



Prevalence of Bovine Subclinical Mastitis and Antibigram Pattern of Isolated Organisms from Mastitic Milk in Chattogram, Bangladesh

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JA and SC conceptualization, investigated the work and wrote and prepared the original draft and did the project administration. Authors SC and GMC wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: This research investigated the prevalence and risk factors of subclinical mastitis (SCM) in dairy cows within the Chattogram, Bangladesh, focusing on its effects on milk production and quality, as well as the antibiotic resistance profiles of *Staphylococcus* spp. and *Escherichia coli* (*E. coli*).

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Materials and Methods: Fifty lactating cows from five dairy farms were studied over eight months (January to August 2018). Milk samples were collected aseptically and screened using the California Mastitis Test (CMT) to identify SCM. Bacterial cultures were conducted to isolate *Staphylococcus spp.* and *E. coli*, and their antibiotic susceptibility was assessed using the Bauer-Kirby disk diffusion method. Farm management data were collected via structured questionnaires.

Results: The SCM prevalence was 50%, with *Staphylococcus spp.* and *E. coli* each found in 32% of cases. *Staphylococcus spp.* showed 100% resistance to amoxicillin, enrofloxacin, and azithromycin, while *E. coli* demonstrated similar resistance patterns. Gentamycin was more effective, with only 25% resistance against *Staphylococcus spp.* Key factors influencing infection included farm size, housing system, lactation stage, and udder cleanliness. Farms with poor hygiene practices, such as infrequent floor cleaning without disinfectant, had higher infection rates.

Conclusion: The findings highlight the necessity for routine SCM screening and improved farm hygiene practices. Combating antibiotic resistance demands careful antibiotic use guided by sensitivity testing. Effective management requires regular monitoring, hygiene enhancements, and strategic treatments to protect dairy production.

Keywords: Subclinical mastitis, antibiotic resistance, *Staphylococcus spp.* and *Escherichia coli*.

1. INTRODUCTION

In Bangladesh, dairy milk production is one of the major sectors for the national income development. Currently Bangladesh is producing 150.44 lakh metric ton (average) milk yearly and creating great contribution to GDP [1]. However, milk production often does not satisfy the country's milk requirements due to a multitude of associated factors. Mastitis is one of the complex and costly diseases of dairy cows that results from the interaction of the cow and its surrounding environment. Mastitis, an inflammation affecting the mammary glands, ranks among the most common and economically significant diseases impacting dairy cows globally. This condition reduces milk production, alters milk composition, and increases both veterinary care and management expenses, leading to considerable financial losses within the dairy sector. Mastitis manifests in two primary forms: clinical mastitis, where symptoms are visibly apparent, and subclinical mastitis, which is asymptomatic but still adversely affects milk yield and quality [2].

SCM is a widespread and economically detrimental condition affecting dairy herds. Unlike clinical mastitis, SCM does not show outward signs of infection, such as swelling or abnormal milk appearance, making it a silent threat that often goes unnoticed. It is a hidden inflammation of the mammary glands and causes reduced milk yield and lower milk quality. It is the most economically significant form of mastitis, occurring 15–40 times more frequently than clinical mastitis and affecting dairy farms globally [3].

Accurate and early detection of subclinical mastitis is therefore crucial for effective disease management and prevention. The CMT is a simple, cost-effective, and widely-used field test that detects subclinical mastitis by estimating the somatic cell count (SCC) in milk. Elevated SCC levels indicate an immune response to infection within the udder, allowing the CMT to serve as an indirect measure of udder health. The test involves mixing a reagent with a milk sample; the reagent interacts with somatic cell DNA, forming a gel-like consistency proportional to the infection's severity [4]. The CMT's accessibility and reliability have made it a valuable tool for dairy farmers and veterinarians to identify and monitor subclinical mastitis, facilitating timely intervention and helping to reduce the risk of infection spreading throughout the herd.

