OPEN ACCESS
Journal of Animal Health and Production

Short Communication

Bacterial Contamination of Raw Meat and Butchers' Equipment in Retail Shops in Tando-Allahyar, Pakistan

Attaullah Bughti[1], Shahid Hussian Abro[1*], Asghar Ali Kamboh[1], Riaz Ahmed Leghari[2], Chandar Kumar[3], Shafique Ahmed Koondhar[1]

[1]Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan; [2]Department of Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan; [3]Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), No. 2 Yuanmingyuan West Road, Beijing, 100193, China.

Abstract | The present study was performed to evaluate the aerobic bacterial contamination in meat and butchers' equipment in retail shops in Tando-Allahyar, Pakistan. A total of 100 samples (40 beef, 40 mutton and 20 butchers' meat-cutting equipment) were obtained from butcher shops from local vendors of different regions of Tando-Allahyar. The samples were collected randomly in sterile labelled polythene bags or bijoux bottles individually and were transferred to laboratory at 4°C within 3-4 hours of collection. All the samples were subjected to aerobic plate count method that followed by the standard method of isolation and identification. The bacterial load in (g^{-1}) meat samples was recorded higher ($p< 0.05$) in beef samples ($4.1x10^9$) than mutton ($3.9x10^7$) and butchers' meat cutting equipment samples ($3.7x10^6$). The bacterial organisms including *Escherichia coli*, *Bacillus cereus, Staphylococcus aureus, Shigella dysenteriae* and *Salmonella enteritidis* were recorded as individual or mixed contaminants in meat and meat-cutting equipment samples. From the results, it could be concluded that in Tando-Allahyar, meat samples of cattle beef and sheep mutton, as well as butcher's meat-cutting equipment are highly contaminated. The contamination level of aerobic spoilage bacteria was found higher in cattle beef as compared to sheep mutton.

Keywords | Sheep mutton, Cattle beef, Butcher equipment, Bacterial contamination, Tando-Allahyar

Editor | Sanjay Kumar Singh, Indian Veterinary Research Institute, Bareilly, India.
Received | August 18, 2017; **Accepted** | September 26, 2017; **Published** | September 30, 2017
***Correspondence** | Shahid Hussain Abro, Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan; **Email:** shahidabro9@yahoo.com
Citation | Bughti A, Abro SH, Kamboh AA, Leghari RA, Kumar C, Koondhar SA (2017). Bacterial contamination of raw meat and butchers' equipment in retail shops in Tando-Allahyar, Pakistan. J. Anim. Health Prod. 5(3): 115-119.
DOI | http://dx.doi.org/10.17582/journal.jahp/2017/5.3.115.119
ISSN (Online) | 2308-2801; **ISSN (Print)** | 2309-3331

INTRODUCTION

Meat is consumed in different parts of the world as a source of animal proteins (Food and Agriculture Organization, 2013). The chemical composition of meat is favorable for the proliferation of a wide range of microbial populations which makes raw meat to be one of the vehicles of foodborne infections in humans (Doulgeraki et al., 2012). The food such as milk and meat products are generally regarded as high threat, if these contain microbes. Approximately 75% of pathogens affecting humans acquired from animals and animal origin products since last few years (WHO, 2011).

Contaminated bovine meat is considered to be one of the sources of foodborne *Salmonella* and pathogenic *Escherichia coli* infections in humans. The reported prevalence of *Salmonella* and pathogenic *E. coli* in bovine meat and products thereof varies from one product to another, but wide variability is also observed amongst different countries. The prevalence is lower in bovine carcasses, when the proper hygienic and slaughter conditions maintained (Stevens et al., 2008). The availability of wholesome and safe food is a basic requirement for human health. Pakistan is a tropical country and environmental conditions are favorable for the growth of microbes, which can rapidly render the meat insecure for human consumption. Episodes of

food borne illness are reported in the Pakistan, but due to lack of investigation network, the exact magnitude of the problem in the country remains unknown, particularly in the Tando-Allahyar region. The majority of the population consume meat slaughtered and sold in small local shops, where the maintenance of hygiene is always questionable (Akhtar, 2015; Leghari et al., 2016).

