Research Article

Microbiological Contamination of Cattle and Buffalo Meat in Peshawar, Pakistan

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Abstract | In present study, cattle and buffalo carcasses were microbiologically sampled, from slaughter house and open market to assess the hygienic practices followed in Peshawar. During sampling two different conditions were considered, one condition was hygienic and found in slaughter houses and butcher shops located in clean and covered markets where meat was displayed in covered showcase; while under un-hygienic conditions meat stalls were sampled at open streets/vegetable market where meat was kept open to dust and flies. A total of 52 meat samples, 26 each from cattle and buffaloes were collected for microbiological analysis. The higher prevalence (100%) of bacterial organisms was recorded in the meat of buffaloes. However, relatively lower prevalence (96.2%) was recorded in the meat samples of cattle. A large number of colonies (g^{-1}) and the mean bacterial counts (g^{-1}) were recorded in the meat samples of buffaloes. In g^{-1} meat sample of buffaloes, the mean number of 330 colonies was counted while quite higher number (6.6×10^8) of bacterial cells was also counted in the meat samples of buffaloes as well. However, comparatively lower number of mean colonies (195) and bacterial counts (3.9×10^8) in g^{-1} were detected in the meat samples of cattle. In the case of un-hygienic conditions, significantly higher population of bacterial cells $(4.0 \times 10^5\ g^{-1})$ was recorded in buffaloes than cattle meat $(3.7 \times 10^5\ g^{-1})$. Furthermore, in hygienic conditions, significantly higher bacterial cells $(3.2 \times 10^5\ g^{-1})$ was counted in the meat samples of buffaloes as compared to cattle meat $(3.1 \times 10^5\ /g^{-1})$. Irrespective of animal species, the higher prevalence of bacterial contamination was evident probably because of environmental conditions prevailing in the area. This study confirms the need for use of Sanitation Standard Operating Procedures and Good Meat Handling Practices for safe and hygiene meat production in the area.

Keywords | Contamination, Meat contamination, Open Market, Peshawar, Slaughter House

INTRODUCTION

Pakistan is a tropical country, with ambient temperatures which are conducive for the growth of microorganisms, which can rapidly render the meat unsafe for human consumption (Bughti et al., 2017; Doulgeraki et al., 2012). Episodes of food borne illnesses are frequently been reported in the Pakistan, but due to lack of a surveillance network, the exact magnitude of the problem in the country remains unknown. Commonly, cow, buffalo, sheep, goat, camel, and poultry meat are used as a source of protein (FAO, 2013). As general practice, raw meat is sold in open market. Particularly in Peshawar and country as a whole, majority of the population consumes meat slaughtered and butchered in small local shops where the maintenance of hygiene is always questionable (Akhtar, 2015). Meat produced for the domestic market is sold as hot meat directly to the consumers on retail meat shops. The poor

hygiene and sanitation prevailing in the abattoirs as well as the shops encourage microbial contamination, survival and growth in meat as well. The higher microbial load in the meat shops is due to floor dressing and neglect of hygiene (Bhandare et al., 2007).