Staphylococcus spp. is a major pathogen responsible for causing subclinical mastitis in dairy cows. It is the most commonly associated bacterium with intramammary infections, often leading to persistent, chronic infections [5]. The infection often shows poor response to antimicrobial treatment, potentially due to antimicrobial resistance (AMR), biofilm formation, and the bacteria's ability to invade bovine mammary cells, resulting in treatment failure [6].

E. coli is another significant pathogen implicated in subclinical mastitis in dairy herds. *E. coli* is an environmental pathogen, meaning it is commonly found in the cow's surroundings, including bedding, manure, and contaminated water. Poor hygiene practices and improper management, such as inadequate barn cleanliness or overcrowding, increase the risk of cows

becoming infected. The bacteria can enter the mammary gland through the teat canal, especially if the udder is exposed to high levels of environmental contamination. Once inside, *E. coli* triggers an immune response, leading to an elevated somatic cell count, even if visible symptoms are not present.

Research has shown that inadequate husbandry practices—such as microbial contamination, insufficient nutrition, poor hygiene, suboptimal transition management, and shifting climate conditions—are key contributors to the rise in SCM cases [7].

Antibiotics have played a crucial role in improving growth, productivity, and treating infections within the dairy industry. However, antibiotic treatment is often initiated only once cows show clinical symptoms, frequently without prior screening or accurate diagnosis, resulting in inconsistent and sometimes excessive antibiotic usage. Detecting subclinical mastitis presents a unique challenge, as it lacks visible symptoms, yet it significantly impacts production costs due to reduced milk yield. This silent yet costly condition highlights the need for proactive screening measures to minimize unnecessary antibiotic application and ensure effective herd health management. Therefore, appropriate drug selection and antibiotic susceptibility testing should be conducted prior to prescribing antibiotics.

The objectives of this study are to assess the prevalence of subclinical mastitis in dairy cows within Chattogram, identify key pathogens, and evaluate their antibiotic resistance patterns. This research aims to highlight the economic burden posed by subclinical mastitis due to decreased milk quality and yield. Additionally, it emphasizes the importance of improved management practices, such as routine screening and targeted antibiotic use, to mitigate the spread of antibiotic-resistant pathogens in dairy herds.

2. MATERIALS AND METHODS

2.1 Study Population and Sample Collection

The study was undertaken on 50 lactating cows for the periods of 8 months from January to August, 2018. Five dairy farms of Chattogram area, Bangladesh (Kalurghat,

Shikalbaha, Bakolia, Patiya, Karnaphuli area) were visited for sample collection. The sample were collected from healthy cows in 15 ml falcon tubes. Maintaining cool chain, samples were sent to the clinical pathology lab, Chattogram Veterinary and Animal Sciences University (CVASU) for laboratory analysis.

2.2 Data Collection via Questionnaire

Data collection was conducted using a structured questionnaire administered throughout the study. The questionnaire gathered information on farm address, farm size, housing and rearing systems, breed type, body condition score, milk yield, lactation number, udder cleanliness, floor cleanliness, lactation stage, history of lameness, reproductive disease history, and medication use. It included a combination of open- and close-ended questions.

2.3 Experimental Design

A total of 50 lactating cows were randomly selected from the five dairy farms. During milk sample collection, aseptic procedures were applied by disinfecting teats with 70% ethyl alcohol, drying them with cotton, and discarding initial milk drops. Milk samples from each quarter underwent the CMT. In case of CMT positive samples, 5 ml of milk were collected in sterilized tubes, labeled, and stored at 4°C until laboratory analysis. Bacterial isolates were identified based on cultural characteristics, such as pigment production and hemolytic activity.

2.4 Determination of Subclinical Mastitis

SCM was evaluated using the CMT which estimates somatic cell concentration. Two milliliters of milk and CMT solution (1:1) were mixed on a test paddle/tray, rotated for thorough mixing, and observed for changes in color and gel formation within 10–15 seconds. Milk quality and mastitis status were assessed using the criteria in Table-1[8].

