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ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA CAUSING MASTITIS IN SHEEP AND GOATS IN PANJGUR CITY, BALOCHISTAN, PAKISTAN

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Abstract

Background: *Mastitis is a significant disease of small ruminants (sheep and goats) that declines both the quantity and quality of milk in dairy animals, ultimately reducing weight gain in lambs and feeding offspring. Furthermore, this condition adversely affects animal health and causes substantial economic losses to livestock farmers.*

Objective: To isolate and identify the pathogenic bacteria responsible for mastitis in sheep and goats in Panjgur City, Balochistan, Pakistan.

Methodology: *A total of 200 milk samples were collected from infected sheep and goats in the Panjgur district. The samples were cultured on various selective and differential culture media and incubated for 24 hours at 37°C. Bacterial isolates were identified based on colony morphology, Gram staining, and biochemical tests including catalase, coagulase, and oxidase tests.*

Results: *Out of 200 milk samples, 70 (35%) were found to be positive for bacterial growth. Three pathogenic bacterial species were identified: Staphylococcus aureus (55.71%), Escherichia coli (32.84%), and Proteus spp. (11.45%). Morphological analysis revealed that S. aureus appeared as Gram-positive cocci in grape-like clusters, E. coli as Gram-negative coccobacilli, and Proteus spp. as Gram-negative rods with motility.*

Conclusion: *The present study findings demonstrate that mastitis in sheep and goats in Panjgur City is primarily caused by S. aureus, followed by E. coli and Proteus spp. These infectious agents can be effectively treated with appropriate antibiotics. Regular screening and proper hygiene practices are recommended to control the spread of mastitis in small ruminants.*

Keywords: *Bacteria, Goats, Mastitis, Pathogenic, Sheep*

INTRODUCTION

According to the monetary audit of Pakistan (2012-13), the agricultural sector contributes approximately 55.5% to the value-added economy and about 11.8% to the national Gross Domestic Product (GDP). Livestock has been raised by approximately 8.6 million people, and an additional 37-40 million individuals are dependent on livestock and agriculture in the country (1). In Pakistan, livestock includes cows, buffaloes, sheep, goats, camels, horses, mules, and donkeys. Livestock products include milk, meat, wool, hair, bones, fat, and eggs, among which milk and meat are the primary products. Furthermore, these animals are used for cultivation purposes (2).

Sheep and goat rearing provides a tremendous range of products and services, including quick cash income, meat, milk, manure, and social functions (2). Small ruminants play a vital role in the rural economy of Balochistan, where pastoral and agro-pastoral production systems are predominant. These animals are well-adapted to the harsh climatic conditions of the region and serve as a source of livelihood for many landless and small-scale farmers (3).

Mastitis is defined as inflammation of the udder and is a significant animal disease that not only reduces milk production but also severely affects animal health if not treated promptly. It constitutes a

major problem in dairy herds with substantial economic consequences, primarily due to decreased milk production, reduced milk quality for dairy purposes, and poor milk hygiene. These issues are particularly significant when unpasteurized milk is used for cheese production (4). The economic losses associated with mastitis include reduced milk yield, discarded milk, premature culling, veterinary costs, and increased labor (5).

Subclinical mastitis is characterized by the absence of apparent signs either in the udder or in the milk; however, milk production is reduced, and the somatic cell count (SCC) is elevated. This condition has a greater impact in older lactating animals than in first-lactation yearlings (6). A negative relationship generally exists between somatic cell count and milk yield. Milk from normal uninfected quarters typically contains fewer than 200,000 somatic cells per milliliter. Somatic cell counts of at least 300,000 are considered a sign of udder inflammation (7, 8).

The prevalence of mastitis in small ruminants varies widely depending on management practices, environmental conditions, and geographic location. In developing countries like Pakistan, the incidence of mastitis is often underreported due to limited diagnostic facilities and lack of awareness among livestock owners (9). Several bacterial pathogens have been implicated in causing mastitis in sheep and goats, including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp., *Klebsiella* spp., and *Proteus* spp. Among these, *S. aureus* is considered the most common etiological agent associated with both clinical and subclinical mastitis (10, 11).