The most common pathogenic bacterial species found in meat are *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella, Aeromonas* spp., *Arobacter* sppp., *Bacillus cereus, Campylobacter* spp. and *Clostridium botulinum* (Cho et al., 2012; Javed, 2016; Kamboh et al., 2017). These organisms are involved in meat poisoning. Meat may become contaminated by these pathogenic bacteria either endogenously or by subsequent postmortem contamination from blood, gastrointestinal contents, feet, hide or skin, water, knives, instruments used in slaughter hall, vehicles, personals and airborne materials (Sheridan et al., 1992). The unhygienic conditions of the slaughter houses, butcher shops, handling of meat, hot environmental condition and packing of meat further provide the source of contamination (Bhandare et al., 2007). It is generally agreed that the internal tissues of healthy slaughtered animals are free of bacteria at the time of slaughter, assuming that the animals are not in a state of exhaustion. On examination of fresh meat and poultry products at the retail level, various numbers and types of microorganisms are found (Ahmad et al. 2013). The intestinal contents along with the usual heavy load of microorganisms may be deposited onto the surface of freshly dressed carcasses. Especially important in this regard is the paunch or rumen of ruminant animals, which typically contains 10^{10} bacteria per gram. In the case of red meats, lymph nodes are usually embedded in fat often contains large numbers of organisms, especially bacteria. If they are cut through or added to portions that are ground, one may expect this biota to become prominent. Hands of handlers are the source of human pathogens to freshly slaughtered meat. Even when gloves are worn, organisms from one carcass can be passed onto other carcasses (FAO, 2013). Further that containers, where meat cuts are placed may be expected to become contaminated with the organisms. This tends to be a primary source of microorganisms to ground or minced meats. Further the handling and storage environment circulating air are significant sources of organisms to contaminate meat surfaces of all slaughtered animals (Ozlem, 2005).

Keeping in view the above facts, the present study was therefore designed to determine the microbiological load in meat obtained from local open market in Peshawar. In this study we determined whether pathogenic bacteria are present or not. And to compare the bacterial load, among the meat of different animal species viz., cattle and buffalo. Further that, the present investigation regarding the meat, provide basic knowledge about the pathogenic bacteria and their harmful activities against public health. The study also helps in recognition of bacterial species which are responsible for spoilage of meat in study area.

MATERIAL AND METHODS

COLLECTION OF MEAT SAMPLES

A total of 52 meat samples, 26 each from cattle and buffaloes were collected from different retail shops and slaughter houses of Peshawar under sterile conditions. Each sample represents the cumulative sampling of various carcass sites collected randomly in polythene bags and then brought to the Veterinary Research Institute (VRI), Peshawar, Khyber Pakhtunkhwa, Pakistan for further processing of bacteriological investigations. Furthermore, during sample collection, cleanliness status of retail shop and its surroundings was also noted, and it was categorized either hygienic on unhygienic.

PROCESSING OF MEAT SAMPLES

The collected meat samples were minced/grinded individually by using grinder/ scissor into very fine pieces, then 10g of the minced meat were properly mixed and added to 90 ml peptone water (Difco™) by stirring with a stirrer or shaking in vortex mixer. The above solution was further prepared into tenfold dilutions. The dilutions 10^{-4}, 10^{-5} and 10^{-6} were used for bacteriological investigation. Three samples, each comprising of 50µl from each dilution were streaked over on individual plates of general and selective media for isolation, characterization and quantitative study of bacterial organisms according to procedures of Haque et al. (2008). The identification of isolates was made through morphological, staining and cultural characteristics on culture media under the microscope with the help of oil immersion objective (X100). Further recognition of the bacterial species was made through different biochemical tests (Manoj et al., 2017).

STATISTICAL ANALYSIS

With the help of Microsoft Excel and Analytical Software "Statistix8.1" data was processed to calculate the means; while Duncan Multiple Rang Test was applied to compare the differences between cattle and buffalo meat for bacterial load and bacterial isolates.

RESULTS

A study was carried out on isolation and characterization of pathogenic bacterial species from meat of domestic animals. In this regard, seven bacterial species were recognized. The bacterial species identified from meat samples of animals in Peshawar were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Listeria monocytogenes, Salmonella enteritidis* and *Campylobacter jejuni*. Morphologically, the bacterial species varied from co-

Table 1: The percentage incidence of bacterial organisms in meat samples of cattle and buffaloes

Meat samples obtained from animal species	Total No. of samples examined	No. of Positive samples	% of positive samples	No of negative samples	% of negative samples
Cattle	26	25	96.2	1.0	3.8
Buffaloes	26	26	100	0.0	0.0

cci to rod shape, short rods to long rods and from short chains to long chains, Gram-positive and Gram-negative. Some bacterial species possessed flagella on their membrane. Bacterial species produced different colonies on solid and broth media. Few of them produced β and α hemolysis of red blood cells on blood agar medium. During study a number of biochemical tests were also carried-out to confirm their biochemical properties for real identification of bacterial species.