2.5 Bacteriological Investigation

The identification of *Staphylococcus spp.* and *E. coli* bacterial species was carried out using standard bacteriological methods outlined by Gao et al., 2017 and Ali et al., 2017 respectively [9,10].

Table 1. Evaluation of Milk Quality and Mastitis Status Based on CMT Scores

CMT Score	Observation	Somatic Cell Level	Interpretation
1	No thickening of mixture	<200,000 cells/ml	Healthy quarter
2	Slight thickening; trace reaction fades with rotation	150,000–500,000 cells/ml	Suspicious
3	Distinct thickening; no gel formation	400,000–1,500,000 cells/ml	Mastitis positive
4	Immediate thickening with slight gel formation	800,000–5,000,000 cells/ml	Mastitis positive
5	Gel forms, elevated surface	>5,000,000 cells/ml	Mastitis positive

2.6 Isolation and Identification of *Staphylococcus spp.*

Isolation of *Staphylococcus spp.* was done following a modified method according to Singh et al., 2008 [11]. Peptone water (PW) was used for enrichment by homogenizing 5 ml of the sample with 45 ml sterile broth and incubating for 24 hours at 37°C [12]. Samples were then streaked on Mannitol Salt Agar (MSA) and incubated at 37°C. Bright yellow colonies indicated *Staphylococcus spp.*, which were further cultured on 5% Bovine Blood Agar to observe hemolysis. Then *Staphylococcus spp.*, with α - and β -hemolytic activity on blood agar was isolated for antibiogram.

2.7 Isolation and Identification of *E. coli*

Samples were initially cultured on MacConkey Agar, where *E. coli* produced pink colonies after 24 hours at 37°C. Presumptive colonies were transferred to Eosin Methylene Blue (EMB) Agar, where metallic sheen colonies were then cultured on 5% bovine blood agar to detect pathogen

activity, with incubation at 37°C to isolate pure culture for further antibiotic sensitivity test.

2.8 Antibiotic Sensitivity Test

The sensitivity of isolated bacteria to antibiotics was evaluated using the Bauer-Kirby disk diffusion method [13] on Muller-Hinton (M173) agar. Bacterial turbidity was standardized using a 0.5 McFarland standard, and a range of antibiotics was tested. Interpretation of results—resistant (R), intermediate (I), and sensitive (S)—followed CLSI 2020 guidelines [14], as shown in Table 2.

2.9 Statistical Analysis

Data from questionnaires and laboratory results were entered into Microsoft Excel (2010) and analyzed using STATA version 13. The prevalence of subclinical mastitis, *E. coli*, and *Staphylococcus spp.* was calculated by dividing the number of cases by the study population. Frequency and percentages of various variables were also calculated based on infection status.

Table 2. CLSI Antibiotic Zone Interpretation for Bacterial Susceptibility

Antimicrobial agent	Disc code	Potency (μ gm)	Bacteria	Test cultures (zone diameters in mm)		
				Resistant	Intermediate	Susceptible
Amoxycilin	AML	10	<i>E. coli</i>	≤ 13	14-17	≥ 18
			<i>Staphylococcus</i>	≤ 19	-	≥ 20
Azithromycin	AZM	15	<i>E. coli</i>	-	-	-
			<i>Staphylococcus</i>	≤ 13	14-17	≥ 18
Pefloxacin	PEF	5	<i>E. coli</i>	≤ 23	-	≥ 24
			<i>Staphylococcus</i>	≤ 23	-	≥ 24
Enrofloxacin	ENR	5	<i>E. coli</i>	≤ 20	21-30	≤ 31
			<i>Staphylococcus</i>	≤ 15	16-20	≥ 21
Doxycyclin	DO	30	<i>E. coli</i>	≤ 10	11-13	≥ 14
			<i>Staphylococcus</i>	≤ 12	13-15	≥ 16
Gentamycin	CN	10	<i>E. coli</i>	≤ 12	13-14	≥ 15
			<i>Staphylococcus</i>	≤ 12	13-14	≥ 15
Streptomycin	S	10	<i>E. coli</i>	≤ 11	12-14	≥ 15
			<i>Staphylococcus</i>	-	-	-

