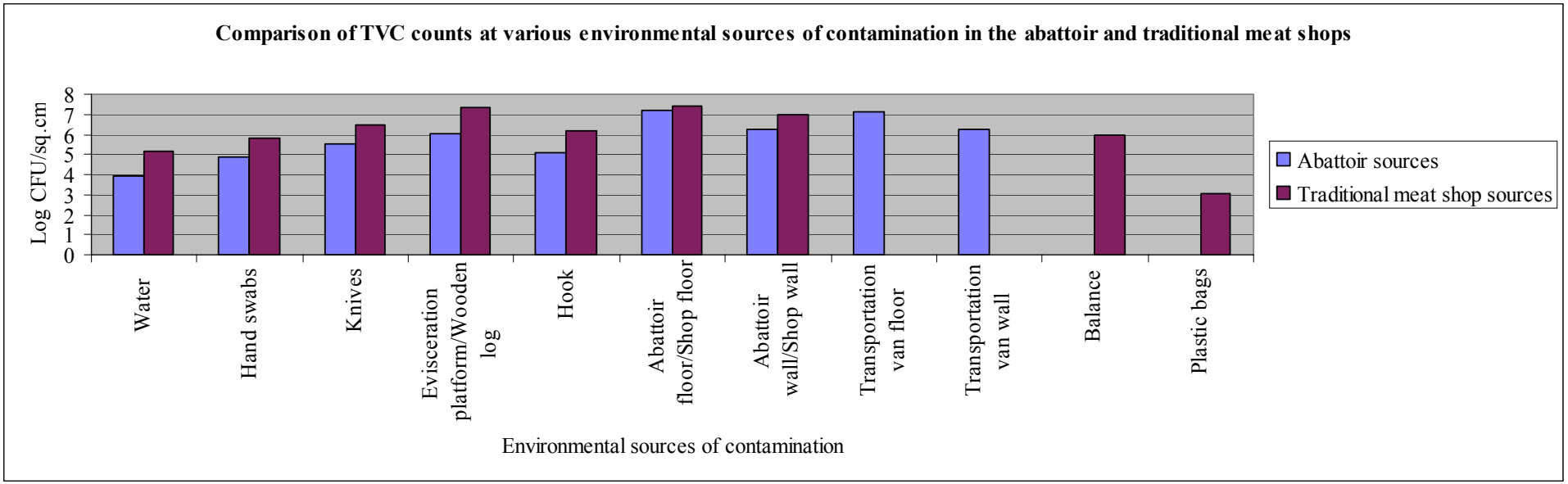
Figures in parenthesis indicate number of positive samples
[-] = Not detected
** = Significant at 1% level (P<0.01)
^ = Counts are expressed in log CFU/ml ± S. D.

Table 2. Mean bacterial counts (log CFU/sq.cm. ± S. D.), analysis of variance between environmental contaminants and % prevalence of various organisms in the shops.

