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BACTERIA ASSOCIATED WITH FIVE BREEDS OF SHEEP MILK IN SINDH AND BALOCHISTAN, PAKISTAN

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Abstract

Milk can serve as a source of bacterial contamination. Being a suitable medium for different bacterial growth, improperly handled sheep milk improperly dealt with using contaminated utensils or dirty hands, can pose serious health hazards. Infectious diseases that occur due to pathogenic bacteria are the most common and widespread in areas where sheep milk is consumed. Some pathogenic bacteria in milk are capable of breaking of important milk components and their utilization, such as lactose, which can disturb the nutritional profile of milk. Disseminated through contaminated sheep milk, some of these pathogens can be life threatening. Therefore, the objective of the study was to assess the prevalence of bacterial contamination and associated factors in the milk collected from different sheep breeds. The five breeds of sheep (Ovis aries) were selected (Dumbi, Kochi, Kooka, Balochi and Mengali) from fifteen locations of Sindh and nine Locations of Balochistan, Pakistan. 2 ml of milk was collected in sterile plastic vials and kept at 2-3 ℃ during the main lactation period of ewes. The pH of sheep milk ranged from 5.32 to 6.70. Milk of Dumbi and Kochi was observed to be the most contaminated, whereas Mengali's milk was observed to be the least contaminated. The isolated bacteria belonged to E. coli, Pseudomonas and Listeria species.

Keywords: *Bacterial contamination, E. coli, Milk, Pseudomonas sp., Sheep breeds*

INTRODUCTION

Milk is considered a highly nutritious food but also serves as an excellent medium for microbial growth, making it prone to bacterial contamination. Contaminated milk and dairy products are important sources of foodborne illnesses worldwide, particularly in developing countries where hygienic practices during milking, handling, and storage are often inadequate. Pathogenic bacteria such as *Escherichia coli*, *Listeria monocytogenes*, and *Pseudomonas spp.* are among the most frequently reported contaminants of raw milk, leading to gastrointestinal infections, systemic illnesses, and spoilage of dairy products (1–3).

Sheep are an important livestock species in Pakistan, reared primarily for milk, meat, and wool production. In Sindh and Balochistan, smallholder and landless farmers commonly keep sheep as a vital source of livelihood. Sheep milk is rich in proteins, fats, amino acids, and minerals, and is considered nutritionally superior to cow or goat milk. However, its high nutrient content also provides favorable conditions for the proliferation of pathogenic and spoilage microorganisms if hygienic standards are not maintained (4, 5).

Contamination of sheep milk can occur through direct contact with infected animals, use of contaminated utensils, or exposure to unhygienic environments during milking. For instance, *E. coli* can persist through the processing of traditional milk products such as cheese, while Listeria poses a significant threat to immunocompromised individuals consuming unpasteurized milk. In addition, *Pseudomonas spp.* are commonly associated with post-pasteurization contamination, resulting in milk spoilage and potential human infections (6-8).

Despite the importance of sheep farming in Sindh and Balochistan, limited studies have investigated the microbial quality of sheep milk in these regions. Evaluating bacterial contamination in relation to

different sheep breeds and localities is essential for understanding the extent of microbial risks and for developing strategies to ensure milk safety.

The present study was therefore designed to investigate the bacterial load and diversity associated with the milk of five sheep breeds reared across fifteen localities of Sindh and Balochistan. The study also analyzed milk pH and colony-forming unit (CFU) counts to assess the prevalence of bacterial contamination.

METHODOLOGY

The investigation was conducted between February and April 2024, coinciding with the peak lactation period of ewes in Sindh and Balochistan. From each locality, 2 mL of milk was aseptically collected from randomly selected ewes into sterile plastic vials and immediately stored at 2–3°C. The flock sizes in the surveyed areas ranged from 11 to 106 animals, and all ewes were milked manually. Prior to milking, the teats were thoroughly washed to minimize contamination.