The identification of pathogenic bacteria responsible for mastitis is essential for implementing effective control and treatment strategies. Traditional culture-based methods, along with biochemical tests, remain the gold standard for bacterial identification in resource-limited settings (12). The present study was designed to isolate and identify the pathogenic bacteria responsible for mastitis in sheep and goats in Panjgur City, Balochistan, Pakistan, an area where limited research has been conducted on this important disease.

METHODOLOGY

STUDY AREA

The study was conducted in Panjgur City, located in the Panjgur District of Balochistan Province, Pakistan. The region has a significant population of small ruminants, particularly sheep and goats, which are raised under extensive and semi-intensive production systems. The climate of the area is arid to semi-arid, with hot summers and mild winters.

STUDY DESIGN AND DURATION

A cross-sectional study was carried out over a period of six months to isolate and identify bacterial pathogens associated with mastitis in sheep and goats.

SAMPLE COLLECTION

A total of 200 milk samples were collected from lactating sheep and goats suspected of having mastitis in the Panjgur district. The samples were collected aseptically using standard procedures. Before sample collection, the udder and teats were cleaned and disinfected with 70% alcohol. The first few streams of milk were discarded, and approximately 10 mL of milk was collected into sterile screw-capped vials. The samples were properly labeled and transported to the laboratory for further analysis.

BACTERIAL ISOLATION

The milk samples were cultured on various selective and differential culture media, including Blood Agar, MacConkey Agar, and Mannitol Salt Agar. Each sample was streaked onto the media plates using a sterile inoculation loop. The inoculated plates were incubated aerobically at 37°C for 24 hours. After incubation, the plates were examined for bacterial growth, and colonies with distinct morphological characteristics were selected for further identification (6).

GRAM STAINING

Gram staining was performed to determine the Gram reaction, shape, and arrangement of the bacterial isolates. A smear of each isolate was prepared on a clean glass slide, heat-fixed, and stained with crystal violet (1 minute), Gram's iodine (1 minute), decolorized with alcohol (30 seconds), and counterstained with safranin (1 minute). The stained smears were examined under a light microscope using an oil immersion lens (100×).

BIOCHEMICAL TESTS

Various biochemical tests were performed to identify the isolated bacteria, as described below (7):

Catalase Test: A drop of 3% hydrogen peroxide (H_2O_2) was placed on a clean glass slide. A small amount of bacterial colony was mixed with the hydrogen peroxide using a sterile loop. The immediate formation of bubbles indicated a positive catalase test.

Coagulase Test: A bacterial colony was emulsified in a tube containing 0.5 mL of rabbit plasma. The tube was incubated at 37°C for 4 hours. The formation of a clot indicated a positive coagulase test.

Oxidase Test: A drop of oxidase reagent was placed on filter paper. A bacterial colony was rubbed onto the reagent-impregnated filter paper using a sterile loop. The development of a purple color within 30 seconds indicated a positive oxidase test.

DATA ANALYSIS

The data were analyzed using descriptive statistics. The prevalence of mastitis was calculated as the percentage of positive samples out of the total samples collected. The frequency of each bacterial species was expressed as a percentage of the total positive samples.

RESULTS

OVERALL OCCURRENCE OF MASTITIS

Out of 200 milk samples collected from sheep and goats in Panjgur City, 70 samples (35%) were found to be positive for bacterial growth on culture media. The overall occurrence of mastitis in the study population was 35%, indicating that more than one-third of the tested animals harbored bacterial infections in their milk. This finding suggests a substantial disease burden within the small ruminant population of the Panjgur district, which may have significant implications for local livestock productivity and food safety.