PERCENTAGE INCIDENCE OF BACTERIAL ORGANISMS IN THE MEAT

The data regarding percentage prevalence of bacterial species in meat samples of cattle and buffaloes are presented in Table 1. Of the 52 samples studied, 51 samples were found positive for bacterial contamination. The higher prevalence (100%) of bacterial organisms was recorded in the meat of buffaloes.

BACTERIAL LOAD IN MEAT SAMPLES OF CATTLE AND BUFFALOES

The data regarding bacterial mean population in meat samples of different animal species are given in Table 2. The large number of colonies (g^{-1}) and mean bacterial counts (g^{-1}) were recorded in the meat samples of buffaloes. In g^{-1} meat sample of buffaloes, the mean number of 330 colonies was counted while quite higher number of bacterial cells (6.6×10^8) was also counted in the meat samples of buffaloes as well. Comparatively lower mean number of colonies and bacterial counts g^{-1} were detected in the meat samples of cattle.

Table 2: The mean bacterial population (bacterial load) in meat samples of cattle and buffaloes.

Meat samples obtained from animal species	Total No. of positive samples	Mean No. of colonies	Total bacterial count g^{-1}
Cattle	25	195	$3.9\times10^{8\ b}$
Buffaloes	26	330	$6.6\times10^{8\ a}$

a-b different lettering in a column showing significant difference (p < 0.05)

Comparison of mean population of individual bacterial species in meat samples: The comparison of mean population of individual bacterial species was demonstrated among the meat samples of the animals (Table 3). Non-significant differences (p > 0.05) in the mean populations of *Escherichia coli* and *Pseudomonas aeruginosa* were observed

between the meat samples of cattle and buffaloes. However, the mean population of *Staphylococcus aureus, Salmonella enteritidis, Bacillus cereus* and *Campylobacter jejuni* were recorded higher (p < 0.05) in the meat samples of buffaloes as compared to cattle.

Table 3: The mean comparison of population of individual bacterial species in meat samples of cattle and buffaloes obtained by log cfu g^{-1}

Bacteria species	Cattle	Buffaloes
Escherichia coli	7.5 ^{A}	7.8 ^{A}
Staphylococcus aureus	5.3 ^{Bb}	6.4 ^{Aa}
Pseudomonas aeruginosa	4.7 ^{C}	4.8 ^{C}
Bacillus cereus	3.9 ^{Db}	4.2 ^{Ca}
Listeria monocytogenes	3.5 ^{Db}	4.3 ^{Ca}
Salmonella enteritidis	4.9 ^{Cb}	5.1 ^{Ba}
Campylobacter jejuni	3.9 ^{Db}	4.6 ^{Ca}

A-D Means log followed by different lettering in a column showing significant difference (p < 0.05).
a-b different lettering in a row showing significant difference (p < 0.05).

Table 4: The mean bacterial load in meat samples (g^{-1}) of cattle and buffaloes investigated under different conditions

Animals species	Hygienic	Un-hygienic	Total bacterial Load
Cattle	$3.1923\times10^{5\ Abb}$	$3.7308\times10^{5\ Ba}$	$3.4654\times10^{5\ A}$
Buffalo	$3.2385\times10^{5\ Ab}$	$4.0000\times10^{5\ Aa}$	$3.6192\times10^{5\ A}$

A-D Means followed by different lettering in a column showing significant difference (p < 0.05).
a-b different lettering in a row showing significant difference (p < 0.05).