Meat may be contaminated by bacteria either endogenously or by subsequent contamination from blood, gastrointestinal contents, feet, hide or skin, water, knives, instruments used in slaughter house vehicles and working personals. The most abundant bacterial species found in meat are *E. coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella, Aeromonas spp., Arobacter spp., Bacillus cereus, Campylobacter spp., Clostridium botulinum* and *Helicobacter* species. The contaminated animal meat with *Bacillus anthracis, Mycobacterium tuberculosis* and *Brucella abortus* should be condemned by veterinary inspectors before it reaches to consumers (Cho et al., 2012; Javed, 2016; Kamboh et al., 2017).

Most of these organisms were found to cause serious food borne diseases and also involved in spoilage of foods eventually imposing a great threat to human health as well as in country's economy. The insanitary conditions of the slaughter houses, butcher shops, handling of meat, environmental condition and improper packing and selling of meat further provide the source of contamination. Contaminated raw meat is one of the main sources of foodborne illnesses (Bhandare et al., 2012). Approximately 250 food borne diseases are identified till to date and most of them are caused by various types of bacteria, virus, parasites and prions (Robert et al., 2014). Approximately, one in three peoples in the world suffers from a food borne diseases, and majority of these diseases caused due to consumption of contaminated food and water (Anonymous, 2007; Othman, 2007).

To control the food-borne illnesses and to keep the microbial load of raw meat in check, the food safety requirements should be followed strictly in accordance with HACCP (Hazard analysis critical control point), but in developing countries like Pakistan, the abattoir environment, its sanitary level, and transportation and storage conditions not only contaminated but also enhance the growth of different types of spoilage as well as pathogenic bacteria in meat (Javed, 2016). Considering the slaughter and market condition in local environment, the current study was designed to evaluate the microbial contamination in raw meat (cattle beef and sheep mutton) and butchers' meat-cutting equipment in Tando-Allahyar, Pakistan.

MATERIALS AND METHODS

Collection of Samples

A total of 100 samples (each 40 for cattle beef and sheep mutton, and 20 for various meat-cutting equipment) were collected from butcher shops from local vendors of different regions of Tando-Allahyar. Four regions of Tando-Allahyar were chosen for sample collection viz., Station road, Meat market, Nasarpur and Bukera Sharif. Each of 10 raw meat samples and 5 equipment samples were collected from each area. Approximately, five meat sample and two to three equipment sample were collected from each outlet. Meat samples were collected randomly within 4 hours post-slaughtering in sterile labelled polythene bags and transported to the laboratory at 4°C. For meat-cutting equipment (knives, weigh scale, bone cutter, wooden board, and meat mincer), swab samples were collected in sterile labeled peptone water containing bijoux bottles. In the laboratory samples were processed within 2-3 hours to avoid any further growth of bacteria.

Processing of Meat Samples

Each meat sample (10g) was minced individually by using grinder /scissor, cut into very fine pieces, then the minced meat was properly mixed and added to 90ml peptone water by stirring with a stirrer or shaking in vortex mixer under strict aseptic conditions. The meat extracts were prepared into tenfold dilutions. The bacterial counts were expressed as colony forming unit per gram (CFU/g) of meat (Kamboh et al., 2017).

Isolation and Identification of Bacteria

Isolation and identification of aerobic bacterial contaminants was done according to ISO 4833(ISO:4833, 2003) In brief, diluted meat extracts (5 µl) were streaked over on individual plates of general (Nutrient agar), differential (MacConkey's agar) and selective media *Salmonella- Shigella* agar, Mannitol salt agar and Blood agar) for isolation, characterization and quantitative study of bacterial organisms. Equipment swabs, kept in sterile peptone water, were also inoculated on general and selective agar plates by direct swabbing. The identification of isolates was made through cultural, staining and morphological characteristics under microscope with the help of oil immersion objective (100X). Further identification of the bacterial species was made through different biochemical tests according to the standards methods recommended by Cruickshank et al. (1973) with slight modification (Nazia et al., 2015). All culture media were obtained from Oxoid (Hamshire, UK).

Data Analysis

The results for occurrence of bacterial species in meat and equipment samples were calculated and presented in percentage format using the Microsoft Excel Spreadsheets. While, aerobic bacterial load was presented as CFU/g and

Table 1: The occurrence of individual and mixed bacterial species in cattle beef, sheep mutton and various equipment used in retailed butcher shop.