Five sheep breeds—Dumbi, Kochi, Koka, Balochi, and Mengali—were included in the study. Sampling in Sindh was carried out in Umerkot, Mithi, Jamshoro, Mirabad, Badin, Tando Muhammad Khan, Sikhat, Tandojam, Nasrpur, Gharo, and Oderolal Station. In Balochistan, samples were collected from Hub Chowki, Wadh, Lasbela, Winder, Surab, Khad Kocha, Baghbana, Kalat, and Mastung.

The pH of milk samples was determined using a portable pH meter (HI 98162, Hanna Instruments), equipped with an FC 1013 FoodCare pH and temperature electrode designed for dairy products, offering resistance to fungal growth and ensuring accurate measurement.

MICROBIOLOGICAL ANALYSIS

To determine bacterial presence and counts, milk samples were directly inoculated onto selective microbiological media specific for *Escherichia coli*, *Pseudomonas spp.* and *Listeria spp*. Eosin Methylene Blue (EMB; Oxoid) agar was used for the detection of *E. coli*, Cetrimide agar for *Pseudomonas spp*. and Listeria Selective Agar for *Listeria spp.* For each sample, 1 mL of milk was inoculated into the selective agar medium using the pour plate method. Following solidification, the plates were incubated at 35 ± 2°C for 24–48 hours (Fig. 1a–c).

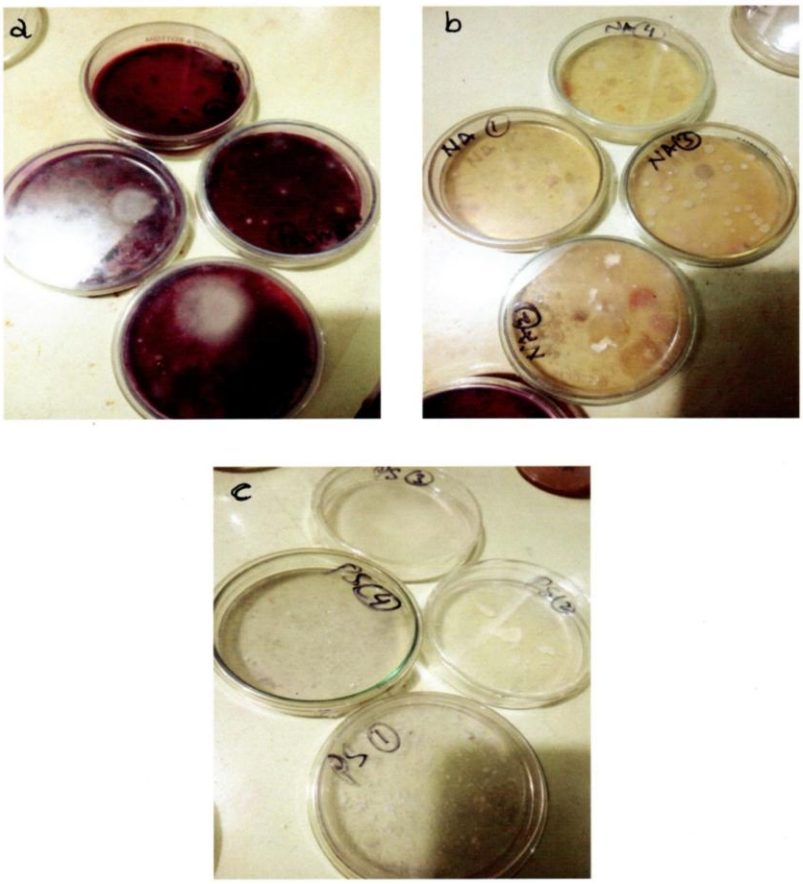

Fig 1 (a-c). Isolated bacterial colonies obtained from different sheep milk.
(a = *E. coli* on EMB agar, b = *Listeria* sp. on Listeria Selective medium, c = *Pseudomonas* sp. on citramide medium)

After incubation, characteristic colonies of *E. coli*, *Pseudomonas spp.* and *Listeria spp.* were identified and counted on the respective selective media. Results were expressed as colony-forming units (CFU) per milliliter of milk. Each sample was analyzed in quadruplicate, and the mean value was calculated to obtain the CFU/mL.