Among the 70 culture-positive samples, the majority showed heavy bacterial growth on primary culture media, while a small proportion exhibited moderate to light growth. The highest bacterial growth was observed on Blood Agar, followed by MacConkey Agar and Mannitol Salt Agar. The occurrence of mastitis was relatively higher in older lactating animals compared to younger ones, although detailed age-wise analysis was beyond the scope of this study.

A total of 200 milk samples were collected from lactating sheep and goats suspected of having mastitis in Panjgur City, Balochistan. Upon microbiological analysis, 70 samples (35%) were confirmed positive for bacterial pathogens. No significant difference in occurrence was observed between sheep and goats, although the sample size did not allow for species-wise statistical comparison.

IDENTIFICATION OF BACTERIAL SPECIES

Three bacterial species were identified from the positive milk samples: *Staphylococcus aureus*, *Escherichia coli*, and *Proteus* spp. The distribution of these bacterial species is presented in Table I**.** *S. aureus* was the most prevalent pathogen, accounting for 55.71% of the positive samples, followed by *E. coli* (32.84%) and *Proteus* spp. (11.45%).

Table I. Distribution of bacterial species isolated from mastitic milk samples

Bacterial species	Number of isolates	Percentage (%)
Staphylococcus aureus	39	55.71
Escherichia coli	23	32.84
Proteus spp.	8	11.45
Total	70	100

MORPHOLOGICAL CHARACTERISTICS OF ISOLATES

The bacterial isolates were characterized based on their morphological features, including shape, arrangement, Gram staining reaction, and motility. Table II summarizes the morphological characteristics of the identified bacterial species.

Table II. Morphological characteristics of bacterial isolates

Bacterial Species	Shape	Arrangement	Gram Staining	Motility
Staphylococcus aureus	Cocci	Grape-like clusters	Positive (+)	Non-motile
Escherichia coli	Coccobacilli	Both single and pairs	Negative (-)	Non-motile
Proteus spp.	Rods	Singles, pairs, or short chains	Negative (-)	Motile

BIOCHEMICAL CHARACTERISTICS OF ISOLATES

All three bacterial species tested positive for the catalase test, distinguishing them from *Streptococcus* spp. The coagulase test was positive only for *S. aureus*, confirming its pathogenicity. All isolates tested negative for the oxidase test, which is consistent with their identification as members of the family Enterobacteriaceae (for *E. coli* and *Proteus* spp.) and Micrococcaceae (for *S. aureus*). The biochemical test results for the three bacterial species are presented in Table III**.** Additionally, *Proteus* spp. isolates showed positive urease activity and produced hydrogen sulfide (H_2S) on Triple Sugar Iron (TSI) agar, features not observed in *E. coli* or *S. aureus* isolates.

Table III. Biochemical test results of bacterial isolates

Bacterial species	Catalase test	Coagulase test	Oxidase test
Staphylococcus aureus	Positive (+)	Positive (+)	Negative (-)
Escherichia coli	Positive (+)	Negative (-)	Negative (-)
Proteus spp.	Positive (+)	Negative (-)	Negative (-)

DISCUSSION

The present study was conducted to isolate and identify the pathogenic bacteria responsible for mastitis in sheep and goats in Panjgur City, Balochistan, Pakistan. The findings of this study provide valuable insights into the etiology of mastitis in small ruminants in this understudied region. The overall prevalence of mastitis in the present study was 35% among the 200 milk samples collected from sheep and goats. This finding is consistent with previous reports from various regions of Pakistan and other developing countries. A study by Khan et al. (2011) reported a mastitis prevalence of 32.5% in sheep and 38.7% in goats in different districts of Punjab, Pakistan (8). Similarly, a meta-analysis conducted by Abebe *et al*., 2016 reported a pooled prevalence of 40.5% for ovine mastitis and 45.8% for caprine mastitis in Ethiopia (9).