Sample	Percentage of samples contained individual bacterial species	Percentage of samples contained mixed bacterial species	Bacterial species found individually in samples	Bacterial species found mixed in samples
Cattle Beef	30%	70%	*Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Corynebacterium pyogenes, Salmonella enteritidis*	*Escherichia coli + Bacillus cereus + Salmonella enteritidis; Escherichia coli + Bacillus cereus; Escherichia coli + Salmonella enteritidis*
Sheep Mutton	37.5%	62.5%	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella enteritidis*	*Salmonella + Shigella Staphylococcus aureus + Escherichia coli*
Butchers' Equipment	66.6%	33.3%	*Escherichia coli, Staphylococcus aureus, Campylobacter jejuni, Shigella dysenteriae*	*Escherichia coli + Staphylococcus aureus; Staphylococcus aureus + Shigella dysenteriae*

compared by ANOVA using JMP software (version 5.0.1a; SAS Institute, 2000).

RESULTS

Total Aerobic Bacterial Load in Meat and Butchers' Equipment

The data regarding aerobic bacterial load or viable count in cattle beef, sheep mutton and butchers' meat-cutting equipment samples is presented in Figure 1. The bacterial load in (g^{-1}) meat samples was recorded higher ($p< 0.05$) in beef samples ($4.1x10^9$) than mutton ($3.9x10^7$) and butchers' meat cutting equipment samples ($3.7x10^6$) obtained from different places of Tando-Allahyar.

The Occurrence of Individual and Mixed Bacterial Species in Meat and Various Equipment Samples

As shown in Table 1, the 30% cattle beef samples were contained individual bacterial species such as *Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Corynebacterium pyogenes* and *Salmonella enteritidis*. While 70% samples were contaminated with mixed species i.e., *E. coli*, *B. cereus* and *S. enteritidis*. However, 37.5% sheep mutton samples were contaminated with individual bacterial species such as *E. coli, S. aureus, Pseudomonas aeruginosa, Listeria monocytogenes* and *S. enteritidis;* whereas 62.5% samples were contaminated with mixed species including *Salmonella, Shigella, S. aureus* and *E. coli*. The samples collected from various equipment used in butcher shops revealed that 66.6% samples were contained individual bacterial species i.e., *E. coli, S. aureus, Campylobacter jejuni* and *Shigella dysenteriae*. While 33.3% samples were contaminated with mixed species including *E. coli, S. aureus* and *S. dysenteriae*.

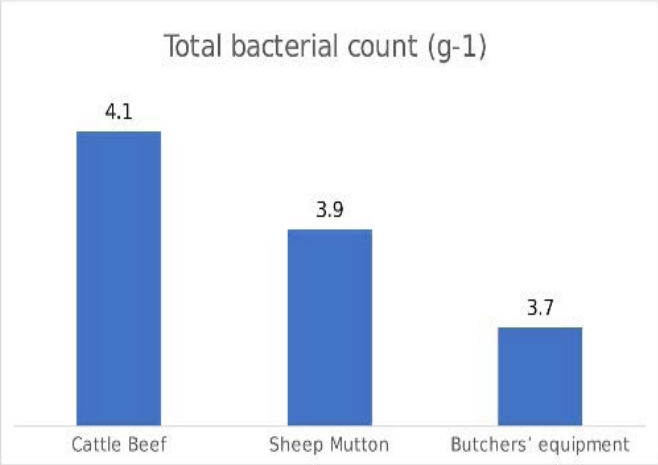

Figure 1: The mean bacterial population (bacterial load) in cattle beef, sheep mutton and butchers' equipment samples retailed at Tando-Allahyar.

DISCUSSION

Tando-Allahyar is a district of Sindh according to the 2011 census of Pakistan, the district had a population of 0.4 million of which 20% were urban live areas and 80% were live in rural areas. Meat produced for the domestic market is also sold as a hot meat directly to the consumer on retail meat shops (Khan et al., 2016).

