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Comparative Assessment of Microbiological Quality and Safety of Raw Mutton Meat Sold in Different Retail Shops of Multan, Punjab, Pakistan

Original Article

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ABSTRACT

This study investigates the quality and safety of raw mutton meat influenced by microorganisms and meat is sold in various retail shops in Multan, Pakistan. Meat is highly favorable to microbial growth due to its critical protein portion as meat considered as rich source of protein, leading to spoilage and foodborne illnesses. A total of 270 samples, comprising 108 meat samples (breast and shank) and 162 environmental swabs from butcher equipment, were collected from 18 retail outlets. The microbial load, expressed in log 10 CFU/g ± SD, revealed significant contamination in both meat and equipment. Escherichia coli, Staphylococcus aureus, Salmonella spp., Brucella, and Listeria species were identified, with E. coli being the most prevalent. The highest bacterial count was observed in mutton shank (3.59 ± 0.23 log CFU/g). The study highlights the substantial risk of meat spoilage and consumer health threats posed by microbial contamination. These findings underscore the need for stringent hygiene practices in meat handling and equipment sanitation to ensure food safety. Similar contamination trends have been noted in other developing countries, emphasizing the global relevance of this issue.

Introduction:

Meat serves as an essential protein source in the human diet; however, it is highly risky to microbial contamination, which can lead to spoilage and foodborne diseases. These issues pose both economic and public health challenges (1). Although muscle tissues in healthy animals are sterile, contamination can occur at multiple points, including slaughter, processing, and transportation (2). Due to its high protein and fat content, along with adequate water activity, raw meat provides optimum conditions for the growth of both spoilage and pathogenic bacteria (3).

In many developing countries, foodborne pathogens are a leading cause of illness and infection. Poor hygiene practices, improper handling, and inadequate sanitation in slaughterhouses and retail meat shops significantly contribute to microbial contamination (4). The Food and Agriculture Organization (FAO) as well as the World Health Organization (WHO) have identified foodborne diseases as a major global health concern, impacting both public well-being and economic productivity (5).

Raw meat may contain harmful microorganisms such as *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter jejuni/coli*, *Yersinia enterocolitica*, and, to a lesser extent, *Listeria monocytogenes*. If meat is not handled properly, these pathogens can cause serious foodborne illnesses (6). The processing and distribution of meat from slaughterhouses to retail outlets involve multiple stages, each presenting opportunities for contamination. The sanitary conditions of abattoirs and surrounding environments play a crucial role in determining the microbial load present in meat products (7).

Additional contamination risks arise during transportation, storage, and handling at butcher shops. Implementing strict hygiene practices and other food safety protocols based on International Safety Standards and Hazard Analysis and Critical Control Points (HACCP) is crucial for reducing foodborne infections and ensuring meat safety. However, in developing countries such as Pakistan, inadequate sanitation in slaughterhouses, poor storage conditions, and improper meat handling not only lead to contamination but also create conditions that support bacterial proliferation, further increasing the risk of foodborne diseases.

By keeping in mind all the factors present study was assigned to assess the microbiological quality and safety of raw mutton meat sold in different retail shops in different areas of Multan.

Materials and Methods

Sampling

Samples were randomly collected in the Main Saddar market from 18 different retail outlets of Multan, Pakistan. A total of 270 samples, including both meat and surface swabs, were gathered. Among these, 108 were retail meat samples, consisting of 54 breast portions and 54 thigh portions. To minimize microbial variations caused by environmental factors and post-slaughter duration, after slaughtering immediately samples were collected within 12 hours of activity, primarily during early afternoon hours. Each retail outlet contributed approximately six meat samples.

Additionally, 162 preexisting environmental samples were collected from knives, tables, and butchers' hands comprising surface swabs as outlined in Table 1. Each outlet provided between seven and nine surface swabs.

Sample Collection and processing:

The collection process was influenced by the size of the shop and the level of cooperation from the butchers. Many shop owners lacked awareness regarding the significance of proper disinfection and sanitation. Typically, these retail meat shops were cleaned only once every 24 hours using detergent and water, without incorporating sanitizing agents.