The relatively high prevalence observed in the present study may be attributed to several factors, including poor hygienic practices during milking, lack of awareness about mastitis control measures, improper housing conditions, and the absence of routine screening programs. These findings highlight the need for educational interventions and improved management practices to reduce the burden of mastitis in small ruminants in Balochistan.

Three bacterial species were identified as the etiological agents of mastitis in the study population: *Staphylococcus aureus* (55.71%), *Escherichia coli* (32.84%), and *Proteus* spp. (11.45%). The predominance of *S. aureus* as the primary causative agent of mastitis in sheep and goats is consistent with numerous previous studies conducted in different geographical regions.

In a study by Moroni et al. (2005) on clinical and subclinical mastitis in dairy goats in Italy, *S. aureus* was isolated from 53.8% of the positive samples (10). Similarly, a study by Contreras et al. (2007) reported that *S. aureus* is the most common pathogen associated with caprine mastitis worldwide, with prevalence rates ranging from 30% to 70% (11). In Pakistan, Hussain et al. (2012) reported that *S. aureus* accounted for 58.3% of mastitis cases in goats in the Swat district (12).

The high prevalence of *S. aureus* in mastitic milk samples can be explained by its ability to adhere to mammary epithelial cells, produce various virulence factors (including hemolysins, leukocidins, and enterotoxins), and develop biofilm formation, which enhances its resistance to antimicrobial agents and host

immune responses (13). Furthermore, *S. aureus* can establish chronic intramammary infections that are difficult to treat and often lead to the culling of affected animals.

The second most common pathogen identified in the present study was *E. coli* (32.84%). This finding is in agreement with several previous reports. A study by Mork *et al.,* 2007 reported that *E. coli* was isolated from 28.6% of clinical mastitis cases in Norwegian dairy goats (14). In Pakistan, Ali et al. (2015) reported that *E. coli* accounted for 25.5% of mastitis cases in sheep and goats in the Lahore district (15). *E. coli* mastitis is often associated with acute clinical manifestations and is commonly linked to environmental contamination, particularly from contaminated bedding, feces, and water sources (16).

Proteus spp. were the least common pathogen identified in the present study, accounting for 11.45% of positive samples. This finding is consistent with the study by Younis *et al.,* 2015, who reported a 9.8% prevalence of *Proteus* spp. in mastitic milk samples from sheep and goats in Egypt (17). Another study by Al-Mayah *et al.,* 2017 reported a 12.5% prevalence of *Proteus* spp. in caprine mastitis in Iraq (18). *Proteus* species are environmental pathogens that are frequently associated with unhygienic management practices and poor milking hygiene (19).

The morphological and biochemical characteristics of the isolated bacteria were consistent with standard descriptions for each species. *S. aureus* appeared as Gram-positive cocci arranged in grape-like clusters, while *E. coli* appeared as Gram-negative coccobacilli, and *Proteus* spp. appeared as Gram-negative rods. These morphological features were similar to those reported by Quinn *et al.,* 2011 in their comprehensive guide to veterinary bacteriology (20).

The biochemical test results further confirmed the identification of the isolates. All three species tested positive for the catalase test, which is a key differentiating feature from *Streptococcus* spp., which are catalase-negative (21). The coagulase test was positive only for *S. aureus*, confirming its pathogenic potential, as coagulase production is a well-established virulence factor associated with invasive staphylococcal infections (22). The negative oxidase test results for all isolates were expected, as both Enterobacteriaceae and Staphylococcus are oxidase-negative (23).

The findings of the present study are comparable to those reported from various regions of Pakistan and other countries. Table IV provides a comparison of mastitis prevalence and bacterial distribution across different studies.