In (g^{-1}), the mean number colonies were counted for bacterial organisms and 10^6 - 10^9 CFU were recorded in cattle beef, sheep mutton and butchers' meat cutting equipment. An earlier study done by Ali et al. (2010) has also reported the similar trend (10^6 – 10^{10} CFU/g) of bacteriological load in meat and butchers' surrounding-environment samples in Karachi region of Pakistan. Contrary to our results, Saleh et al. (2013) reported that mutton meat samples had more contamination level than beef meat samples collected from El-Beheria province. However, in agreement to our results Ahmad et al. (2013) reported a comparatively high-

er contamination level in beef than mutton in both abattoir (5.35 vs. 4.84 log10 CFU/cm^2) and retail outlet samples (7.15 vs. 6.92 log10 CFU/cm^2) in Lahore, Pakistan. In advanced countries, regulatory bodies have set a spoilage limit (i.e., 10^6 CFU/g) for meat that must not be present for its sell to consumers (Nieto et al., 2010). The high level of bacterial load in meat (10^6 - 10^9 CFU/g) that have observed in our study and also in investigations of other workers indicated that meat sold in our local markets with open retail outlets contains high number of viable spoilage organism that could be potential threat to meat spoilage as well as consumers' health (Ali et al., 2010).

E. coli are the most common contaminants of food items that also been considered as an indicator of food quality (Khan et al., 2016). In current investigation, *E. coli* have been found in all meat and meat-cutting equipment samples both as an individual and mixed contaminant. In previous studies (Ahmad et al., 2013; Ali et al., 2010), this bacterium has also been recorded almost with a similar trend and frequency as recorded in our current investigation. In addition to that, we have observed many other bacterial organisms either singly or in combined form with other organisms including, *S. aureus, B. cereus, S. enteritidis, P. aurginosa, L. monocytogenes* and *S. dysenteriae*. A recent Egyptian study also reported the mixed microbial contamination including *E. coli, Citrobacter diversus, Citrobacter freundii, Klebsiella pneumoniae, Enterobacter aerogenes, S. aureus*, mold and yeast in beef and mutton meat samples (Saleh et al., 2013). The microbial contaminants in meat samples were also noted by Bradeeba and Sivakumaar, (2013). In their study, 29.66% meat samples were found to be exceeding the limit of total viable count set by regulations 2011. A 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 was 28% pork, 18% mutton and 14% each of chevon and chicken (Bradeeba and Sivakumaar, 2013).

CONCLUSION

From the present study, it is concluded that in Tando-Allahyar, meat samples of cattle beef and sheep mutton, and butcher's meat-cutting equipment are highly contaminated, probably because of peoples are slaughtering and selling meat besides roads, small streets, open shops, small cots and semi- open slaughter houses. The contamination of aerobic spoilage bacteria was found higher in cattle beef as compared to sheep mutton.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge the butchers for their kind cooperation in sample collection.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have worked uniformly and read and approved the contents of manuscript.

REFERENCES

- Ahmad MUD, Sarwar A, Najeeb MI, Nawaz M, Anjum AA, Ali MA, Mansur N (2013). Assessment of microbial load of raw meat at abattoirs and retail outlets. J. Anim. Plant Sci. 23(3): 745-748.
- Akhtar S (2015). Food safety challenges—a Pakistan's perspective. Critic. Rev. Food Sci. Nutr. 55(2): 219-226. https://doi.org/10.1080/10408398.2011.650801
- Ali NH, Farooqui A, Khan A, Khan AY, Kazmi SU (2010). Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J. Infect. Develop. Countries. 4(06): 382-388.
- Anonymous (2007). "Food safety and food borne illness". WHO Fact sheet No. 237. March http: //www.who.int/medicare/factsheets /fs237/en/.
- Bhandare SG, Sherikarv AT, PaturkarAM, Waskar VS, Zende RJ (2012). A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food Control. 18: 854-868. 275.
- Bradeeba K, PK Sivakumaar (2013). Assessment Of microbiological quality of beef, mutton and pork and its environment in retail shops in Chidambaram, Tamil Nadu 3(1): 12-18
- Cho JI, Joo IS, Choi JH, Jung KH, Choi EJ, Lee SH, Hwang IG (2012). Prevalence and characterization of foodborne bacteria from meat products in Korea. Food Sci. Biotechnol. 21(5):1257-1261. https://doi.org/10.1007/s10068-012-0165-3
- Cruickshank R, Dugaid JP, Marmion BP, Swain RHA (1973). Medical microbiology 12th ed. Edinburg, Livings tone.
- Doulgeraki AI, Ercolini D, Villani F, Nychas GJE (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. Int. J. Food Microbiol. 157(2): 130-41. https://doi.org/10.1016/j.ijfoodmicro.2012.05.020
- Food and Agriculture Organization (2013). Food Outlook: Biannual report on global food markets (GIEWS.). Rome Italy: Trade and Market Division - FAO. Retrieved from http://www.fao.org/Giews/english/fo/index.html
- ISO:4833 (2003). Microbiology of food animal feeding stuff: Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 ◦C. Geneva, Switzerland: International Organization for Standardization.
- Javed A (2016). Food Borne Health Issues and Their Relevance to Pakistani Society. American Scient. Res. J. Engin. Technol. Sci. (ASRJETS). 26(4): 235-251.
- Khan A, Rind R, Shoaib M, Kamboh AA, Mughal GA, Lakho SA, Malhi KK, Nizamani AR, Yousaf A (2016). Isolation, identification and antibiogram of *Escherichia coli* from table eggs. J. Anim. Health Prod. 4(1): 1-5. https://doi.org/10.14737/journal.jahp/2016/4.1.1.5