STATISTICAL ANALYSIS

For statistical analysis, Analysis of Variance (ANOVA) and regression modeling were performed (9). The regression equation explained 52.09% of the total variation in bacterial counts (CFU). The model was expressed as: CFU (response variable) = Constant + β_1(Breed, categorical variable) + β_2(pH, continuous variable).

RESULTS

The recorded data revealed considerable variation in bacterial colony-forming units (CFU) among the five sheep breeds. Milk from the Dumbi breed showed the highest bacterial load, ranging from 308.24 to 483.30 CFU/mL. In contrast, the Kochi breed ranged from 231.40 to 306.64 CFU/mL, while the Kooka breed ranged from 243.24 to 280.86 CFU/mL. The Balochi breed showed a wider range of 74.62 to 388.12 CFU/mL, whereas the Mengali breed exhibited the lowest bacterial counts, ranging from 7.72 to 32.80 CFU/mL. Overall, Dumbi milk samples had the highest bacterial contamination, while Mengali samples consistently showed the lowest levels (Fig. 2).

The pH values of milk samples also varied across localities and breeds. The lowest pH (5.70) was recorded in milk collected from Dumbi sheep in Umerkot, Sindh, while the highest pH (6.78) was observed in Balochi sheep milk collected from Winder, Balochistan (Table I). Statistical analysis indicated that pH had a significant effect on bacterial counts at the 5% level of significance (Table II), whereas the variation in CFU among breeds was found to be non-significant (Fig. 2).

Comparatively, the higher bacterial counts observed in Dumbi milk may be attributed to management practices, environmental conditions, or differences in teat morphology that facilitate bacterial entry. In contrast, the consistently low CFU in Mengali milk suggests either better resistance to bacterial colonization or more hygienic rearing conditions in the surveyed areas. These breed-related variations highlight the potential influence of both animal factors and farm management on milk quality (Fig. 2).