Sterile swabs immersed in peptone water (3ml) environmental samples were collected. After collecting sample, these were

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brought to the laboratory immediately and processed within two hours approximately. Each sample of meat was of 25 grams was transferred into sterile bottles having 100 ml of saline solution of buffed phosphate (PBS). Under sterile conditions, a meat grinder was used to homogenize the samples. Then Environmental swabs, which were stored in sterile glass tubes containing peptone water, were poured on blood agar plates.

To determine the total viable bacterial count, samples were serially diluted in PBS and cultured on nutrient agar plates. Unless otherwise specified, all antibiotic disks and culture media used for growth of listed microorganisms in the study were obtained from Oxoid (Hampshire, UK).

Microbial Analysis

The pour plate method was utilized to determine the total viable bacterial count in diluted meat samples cultured on nutrient agar. The plates were incubated at 37°C. To detect *Salmonella*, one gram of each sample was inoculated into Selenite F medium (Difco, Michigan, USA) and incubated at 37°C for 18 hours. The cultures were then transferred onto xylose-lysine deoxycholate (XLD) agar and incubated for an additional 18 hours at the same temperature. For preliminary screening of *Escherichia coli*, Sorbitol MacConkey agar was specifically used, followed by serotyping of non-sorbitol fermenting, colorless colonies.

To isolate *Brucella* species, one gram of ground meat was inoculated into 50 ml of brain heart infusion (Merck, Darmstadt, Germany) and trypticase soy broth, supplemented with nalidixic acid (5 µg/ml), bacitracin (25 IU/ml), cycloheximide (100 µg/ml), and polymyxin B (5 IU/ml) (Sigma, Hampshire, UK). The broth cultures were sub-cultured weekly on 5% sheep blood agar and incubated at 37°C in a 5% CO_2-enriched environment for up to one month.

For *Listeria* detection, nutrient agar plates were incubated at 37°C for two weeks, with routine observation for characteristic colonies. Additionally, one gram of meat was inoculated into 10 ml of trypticase soy broth containing nalidixic acid (25 µg/ml) and polymyxin B (105 µg/ml), followed by incubation at 37°C for two weeks. Sub-culturing onto 5% sheep blood agar was performed every three days. Standard biochemical methods were employed for bacterial identification.

Data analysis

Bacterial species in meat and equipment samples were counted and displayed by means of percentages using the Microsoft Excel Sheets. In JMP software, the aerobic bacterial load expressed as CFU/g was compared using ANOVA. Cybercurrency valued at CFU/g was compared using ANOVA software.

Results

Meat and surface samples from retail shops in Multan revealed high viable bacterial counts, as illustrated in Table 1. E. coli which is a gram- negative bacteria predominantly contributed to the total viable count, while frequently observed included Bacillus subtilis and Staphylococcus species which are gram-positive bacteria.

Table: 1 Counts of aerobic mesophilic bacteria on environmental and meat samples from retail shops in Multan, Pakistan.

Sampling	Type of Sample	Number of samples (n)	Total viable count (log CFU/g or cm^2)
Meat samples	Meat	108	
	Breast meat	54	5.27 ± 1.31
	Shank meat	54	5.31 ± 1.40
Swabs from meat cutting equipment	Knives	54	4.67 ± 2.31
	Wooden Boards	54	4.54 ± 0.92
Swabs from the Environment	Customer Platforms	54	4.13 ± 0.37

From 270 samples which were collected, total of 430 potential pathogenic bacterial pathogen isolates were obtained from 270 samples collected, with 108 isolates from meat samples (breast and shank) and 162 from environmental swabs (knives, wooden boards, and customer platforms).

Table.2: The presence of bacterial species in mutton and various equipment used in retail butcher shops.

Sample	Percentage of samples with bacterial species	Bacterial species found in samples
Retail Meat (Mutton Breast, Shank)	59.7%	Escherichia coli, Listeria species, Brucella, Salmonella, Staphylococcus aureus.
Equipment's used in butcher's shop	36.4%	Escherichia coli, Listeria species, Salmonella, Staphylococcus aureus.