3. RESULTS

3.1 Descriptive Statistics of the Study Population

This study focused on 50 lactating cows from five dairy farms in Chattogram, Bangladesh. Over the study period, 25 of these cows (50%) tested positive for subclinical mastitis using a commercially available CMT kit. The prevalence of *Staphylococcus* spp. and *E. coli* was observed to be 32% each among the sample population.

The distribution of affected cows across different farm sizes revealed that 32% (n=8) were from farms with 50–80 animals, 20% (n=5) from farms with 81–110 animals, and 48% (n=12) from larger farms housing more than 110 cows.

The data on housing systems highlighted two main categories: "face-out" and "face-in" stanchion barns. Among the positive cases, 68% (n=17) of cows were housed in a face-out system, while 32% (n=8) were housed in a face-in system. Additionally, cows were classified based on their lactation number: 56% (n=14) of the positive cows were within their 1st to 2nd lactation period, 36% (n=9) were in their 3rd lactation, and 8% (n=2) were in a higher lactation period.

Breed data showed that cows with a higher proportion of Holstein Friesian (HF) blood—specifically, 75% HF—had a greater prevalence of subclinical mastitis, accounting for 52% (n=13) of positive cases, compared to 48% (n=12) in cows with less than 75% HF blood.

In terms of body condition score (BCS), 16% (n=4) of affected cows had a BCS of 2, 72% (n=18) had a BCS of 3, and 12% (n=3) had a BCS of 4. Milk yield data indicated that 16% (n=4) of the affected cows produced less than 5 kg of milk daily, 36% (n=9) produced between 5–10 kg, 32% (n=8) produced 10–15 kg, and another 16% (n=4) produced more than 15 kg daily. Cows with an average daily milk yield of 5–10 kg had the highest prevalence of subclinical mastitis.

The body weight distribution showed that 8% (n=2) of affected cows weighed less than 250 kg, 40% (n=10) weighed between 250–300 kg, 44% (n=11) weighed between 300–350 kg, and the remaining 8% (n=2) weighed over 350 kg.

Farm hygiene practices, including udder and floor cleanliness, were also evaluated. The data indicated that 80% of cows affected by mastitis were from farms that practiced floor washing less than twice daily without using disinfectant. Udder cleanliness was also a significant factor, with 68% of mastitic cows having moderately clean udders and 32% having clean udders.

Furthermore, 92% of the cows with mastitis had no recorded reproductive disorders. Regarding the management of mastitis, 20% of cases were managed exclusively by a veterinarian, 52% were managed by both veterinarians and veterinary field assistants (VFA), and 28% were managed solely by a VFA.

The following table (Table: 3) summarizes the demographic data and risk factors among the mastitis-affected farms and cows:

3.2 Antibiotic Sensitivity Testing

The antibiotic sensitivity test results for both *Staphylococcus* spp. and *E. coli* showed varying resistance levels to different antibiotics. Results for each bacterium are detailed below (Fig. 1 and 2; AZM = Azithromycin, ENR = Enrofloxacin, S= Streptomycin, CN= Gentamycin, DO = Doxycycline, PEF = Pefloxacin, AML = Amoxicillin).

These figures highlight that while gentamycin showed the highest sensitivity (75%) against *Staphylococcus* spp., resistance levels were high for amoxicillin, enrofloxacin, and azithromycin in both *Staphylococcus* spp. and *E. coli*, indicating a significant resistance issue.