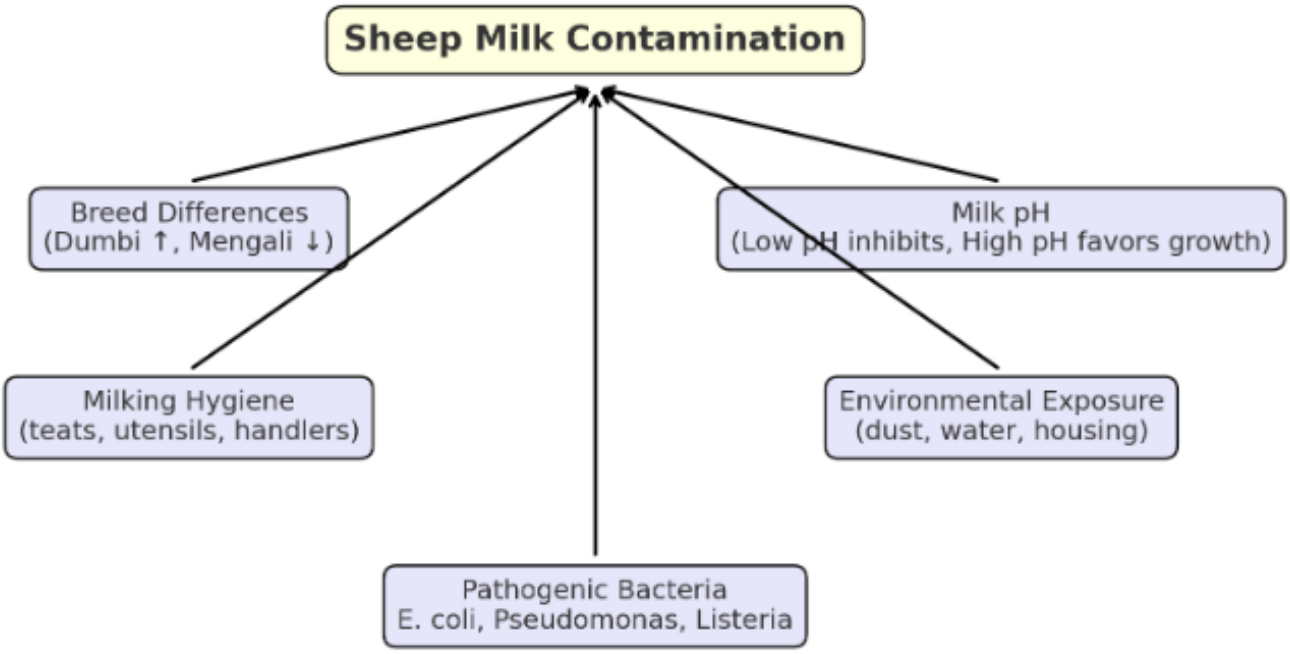

Fig. 2. Diagram illustrating the key factors influencing bacterial contamination in sheep milk, including breed differences, pH, hygiene, environmental exposure, and pathogenic bacteria

Table I. Milk pH along with sheep breed, locality, bacteria isolated and CFU/mL

Breed	pH	Location	Bacteria	Mean CFU/ml
Dumbi	6.56	Hub chawki (B)	*E. coli, Pseudomonas* sp. and *Listeria* sp.	> 500
Dumbi	5.70	Umerkot (S)	*E. coli, Pseudomonas* sp. and *Listeria* sp.	> 500
Dumbi	6.56	Mithi (S)	*E. coli, Pseudomonas* sp. and *Listeria* sp.	> 500
Dumbi	6.56	Jamshoro (S)	*Listeria* sp.	>118
Dumbi	6.56	Mirabad (S)	*Listeria sp.*	> 115
Kochi	6.62	Badin (S)	*E. coli, Pseudomonas* sp. and *Listeria* sp.	> 500
Kochi	6.62	Tando Mohd Khan (S)	*E coli, Pseudomonas* sp. and *Listeria* sp.	> 206
Kochi	6.66	Sikhat (S)	*E. coli, Pseudomonas* sp. and *Listeria* sp.	> 506
Kochi	6.62	Hub chowki (B)	*E. Coli, Pseudomonas* sp. and *Listeria* sp.	> 500
Kochi	6.60	Tando Adam (S)	*Pseudomonas* sp.	> 216
Kooka	6.56	Sikhat (S)	*Pseudomonas* sp.	> 200
Kooka	6.56	Nasrpur (S)	*Listeria* sp.	> 200
Kooka	6.59	Mirabad (S)	*Listeria* sp.	> 185
Kooka	6.58	Tando Adam (S)	*E. coli* sp.	> 180
Kooka	6.56	Tando Allahyar (S)	-	-
Balochi	6.63	Wadh (B)	*E. coli*	> 435
Balochi	6.53	Lasbela (B)	*E. coli*	> 400
Balochi	6.68	Gharo (S)	*Pseudomonas* sp.	> 132
Balochi	6.78	Winder (B)	*Pseudomonas* sp. and *Listeria* sp.	> 225
Balochi	6.54	Surab (B)	-	-
Mengali	6.68	Khad Kocha (B)	-	-
Mengali	6.70	Baghbana (B)	-	-
Mengali	6.70	Kalat (B)	-	-
Mangali	6.70	Oderolal (S)	-	-
Mengali	6.68	Mastung (B)	*E. coli*	> 110

*B=Balochistan; S=Sindh

Table II. ANOVA showing the effect of cfu on sheep breed and pH

Source	Adj	SS	MS	F-Value	P-Value
Regression	5	386173	77235	413	0.010
Error	19	355131	18691	-	-
Total	24	741304			