Table 2, 59.7% of the retail meat samples (mutton breast and shank) contained bacterial species such as Escherichia coli, Listeria species, Brucella, Salmonella, and Staphylococcus aureus. Equipment used in butcher shops showed that 36.4% of samples were contaminated with bacterial species including Escherichia coli, Listeria species, Salmonella, and Staphylococcus aureus. As demonstrated in Table 3, out of the 98 isolates of bacterial pathogens from meat samples, among those pathogens 35 (32%) were marked as Escherichia coli, and 15 (14%) of these were Salmonella. Other potentially pathogenic isolates included 5 (5%) Listeria species, 16 (15%) Brucella and 9 (8%) Staphylococcus aureus. Similarly, in the equipment used in the butcher's shop, 13 pathogens were identified as Escherichia coli, 6 were Salmonella, 2 were Listeria species, 6 were Brucella, and 3 were Staphylococcus aureus.

Table 3: Percentage of Bacterial pathogens in retail meat and Equipment's used in butcher's shop

Bacterial Pathogens	Retail Meat (Mutton Breast, Shank)	Equipment's used in butcher's shop
Escherichia coli	35	13
Salmonella	15	6
Listeria species	5	2
Brucella	16	6
Staphylococcus aureus	9	3

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Table 4: The microbial count in retail meat (breast, shank) and the equipment's (knife, wooden board, table) used in the butcher's shop is expressed in log 10 CFU/g ± standard deviation.

Sample Type	No. of Samples	Bacterial Pathogens found in Meat and Shop				
		Escherichia coli	Salmonella	Listeria species	Brucella	Staphylococcus aureus
	n	Log CFU/g mean ± SD				
Mutton Breast	54	3.45 ± 0.12[a]	3.37 ± 0.23[a]	3.15 ± 0.26[c]	3.81 ± 0.45[d]	2.80 ± 2.35[a]
Mutton Shank	54	3.59 ± 0.23[a]	3.28 ± 0.14[b]	3.78 ± 1.29[d]	3.62 ± 0.04[d]	2.39 ± 1.06[a]
Knifes	54	3.38 ± 0.16[a]	3.18 ± 0.13[a]	3.31 ± 0.75[a]	3.54 ± 1.02[c]	2.14 ± 0.58[a]
Wooden boards	54	3.29 ± 0.27[a]	3.31 ± 0.17[a]	3.05 ± 3.21[c]	3.34 ± 0.75[a]	1.98 ± 1.17[c]
Customers table	54	3.17 ± 0.42[a]	3.24 ± 4.28[b]	--	3.21 ± 0.23[a]	1.86 ± 1.42[b]

Discussion:

The presence of a large number of viable bacteria, which indicates the expected shelf life of meat, raises the likelihood of rapid meat spoilage, as noted by the Agriculture and Consumer Protection Department of the FAO. Similar findings have been reported in neighboring countries, such as India and Bangladesh.

The high bacterial load observed in meat (ranging from 10^6 to 10^9 CFU/g) in our study, as well as in studies by other researchers, indicates that meat sold in our local markets through open retail outlets contains a significant number of viable spoilage organisms. These organisms pose a potential threat to both meat quality and consumer health (8). Meat made for the local market is also available as hot meat straight on retail meat stores (Khan et al., 2016). The average number of colonies were tabulated for bacterial species; they were counted at 106 - 109 CFU in cow beef, sheep mitten, and butcher meats cutting tools in g-1 (9).

The microbial counts expressed in log 10 CFU/g ± standard deviation across various sample types indicate that there are significant levels of bacterial pathogens present in both retail meat and the equipment used in butcher shops. The counts of Escherichia coli were relatively consistent across all sample types, with no significant differences. The highest mean count was observed in mutton shank (3.59 ± 0.23), and the lowest in customer tables (3.17 ± 0.42). And Listeria species are not detected in butchers equipment. The microbial analysis shows that bacterial contamination is present across all samples, but the statistical analysis indicates no significant differences among the sample types for each bacterial pathogen tested (10). This suggests that both meat and butcher shop equipment contribute equally to the microbial load, highlighting the importance of stringent hygiene practices in both meat handling and equipment sanitation to mitigate the risk of bacterial contamination.