Mean bacterial load in meat samples collected under different conditions: The data about mean bacterial load in the meat samples examined under different conditions are summarized in Table 4. Generally, no any suitable places for slaughtering, processing and selling of meat is available in all four provinces of Pakistan. People are slaughtering and selling meat beside the roads, small streets, open shops, small cots, and semi-open slaughter houses, under such conditions, definitely meat could be contaminated with different bacterial population. During present study fewer than two different conditions, the investigation on meat samples contamination with bacterial species was carried out. One condition was considered to be hygienic where minimum hygienic facilities were available in semi-open

slaughter houses while other was considered to be unhygienic, where open streets, sabzi mandi shops, cabins etc were brought in use. In hygienic conditions, significantly higher bacterial cells (3.23×10^5 g^{-1}) was counted in the meat samples of buffaloes while in the same conditions, the lower count of bacterial cells (3.19×10^5 g^{-1}) was recorded from the meat samples of cattle. Furthermore, in the case of un-hygienic conditions, significantly higher differences in the mean population of bacterial cells were recorded as 4.0×10^5 g^{-1} in buffaloes and lower mean population of bacterial cells (3.73×10^5 g^{-1}) was recorded in the meat samples of cattle. Significantly higher bacterial population (3.6×10^5 g^{-1}) was recorded in the meat samples of buffaloes as compared to cattle. While lower mean count of bacterial cells (3.4×10^5 g^{-1}) was recorded in the meat samples of cattle.

DISCUSSION

The meat of cattle is observed to be the least contaminated than buffalo meat sample studied. Among bacterial species isolated from the meat samples of cattle and buffaloes, the higher bacterial load of *Escherichia coli* was recorded in meat samples of animals and the lowest bacterial load of *Bacillus cereus* was recorded in meat samples of all animals investigated. However, some variation in bacterial population was observed among the meat samples of animals (Tables 2 and 3). Roberto et al. (2006) reported the aerobic mesophilic bacteria with values that ranged from 5.5 to 6.9 log^{10} cfu g^{-1}, indicating the higher contamination takes place during the slaughtering and processing stages. Fung et al. (1980) defined the number of bacterial contamination that ranged from 5.0 to 6.0 log^{10} cfu g^{-1} of aerobic microorganism's was recorded as high population of bacterial organisms considered to be not acceptable for consumption while the values up to 4.0 log^{10} cfu g^{-1} of bacterial population in meat samples could be considered to be acceptable for consumption. However, Jay (1996) reported that the total count of aerobic mesophilic bacteria between 5.0 and 7.0 log^{10} cfu g^{-1} for raw meat are considered to be normal and the values above this range could spoil the meat and cause unpleasant odour. All samples presented a total number of *Coliforms* but 96.6% presented as fecal *Coliforms* in between 2.3 and 5.0 log^{10} NMP g^{-1}. Uzeh and Adeniji (2006) reported that *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* isolated from both raw meat and tsire-suya. The total viable bacterial count varied from 20×10^2 to 289×10^2 cfu g^{-1} for the raw meat while 7×10^2 to 171×10^2 cfu g^{-1} for the tsire-suya. The *Coliforms* count varied from 4×10^2 to 71×10^2 cfu g^{-1} for raw meat and 1×10^2 to 42×10^2 cfu g^{-1} for tsire-suya, while *Staphylococcus aureus* count varied from 1×10^2 to 60×10^2 cfu g^{-1} for tsire-suya. In all cases, the bacterial count was higher in raw meat than tsire-suya. Therefore, the bacterial species and their population recorded in meat samples by

the above workers are in line to the numbers measured in the present study. However, the results of the present study regarding bacterial load/population and prevalence of bacterial species in meat samples do agree with the findings of the above authors. Further that we have recorded similar trend and pattern of contamination as recorded by the earlier mentioned workers in their studies.