- Khan A, Bhutto B, Shoaib M, Fahad S, Ahmad A, Khetran IB, Nizamani AR, Zeb A, Rahman M, Khan S (2016). Prevalence of gastro intestinal cestodes in backyard chickens in district Tando Allahyar, Sindh. J. Anim. Health Prod. 4(1): 26-30. https://doi.org/10.14737/journal.jahp/2016/4.1.26.30
- Kamboh AA, Memon AM, Mughal MJ, Memon J, Bakhetgul M (2017). Dietary effects of soy and citrus flavonoid on antioxidation and microbial quality of meat in broilers. J. Anim. Physiol. Anim. Nutr. (in press) https://doi.org/10.1111/jpn.12683
- Leghari A, Kamboh AA, Dewani P, Abro SH, Umrani AP, Malhi KK, Rajput ZI, Lakho SA, Bano I, Shah JM (2016). Isolation of *Mycobacterium bovis* from milk and nasal discharge samples of cattle from Hyderabad and Tando Allahyar districts. J. Anim. Health Prod. 4(4): 105-110. https://doi.org/10.14737/journal.jahp/2016/4.4.105.110
- Nazia, Malhi KK, Durrani NU, Kamboh AA, Lakho SA, Rind R, Abro SH, Soomro NM (2015). Prevalence of septic arthritis caused by *Staphylococcus aureus* in poultry birds at Tandojam, Pakistan. J. Anim. Health Prod. 3(3): 73-77.
- Nieto G, Díaz P, Bañón S, Garrido MD (2010). Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. *gracilis*) leaves in ewes' diet. Meat Sci. 85:82–88. https://doi.org/10.1016/j.meatsci.2009.12.009
- Othmann NM (2007). Food safety in Southeast Asia: Challenges Facing the region. Asian J. Agri. Develop. 4(2): 83-92.
- Robert L, F Shahada, A Matemu (2014). Prevalence of *Salmonella* spp. and *Escherichia coli* in raw milk value chain in Arusha, Tanzania. American J. Res. Commun. 2(9) 1-13.
- Saleh EA, Ibrahim HA, El-Kewaiey IA, Zaqzouq GS (2013). Microbiological aspects of sheep and cattle meats in El-Beheria province. Assiut Vet. Med. J. 59: 138.
- SAS Institute (2000). JMP statistics and graphics guide. Version 5.0.1a. Cary, NC: SAS Institute.
- Stevens A, Kerouanton A, Marault M, Millemann Y, Brisabois A, Cavin JF (2008). Epidemiological analysis of Salmonella enterica from beef sampled in the slaughterhouse and retailers in Dakar (Senegal) using pulsed-field gel electrophoresis and antibiotic susceptibility testing. Int. J. Food Microbiol. 123(3): 191e197.
- WHO (2011). http;//www.who.int/zoonoses/vph/en.