E. coli, Staphylococcus aureus and Salmonella sp., were commonly found in meat samples according to Bhandare et al, 2007. In the present study, a small number of samples from butcher shops tested positive for presumptive Salmonella spp., with a prevalence rate of 15%. This finding aligns with, which reported that about 12% of raw retail meat in Addis Ababa contained Salmonella (11).

The high prevalence of microorganisms in meat indicates significant contamination during handling, posing a considerable risk of meat spoilage. The quantity and variety of bacteria are largely influenced by the specific part of the chicken, as well as the packaging methods and storage conditions after the meat reaches the market.

Conclusion

This study underscores the pressing issue of microbiological contamination in raw mutton meat and associated retail environments in Multan, Pakistan. The detection of significant microbial loads, including pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Brucella*, and *Listeria* species, highlights a critical threat to both food safety and public health. The findings reveal that inadequate hygiene practices in meat handling and equipment sanitation are major contributors to this contamination, posing risks of meat spoilage and foodborne illnesses.

Given the prevalence of *E. coli* and the elevated bacterial counts in mutton shank, targeted interventions are essential. Implementation of stringent hygiene protocols, regular microbial monitoring, and comprehensive training for butchers are imperative measures to mitigate these risks. Moreover, public awareness campaigns on safe meat handling and storage practices are crucial for consumer protection.

The study's findings resonate with similar challenges faced by other developing countries, emphasizing the global need for improved food safety standards and infrastructure. Addressing these issues will not only enhance public health outcomes but also strengthen consumer confidence in meat products, contributing to economic and societal well-being.

REFERENCES:

1. Pereira, P. C., & Vicente, F. (2022). Meat nutritive value and human health. In *New aspects of meat quality* (pp. 561-577). Woodhead Publishing.
2. Rani, Z. T., Hugo, A., Hugo, C. J., Vimiso, P., & Muchenje, V. (2017). Effect of post-slaughter handling during distribution on microbiological quality and safety of meat in the formal and informal sectors of South Africa: A review. *South African Journal of Animal Science*, *47*(3), 255-267.
3. Li, H., Sun, X., Liao, X., & Gänzle, M. (2020). Control of pathogenic and spoilage bacteria in meat and meat products by high pressure: Challenges and future perspectives. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 3476-3500.
4. Abebe, E., Gugsa, G., & Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of tropical medicine*, *2020*.
5. Alum, E. A., Urom, S. M. O. C., & Ben, C. M. A. (2016). Microbiological contamination of food: the mechanisms, impacts and prevention. *Int. J. Sci. Technol. Res*, *5*(3), 65-78.
6. Ali, S., & Alsayeqh, A. F. (2022). Review of major meat-borne zoonotic bacterial pathogens. *Frontiers in Public Health*, *10*, 1045599.
7. Gutema, F. D., Agga, G. E., Abdi, R. D., Jufare, A., Duchateau, L., De Zutter, L., & Gabriël, S. (2021). Assessment of hygienic practices in beef cattle slaughterhouses and retail shops in bishoftu, ethiopia: Implications for public health. *International journal of environmental research and public health*, *18*(5), 2729.

8. Ali, N. H., Farooqui, A., Khan, A., Khan, A. Y., & Kazmi, S. U. (2010). Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. *The Journal of Infection in Developing Countries*, *4*(06), 382-388.
9. Ejeta, G., Molla, B., Alemayehu, D., & Muckle, C. A. (2004). Salmonella serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Revue de médecine vétérinaire*, *155*(11), 547-551.
10. Kozačinski, L., Hadžiosmanović, M., & Zdolec, N. (2006). Microbiological quality of poultry meat on the Croatian market. *Veterinarski arhiv*, *76*(4), 305-313.
11. Sharaf, E. M., & Sabra, S. M. (2012). Microbiological loads for some types of cooked chicken meat products at Al-Taif Governorate, KSA. *World Applied Sciences Journal*, *17*(5), 593-597.

CONFLICT OF INTEREST

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

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