Similar kind of investigation was carried out by Yadav et al. (2006) who recorded the bacterial load in 100 sheep carcasses collected from retail meat shops of domestic markets. The mean log^{10} of aerobic plate was counted as 7.26 cfu g^{-1}, and that of total *Coliforms* count while *Escherichia coli* was counted as 4.11 log^{10} cfu g^{-1} and 3.03 log^{10} cfu g^{-1}, respectively. All samples (100) were found positive for *Coliforms*, however, 49.0% was found positive for *Escherichia coli* and 3.0% for *Salmonella*. Haque et al. (2008) reported the mean values of TVC of slaughter yards and meat stalls were the log of 6.03 and log 6.53 respectively, whereas the TCC showed log of 4.85 and 3.82 respectively and that of TSC were 3.31 and 3.82 respectively. The mean values of TVC in brisket, neck and thigh regions of slaughter yards were log of 6.11, 6.01, and 6.31 while in meat stalls were log of 6.48, log 6.30, log 6.84 respectively. The TCC values of slaughter yards showed the log of 4.77, 4.36, and 5.12 whereas in meat stalls demonstrated logs of 4.94, 4.68, and 5.42 respectively. In the case of TSC values, the mean values were the log of 3.83, 3.07, 4.06, 3.96, 3.37, and 4.22 respectively. The results demonstrated the fact was that the unhygienic and poor sanitary conditions under where the meat and meat products were handled and processed were not acceptable from sanitary point of view. The statistical analysis showed that TVC and TCC obtained from meat samples of different markets and different regions of the carcass exhibited significantly ($P < 0.01$) variation in counts at regional level whereas TSC did not present any remarkable regional variation. A significant correlation ($P < 0.01$) in between TVC and TCC was found and similar significant correlation ($P < 0.01$) was also recorded in TCC and TSC, but surprisingly, no significant correlation was observed in between TVC and TSC.

During present study the bacterial load/population in the meat samples of sheep and goats was also investigated and the results were recorded. However, Sudhakar et al. (2007) investigated microbial load in sheep/goat meat samples, the mean TVC for all carcass sites after flaying in the abattoir was counted as 5.51 ± 0.36 log cfu cm^2, whereas the meat obtained from shops was 5.83 ± 0.42 log cfu cm^2. While Okodugha and Aligba (1991) determined a TVC of 6.11 log cfu cm^2 from beef at retail outlets. Also mentioned that after evisceration, the TVC increased. At the abattoir the mean TVC was recorded as 6.06 ± 0.53 log cfu cm^2 while at shops it was counted as 6.48 ± 0.27 log cfu cm^2. Borse et al. (1998) who reported the mean TVC of 6.35

log cfu cm² after evisceration of sheep carcasses, which increased 0.45 log units as compared to the post flaying level of contamination at the abattoir. Similarly, Gill and Baker (1998) also noted an increase in total counts by 0.30 and 0.64 log units, respectively after evisceration of sheep carcasses. Most butchers and shop keepers used to clean the meat by washing through simple tap water or by rubbing with a piece of cloth, both of these conditions were found to be highly contaminated.

In the light of their study, Sofos et al. (2000) explained that live animals and the environment serve as main sources for pathogenic microorganisms, which contaminate carcasses during the slaughtering process and meat products during processing, storage and handling. The decontamination processes, include animal cleaning, chemical dehairing at slaughter, spot-cleaning of carcasses before evisceration by knife-trimming or steam and vacuum, spraying, rinsing, or deluging of carcasses before evisceration and/or before chilling with water or chemical solutions (e.g., organic acids, trisodium phosphate, etc.) or steam could help to reduce the number of population of bacterial organisms. The processes applied at various concentrations or intensities, pressures (2-20 bar), temperatures (15-80 ℃) for different lengths of time (5-20 sec), individually or in sequential combinations and under hygienic conditions also decrease the bacterial population.

CONCLUSIONS

From the present study, it is concluded that meat samples, irrespective of animal species, have higher prevalence of bacterial contamination hat probably because of poor environmental conditions prevailing in the area. It was further observed that the meat samples of buffaloes were highly contaminated with bacterial organisms as compared to cattle. To avoid high level of bacterial contamination in meat, slaughtering as well as meat handling practices should be improved. To reduce the higher levels of bacterial count in meat, a good infrastructure such as dressing facility, drainage, differentiation between clean and unclean operations and maintenance of hygiene and sanitation should be constructed and provided to every slaughter house.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHOR'S CONTRIBUTION

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