4. DISCUSSION

The prevalence of subclinical mastitis remains a global concern due to its considerable impact on milk yield, animal welfare, and economic losses in the dairy industry. This study, conducted in the Chattogram of Bangladesh, revealed a substantial prevalence of SCM in dairy cows, with *Staphylococcus* spp. and *Escherichia coli* being the predominant pathogens. These findings are consistent with recent global studies that emphasize the role of these pathogens in subclinical mastitis, especially in low and middle-income countries where environmental and management practices significantly influence disease prevalence [15, 16, 17, 18].

Table 3. Demography of Affected Farms and Cows in the Study Area

Variable	Category	Frequency	Percentage
Farm size	50–80	8	32%
	81–110	5	20%
	>110	12	48%
Housing system	Face-out	17	68%
	Face-in	8	32%
Lactation number	1–2	14	56%
	3	9	36%
	>3	2	8%
Breed	75% HF	13	52%
	<75% HF	12	48%
BCS	2	4	16%
	3	18	72%
	4	3	12%
Milk yield	<5 kg	4	16%
	5–10 kg	9	36%
	10–15 kg	8	32%
	>15 kg	4	16%
Body weight	<250 kg	2	8%
	250–300 kg	10	40%
	300–350 kg	11	44%
	>350 kg	2	8%
Udder cleanliness	Clean	8	32%
	Moderately clean	17	68%
Floor cleanliness	Daily washing 2 times w/ disinfectant	5	20%
	<2 times washing w/o disinfectant	20	80%
Any reproductive disorder	No	23	92%
	Yes	2	8%
How mastitis is managed	By vet	5	20%
	Vet and VFA	13	52%
	VFA	7	28%
	2 (gentamycin, amoxicillin, oxytetracycline)	17	68%
<i>Staphylococcus</i> status	Negative	17	68%
	Positive	8	32%
<i>E. coli</i> status	Negative	17	68%
	Positive	8	32%