DISCUSSION

In the present study, bacterial contamination of sheep milk collected from Sindh and Balochistan was analyzed, with particular focus on *Escherichia coli, Pseudomonas spp. and Listeria spp*. The results revealed significant differences in colony-forming units (CFU) among breeds, with Dumbi milk showing the highest bacterial counts, while Mengali milk exhibited the lowest. Furthermore, pH was found to have a significant effect on bacterial load, highlighting its role in microbial proliferation and milk quality. These findings underscore the importance of both intrinsic (breed and milk composition) and extrinsic (hygiene, handling, environment) factors in determining microbial safety of sheep milk.

Sheep milk, though nutritionally superior to cow and goat milk due to its higher protein, fat, and mineral content, is equally susceptible to microbial contamination if hygienic standards are not maintained during milking and storage (10, 11). In the present study, *E. coli* and *Pseudomonas spp.* (Gram-negative bacteria) and *Listeria spp.* (Gram-positive bacteria) were isolated, which are well-known foodborne pathogens of public health concern. These findings are consistent with studies conducted in other regions

where raw sheep and bovine milk were found to harbor enteric and environmental bacteria, reflecting deficiencies in hygiene and farm management (12, 13).

Escherichia coli is often regarded as an indicator organism of fecal contamination, suggesting that its presence in milk may arise from improper cleaning of teats, use of contaminated utensils, or exposure to unhygienic milking environments (14). In some cases, pathogenic strains such as Shiga-toxin producing *E. coli* (STEC) have been implicated in severe outbreaks linked to raw milk consumption (15). Similarly, *Pseudomonas spp.,* frequently associated with post-pasteurization contamination, are notorious for their psychrotrophic nature and ability to thrive under refrigerated conditions, leading to spoilage, off-flavors, and reduced shelf life of milk and dairy products (16). On the other hand, *Listeria monocytogenes* is a zoonotic pathogen with serious public health implications, particularly in immunocompromised individuals, pregnant women, and neonates (17). The isolation of these bacteria from sheep milk in Sindh and Balochistan highlights potential risks to consumers, especially in rural communities where raw milk consumption is common.

The present study revealed substantial differences in bacterial counts among breeds. Dumbi milk exhibited the highest bacterial load, whereas Mengali milk showed the lowest. Several factors may contribute to these variations. Breed-specific differences in udder morphology, teat canal length, and milk composition can influence susceptibility to bacterial entry and colonization (18). For example, shorter teat canals or wider teat openings may predispose certain breeds to higher bacterial contamination. Moreover, differences in management practices flock size, and environmental exposure across localities could further explain the observed variability.

The higher CFU observed in Dumbi milk may be attributed to larger flock sizes and more intensive farming practices in Sindh, where hygiene standards might be compromised. In contrast, the Mengali breed, traditionally reared under semi-intensive or nomadic systems in Balochistan, may have less exposure to contaminated environments, explaining the lower bacterial counts. Similar breed-associated differences in microbial load have been reported in goat and bovine milk, where indigenous breeds often display better resistance to infections compared to commercial breeds (19).

Milk pH is a critical determinant of microbial survival, proliferation, and dairy processing properties. In this study, pH values ranged from 5.70 (Dumbi milk in Umerkot) to 6.78 (Balochi milk in Winder), with statistical analysis confirming a significant effect of pH on bacterial counts. Lower pH values are generally associated with inhibitory effects on pathogenic bacteria, as acidic environments can limit their growth (20). However, in fresh raw milk, variations in pH can reflect not only natural composition but also microbial activity, particularly lactic acid bacteria fermentation that lowers pH during storage (21).