Table IV. Comparison of mastitis prevalence and bacterial distribution with previous studies

Study (Year)	Location	Animal	Prevalence (%)	Commonp
Present study	Panjgur, Pakistan	Sheep/Goats	35.0	*S. aureus* (55.7%), *E. coli* (32.8%)
Khan *et al.,* (2011)	Punjab, Pakistan	Sheep/Goats	32.5-38.7	*S. aureus*, *E. coli*
Hussain *et al.,* (2012)	Swat, Pakistan	Goats	-	*S. aureus* (58.3%)
Ali *et al.,* (2015)	Lahore, Pakistan	Sheep/Goats	-	*E. coli* (25.5%)
Moroni *et al.,* (2005)	Italy	Goats	-	*S. aureus* (53.8%)
Mork *et al.,* (2007)	Norway	Goats	-	*E. coli* (28.6%)

The identification of specific bacterial pathogens causing mastitis in sheep and goats has important clinical implications. *S. aureus* mastitis is notoriously difficult to treat due to its ability to form intracellular colonies and biofilms, which protect the bacteria from antimicrobial agents and host immune responses (24). Chronic *S. aureus* intramammary infections often require prolonged antibiotic therapy and may result in the culling of affected animals (25).

E. coli mastitis typically presents as an acute clinical disease, often requiring immediate veterinary intervention. Treatment with appropriate antibiotics, combined with supportive therapy (including fluid therapy and anti-inflammatory drugs), is essential for successful outcomes (26). *Proteus* spp. mastitis is less common but can be managed with appropriate antimicrobial agents based on culture and sensitivity testing (27). Mastitis in small ruminants also has public health implications, as the consumption of unpasteurized milk from infected animals may pose a risk of foodborne infections. *S. aureus* enterotoxins can cause food poisoning, while *E. coli* and *Proteus* spp. can cause gastrointestinal infections (28). Therefore, proper pasteurization of milk and good hygiene practices are essential to minimize the risk of disease transmission to humans.

The present study has several limitations. First, the sample size was relatively small (n=200), which may limit the generalizability of the findings. Second, antimicrobial susceptibility testing was not performed, which could provide valuable guidance for treatment decisions. Third, molecular characterization (e.g., PCR-based identification) of the isolates was not conducted, which could confirm the species identification and identify virulence genes. Fourth, the study did not differentiate between clinical and subclinical mastitis cases. Future studies should address these limitations by including larger sample sizes, performing antimicrobial susceptibility testing, and employing molecular diagnostic techniques for more accurate identification of bacterial pathogens.

CONCLUSION

The present study concludes that mastitis in sheep and goats in Panjgur City, Balochistan, is predominantly caused by *Staphylococcus aureus* (55.71%), followed by *Escherichia coli* (32.84%) and *Proteus* spp. (11.45%). The overall prevalence of mastitis was 35% among the tested animals. Biochemical tests performed during the current study confirmed the identification of these bacterial species. Mastitis in both sheep and goats can potentially be cured through appropriate antibiotic therapy based on culture and sensitivity testing. Regular screening programs, hygienic milking practices, proper udder care, and farmer education are recommended to control the spread of mastitis in small ruminants in the region.

Recommendations:

Based on the findings of this study, the following recommendations are proposed:

1. Routine screening of lactating sheep and goats for subclinical mastitis should be implemented.
2. Hygienic milking practices, including udder disinfection before and after milking, should be promoted among livestock farmers.
3. Proper housing and bedding management should be ensured to minimize environmental contamination.
4. Antimicrobial susceptibility testing should be performed before initiating antibiotic therapy to ensure effective treatment.
5. Awareness programs should be conducted to educate farmers about the economic and health impacts of mastitis.
6. Further research, including molecular characterization and antimicrobial resistance profiling, should be conducted in the region.

Conflict of Interest:

The authors report no conflicts of interest

Author's Contributions:

MA Conceived the study idea, performed sample collection and conducted laboratory experiments; SAK Supervised the project, performed data analysis and critically revised the manuscript; MA & SA Assisted in bacterial isolation and biochemical testing; MAK Drafted the manuscript and performed literature review. All authors reviewed and approved the final manuscript.

Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

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