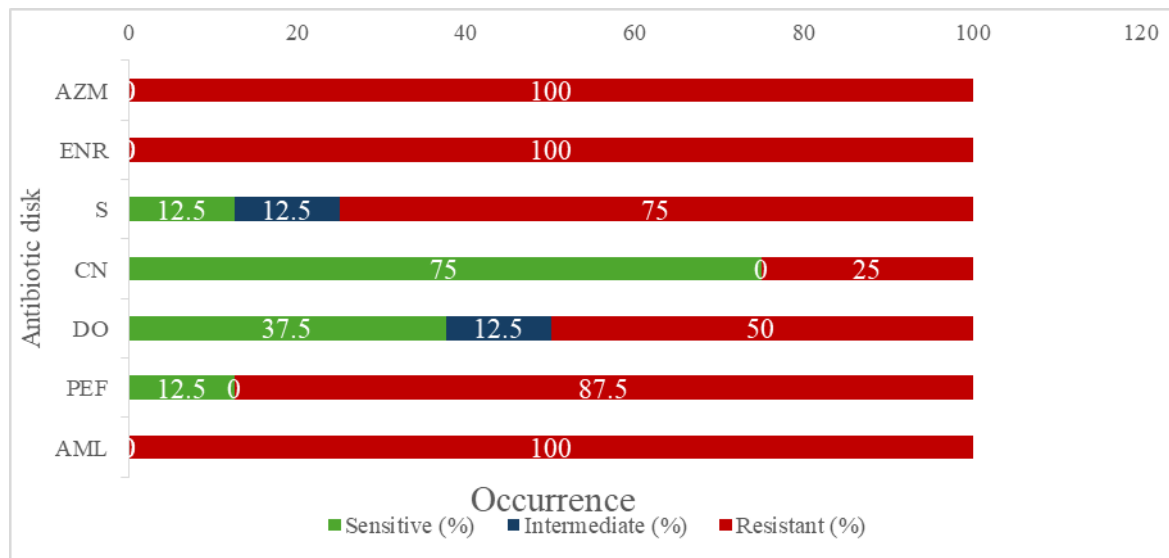


Fig. 1. Antibiotic Sensitivity Test for *Staphylococcus* spp



Fig. 2. Antibiotic Sensitivity Test for *E. coli*

4.1 Prevalence and Risk Factors

The study was undertaken on 50 lactating cows from 5 dairy farms of Chattogram. SCM prevalence in this study population, as indicated by a 50% positivity rate in CMT testing, is comparable to findings in other regional studies. According to Rana et al., 2022 [17], 38% cows were affected with subclinical mastitis screened by CMT test and the study was conducted in Shikalbaha and Bandar thana of Chattogram district. On the other hand, Hussein et al., 2022 [19] reported that 60% cows were affected with subclinical mastitis and recorded with positive CMT result.

4.2 Antibiotic Resistance Patterns

In our study we used 7 antibiotics to evaluate the sensitivity. A remarkable number of antibiotics showed high resistance. In case of *Staphylococcus* spp., resistance observed against amoxyciline (100%), enrofloxacin (100%), azithromycin (100%) followed by pefloxacin (87.5%), streptomycin (75%), doxycycline (50%), gentamycin (25%). Similarly, in case of *E. coli*, highest resistant pattern was recorded against amoxyciline and azithromycin. According to previous findings, *Staphylococcus* spp. and *E. coli* resistance to amoxyciline, azithromycin, ampicillin and tetracyclin is the

most common type of antimicrobial resistance among SCM and mastitis-causing bacteria [20, 21]. The resistant pattern of antibiotics revealed by this study is of concern for dairy farmers as it might lead to non-responsive treatment.

4.3 Management and Prevention Strategies

Risk factors such as housing conditions, lactation stage, and udder cleanliness appear to play a crucial role in susceptibility to infection. Recent studies have recommended control measures that include enhancing cow hygiene and farm sanitation, ensuring clean hands for milkers, implementing dry cow therapy, supplementing with micronutrients, conducting regular SCM screenings and providing appropriate treatment [22, 7].

5. CONCLUSION

This study underscores the high prevalence of subclinical mastitis in dairy farms in Chattogram, driven by environmental conditions and the presence of multidrug-resistant pathogens. Effective mastitis control requires a combination of early detection, informed antibiotic use, and robust farm management practices. Addressing antibiotic resistance will demand continuous surveillance and the exploration of alternative therapies that are both sustainable and cost-effective for local farming contexts.

Statement of novelty: This study uniquely identifies the high prevalence (50%) of subclinical mastitis (SCM) in dairy cows within Chattogram, Bangladesh, highlighting its significant impact on milk production. The novel finding of 100% antibiotic resistance in *Staphylococcus spp.* and *E. coli* to commonly used antibiotics like amoxicillin, enrofloxacin, and azithromycin is critical, as it underscores a severe threat to treatment efficacy. Additionally, the research links farm management practices, such as hygiene and housing systems, to SCM rates, providing evidence-based recommendations for mitigating antibiotic resistance through improved farm hygiene and targeted antibiotic use. These findings emphasize the urgent need for routine SCM screening and tailored interventions in local dairy farms.

6. LIMITATIONS

This study primarily relied on cultural characteristics for the presumptive identification

of *Staphylococcus spp.* and *E. coli*, without employing confirmatory morphological, biochemical, or molecular detection methods. As a result, there remains a possibility that the isolates may not be pure or accurately identified, which could affect the interpretation of the antibiotic sensitivity results. Furthermore, the sample size and geographical coverage of farms may limit the generalizability of the findings to broader populations.

7. RECOMMENDATIONS

Future studies should incorporate more robust identification techniques, such as biochemical tests, PCR-based molecular methods, or whole-genome sequencing, to ensure the accurate and definitive identification of bacterial isolates. Additionally, expanding the sample size and geographic scope would provide a more comprehensive understanding of resistance patterns. Exploring the genetic mechanisms of antibiotic resistance in these isolates could also offer valuable insights for designing targeted interventions to mitigate antimicrobial resistance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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