		Water ^	Hand swabs	Knives	Wooden log	Hook	Shop floor	Shop wall	Balance	Plastic bags	Average counts	F values	% prevalence
1	Total Viable Count	5.18 ± 0.23	5.85 ± 0.16	6.45 ± 0.26	7.33 ± 0.20	6.15 ± 0.16	7.45 ± 0.46	7.01 ± 0.19	5.93 ± 0.31	3.08 ± 0.24	6.05 ± 0.25	52.10**	-
2	*S.aureus*	-	4.12 ± 0.21	3.40 ± 0.23	4.62 ± 0.55	3.63 ± 0.09	4.90 ± 0.34	4.42 ± 0.62	3.48 ± 0.35	3.00 ± 0.00	3.95 ± 0.30	7.80**	49.4
3	*S.epidermidis*	3.42 ± 0.28	4.49 ± 0.28	4.78 ± 0.26	5.61 ± 0.33	4.78 ± 0.35	6.20 ± 0.31	4.86 ± 0.47	4.80 ± 0.24	-	4.87 ± 0.32	33.38**	56.8
4	*Micrococcus* spp.	3.42 ± 0.09	4.45 ± 0.56	5.04 ± 0.69	5.60 ± 0.81	4.21 ± 0.62	5.39 ± 0.32	4.82 ± 0.78	4.42 ± 0.57	2.17 ± 0.13	4.39 ± 0.44	11.40**	63.0
5	*Enterococcus faecalis*	3.44 ± 0.32	3.87 ± 0.44	4.10 ± 0.33	5.00 ± 0.23	3.89 ± 0.31	4.88 ± 0.22	4.71 ± 0.48	4.34 ± 0.70	-	4.28 ± 0.38	7.66**	55.6
6	*B.cereus*	-	3.13 ± 0.12	3.53 ± 0.06	4.57 ± 0.08	-	4.09 ± 0.15	4.36 ± 0.05	-	-	3.93 ± 0.09	16.29**	12.3
7	*B.subtilis*	3.58 ± 0.16	4.04 ± 0.33	4.23 ± 0.40	4.35 ± 0.46	3.90 ± 0.21	4.63 ± 0.30	3.32 ± 0.16	3.09 ± 0.12	2.00 ± 0.00	3.68 ± 0.24	1.62^{ns}	35.8
8	*Clostridium* spp.	-	3.40 ± 0.38	3.43 ± 0.19	4.31 ± 0.08	2.75 ± 0.2	4.39 ± 0.38	3.60 ± 0.38	3.11 ± 0.14	-	3.57 ± 0.24	3.23*	27.2
9	F.coliforms	3.23 ± 0.28	3.58 ± 0.20	3.45 ± 0.31	4.63 ± 0.54	3.23 ± 0.32	4.99 ± 0.43	4.19 ± 0.62	3.96 ± 0.26	-	3.91 ± 0.37	1.66 ^{ns}	59.3
10	*E.coli*	3.62 ± 0.22	3.44 ± 0.45	3.66 ± 0.19	4.10 ± 0.21	3.45 ± 0.34	4.25 ± 0.18	4.10 ± 0.48	3.42 ± 0.31	-	3.76 ± 0.30	1.66^{ns}	48.1
11	*K.aerogenes*	3.48 ± 0.75	4.20 ± 0.60	3.63 ± 0.72	4.54 ± 0.25	3.16 ± 0.27	4.27 ± 0.49	3.72 ± 0.34	3.76 ± 0.82	2.59 ± 0.11	3.70 ± 0.48	6.55**	46.9
12	*Salmonella* spp.	-	-	Present (2)	Present (4)	-	Present(5)	Present (3)	Present (1)	-	-	-	14.8

Figures in the parenthesis indicate number of positive samples
[-] = Not detected
* = Significant at 5% level (P<0.05)
** = Significant at 1% level (P<0.01)
^ = Counts are expressed in log CFU/ml ± S. D.
ns = Non-Significant

Figure 1. Comparison of TVC counts at various environmental sources of contamination in the abattoir and traditional meat shops.



From a public health point of view the isolation of bacteria from *Staphylococcus* spp. were of concern as they can cause food poisoning due to neglect in storage and handling. Similarly *Micrococcus* spp. is an opportunistic pathogen and can cause disease in immunocompromised consumers of meat. The most worrisome isolates were indicator bacteria like *Salmonella* spp., faecal coliforms and *Clostridium* spp. which are food borne pathogens and can cause severe infections in ultimate consumers. In order to avoid cross contamination of carcasses in slaughter areas it is suggested to clean and sanitize the most contaminating points such as floors, walls, evisceration platforms, wooden logs etc. Cleaning should start as soon as the butchering operations are completed so as to prevent residue hardening on the surface of floors, walls and platforms. Further, pressurized hot water spray (55°C) may be employed to clean the slaughterhouse floor, wall and platforms for complete removal of fat and protein material [15]. Intervals during breaks in production should be used for cleaning operations. Floors should slope towards drains and gutters to facilitate easy cleaning. Gutters should have rounded walls to minimize accumulation of dirt and mud. Partitioning of clean and unclean sections should be done to prevent the spread of spoilage or pathogenic organisms. The contamination of the knives and hooks can be controlled by regular cleaning, washing, sterilization and proper maintenance as this equipment comes in direct contact with carcasses and may act as vehicles. A thorough clean up procedure not only prevents contamination but also creates a clean environment and encourages cleanliness amongst workers.

Conclusions

Overall, this study revealed that the microbial load from environmental sources of contamination was much higher in traditional meat shop environments compared to the abattoir. The consumption of meat from traditional meat shops is more common in all developing countries and there are no regulations to maintain hygiene in such shops. Environmental sources of contamination play a major role in rendering the meat unsafe for human consumption. Education of the meat retailers' community regarding proper maintenance of hygiene and sanitation, enforcement of strict regulations for meat production in traditional meat shops and their regular monitoring is needed. However, a periodic surveillance of environmental contamination is required in the abattoir and the shops. Establishment of control measures depending upon the prevailing conditions with an appropriate monitoring system is necessary so that consumers get safe and wholesome meat.

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