Previous studies have shown that a decrease in milk pH leads to solubilization of micellar calcium phosphate, affecting protein stability and coagulation properties (22). This has important implications for cheese-making, where precise control of pH is necessary for optimal curd formation. On the other hand, elevated pH values in milk may indicate bacterial contamination, particularly by proteolytic and lipolytic organisms such as *Pseudomonas spp.*, which can degrade proteins and release alkaline by-products (23). The significant association between pH and CFU observed in this study suggests that monitoring milk pH could serve as a simple indicator of microbial quality at the farm level.

The presence of *E. coli, Pseudomonas spp.* and *Listeria spp*. in sheep milk poses serious public health concerns. Consumption of raw or inadequately processed milk may result in food borne illnesses ranging from mild gastroenteritis to life-threatening conditions such as hemolytic uremic syndrome (caused by STEC) and listeriosis (24, 25). In rural areas of Pakistan, raw milk is often consumed without boiling or pasteurization, which substantially increases health risks. Furthermore, the widespread use of antibiotics in livestock raises the possibility of antibiotic-resistant bacterial strains entering the food chain, as reported in *E. coli* and Listeria isolates from milk in several countries (26).

The findings of this study are comparable to reports from other regions. In Romania, analysis of raw bovine milk showed that 4.2% of samples exceeded the regulatory limit of *E. coli* contamination (6.18 $\log_{10}$ CFU/mL) (18). In Italy, sheep milk used for artisanal cheese production was also found to harbor unusual

bacterial species, suggesting environmental and geographic influences on microbial composition (27). In Pakistan, previous studies have detected high microbial loads in cow and buffalo milk due to unhygienic handling, emphasizing the need for improved dairy management practices (28).

Our results add to this growing body of evidence, demonstrating that sheep milk in Sindh and Balochistan is also vulnerable to contamination, with breed and locality playing important roles in determining microbial quality. Unlike studies in Europe, where milk contamination is often linked to processing, in Pakistan the major sources are poor hygiene during milking, use of contaminated utensils, and lack of cold chain facilities.

Ensuring the microbial safety of sheep milk requires a multi-pronged approach. First, strict adherence to hygienic practices during milking, including washing of teats, sterilization of utensils, and use of clean water, is essential (29). Second, regular monitoring of milk pH and microbial counts should be implemented at the farm level to identify high-risk batches. Third, pasteurization or boiling must be promoted before consumption to eliminate pathogenic bacteria. Public health education campaigns in rural areas could play a vital role in raising awareness about the dangers of consuming raw milk. Additionally, selective breeding for disease-resistant sheep and improved veterinary care could help reduce bacterial transmission from infected udders (30).

While this study provides valuable insights, some limitations must be acknowledged. The sample size, though covering a wide geographic range, may not fully represent seasonal variations or management differences across all farming systems. Moreover, molecular characterization of bacterial isolates was not performed, which could provide further insights into pathogenicity and antimicrobial resistance patterns. Future research should incorporate advanced molecular techniques such as PCR and whole-genome sequencing to identify virulence factors and resistance genes. Comparative studies across species (sheep, goats, cows, and buffaloes) would also help contextualize the risk levels associated with different dairy animals in Pakistan.

CONCLUSION

This study demonstrated that sheep milk from Sindh and Balochistan harbors pathogenic bacteria including *E. coli*, *Pseudomona spp.* and *Listeria spp*., with significant variation in CFU across breeds. Dumbi milk showed the highest contamination, while Mengali milk had the lowest. pH was found to significantly influence bacterial counts, highlighting its importance as an indicator of milk quality. These findings emphasize the urgent need for improved hygiene practices, routine microbial monitoring, and public health awareness to ensure the safety of sheep milk for consumers in Pakistan.

Conflict of interest:

Authors have no conflict of interest.

Authors' contribution:

SAS Conducted the experiment, collected samples and prepared the first draft of the manuscript; AK Supervised the research, guided the methodology and critically reviewed the manuscript; M Assisted in laboratory analysis, data compilation and statistical interpretation; AKh Contributed to literature review, data interpretation and manuscript editing.

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