



Subclinical Mastitis Prevalence and Antimicrobial Susceptibility Profile in Western Algerian Dairy Herds

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ABSTRACT

Background: Bovine mastitis poses a significant challenge for Algeria's dairy industry, impacting cattle health and productivity while threatening animal welfare and economic stability. The study aimed to determine the prevalence of bovine subclinical mastitis, identify the etiological pathogens and assess their susceptibility. The study included 65 dairy cows from seven small dairy farms.

Methods: The California Mastitis Test was used to screen cows with subclinical mastitis and antibiotic susceptibility of the isolates was assessed using the disk diffusion method.

Result: The prevalence of mastitis was 70.77% (46 / 65) and 35% (91/260) respectively. Coagulase-negative staphylococci (CNS) were the predominant strains at 54.84%, followed by *Staphylococcus aureus* at 20.97% and *Escherichia coli* at 11.29%. Antimicrobial susceptibility tests revealed a high level of multidrug resistance, affecting 81.48% of the strains tested. *Escherichia coli* demonstrated a high resistance to ampicillin, tetracycline and trimethoprim-sulfamethoxazole. Coagulase-negative staphylococci exhibited a notable resistance to streptomycin, penicillin G and tetracycline. Additionally, *Staphylococcus aureus* showed considerable resistance to penicillin G and tetracycline. Most strains tested in this study showed a high sensitivity to Kanamycin. The high prevalence of bovine mastitis and significant multidrug resistance highlight the urgent need for better management practices in the dairy industry. Effective monitoring and targeted treatments are essential for animal welfare and the economic viability of dairy farming in Algeria.

Key words: Coagulase-negative staphylococci, *Escherichia coli*, Multidrug resistance, *Staphylococcus aureus*.

INTRODUCTION

Bovine mastitis is a common disease that affects cattle herds globally. It is frequently reported in dairy herds in Algeria and poses a significant financial challenge to the global dairy industry (Meskini *et al.*, 2023). The leading consequences of this issue are primarily a significant decrease in milk production (with 70% resulting from subclinical mastitis), veterinary intervention (treatments and veterinary expenses), devaluation of the animal (compromised quarter function, early culling or death) and milk loss due to changes or antibiotic residues after treatment (Jagielski *et al.*, 2014; Freitas *et al.*, 2018).

Many bacterial species have been found to be responsible for clinical and subclinical manifestations of cow mastitis. However, staphylococci, streptococci and coliforms are usually regarded as the primary pathogens of mastitis worldwide, with *Staphylococcus aureus* considered to be the predominant pathogen in both types of this illness. CNS have emerged as notable bacteria in subclinical mastitis across various countries. Once considered minor, CNS now includes 51 species and 25 subspecies. It is associated with both subclinical and clinical forms of this infection (Schmidt *et al.*, 2015; Thomas *et al.*, 2015). *Escherichia coli*, an environmental bacterial agent, is most prominent during the periparturient period and decreases during lactation. Infected individuals usually experience infections lasting under 10 days, with limited response to antibiotics (Oliver *et al.*, 2011; Goulart and Mellata, 2022).

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For these reasons, antibiotic therapy remains the most regularly used approach for treating bovine mastitis. However, the misuse and abuse of this treatment have resulted in the development of resistant bacterial strains, presenting a significant challenge to animal health and posing a possible danger to human health (Freitas *et al.*, 2018; Verma *et al.*, 2018).

In view of these challenges, the aim of the research was to determine the prevalence of subclinical mastitis, the predominant bacteria responsible for mastitis cases on conventional dairy farms in the province of sidi bel abbes and to assess their susceptibility to widely used antibiotics.

MATERIALS AND METHODS

Study area and sample size

A cross-sectional study was conducted in the province of Sidi Bel Abbes in the northwestern of Algeria. The study took place from January 2021 to June 2023 and involved seven small dairy herds. The sample included 65 lactating cows of various breeds, managed under traditional and semi-intensive farming practices. The farms were selected based on the owners' consent to partake in this research and all lactating dairy cows on these farms were included in the analysis without exception.

Sample collection

Mastitis-affected quarters were identified using the California mastitis test (CMT), following the guidelines outlined by Quinn *et al.* (1999). The assessment of subclinical mastitis begins with the thorough cleaning of the udders using a biocide solution and water, followed by drying each quarter with a cloth. In this study, 260 quarters were assessed by extracting 2 mL of milk from each one and mixing it with an equal amount of commercial CMT reagent (RAIDEX GmbH, Germany). The viscosity of the mixture was rated on a scale from 0 to 4, where scores of 0 and 1 indicated negative and trace results, respectively. Scores of 2, 3 and 4 were considered positive. A cow was determined to have subclinical mastitis if at least one quarter registered a score of 2 or above.

All sampling procedures adhered to the recommendations in the Laboratory handbook on bovine mastitis (Adkins *et al.*, 2017). A total of 91 positive quarters were sampled individually using sterile glass tubes. Before collecting the milk samples, the quarters were cleaned with 70% alcohol and the first few drops of milk were discarded. During the sampling process, the tubes were held at an angle of approximately 45 degrees, with a typical volume of 2 to 3 mL of milk considered sufficient. To ensure that the samples remained at the required refrigeration temperature, they were transported in an isothermal box and processed within 24 hours of sampling.

Bacterial analysis

A small volume of 0.1 mL of milk was inoculated onto Colombia agar supplemented with 5% sheep blood, as well as in Hektoen agar and Mannitol salt agar. The samples were then incubated at a temperature of 37°C for 24 hours, followed by purification in the respective selective media. The identification of the bacteria was carried out by examining their physical characteristics, such as colony morphology and gram stains. Additionally, biochemical tests for catalase and oxidase activity were performed. To ensure precise identification, API Staph and API 20 E strips from Bio Mérieux (France) were utilized, following the manufacturer's technical data instructions.

Antimicrobial susceptibility

An antimicrobial sensitivity test using the disc diffusion method on Muller Hinton agar was carried out on a set of

11 antibiotic disks; nine discs contained a single antibiotic (Ampicillin 10 µg, Penicillin G 10 µg, Kanamycin 30 µg, Streptomycin 300 µg, Erythromycin 15 µg, Spiramycin 100 µg, Tetracycline 30 µg, Colistin 10 µg and Lincomycin 15 µg). Two discs contained a combination of two antibiotics (Amoxicillin 20 µg + Clavulanic acid 10 µg and Trimethoprim 1.25 µg + Sulfamethoxazole 23.75 µg).

The antibiotics were selected based firstly on an online survey feedback sent to veterinarians and secondly on the availability of antibiotic discs on the Algerian market. The test was performed following the protocol described by Hudzicki (2009) cited by Meskini *et al.* (2021) and carried out on the following strains; *Escherichia coli*, *Staphylococcus aureus* and CNS. The zone of inhibition was measured and interpreted as susceptible (S), intermediate (I), or resistant (R) to the exposed agent according to the zone diameter interpretation standards provided by the antibiogram committee of the French Society of Microbiology (SMF 2023): Veterinary recommendations.

Statistical analysis

Data collected from laboratory tests and field questionnaires were recorded in Microsoft Excel 2016. The prevalence of subclinical mastitis was calculated by dividing the number of cows affected by subclinical mastitis by the total number of cows tested.

RESULTS AND DISCUSSION

Prevalence of mastitis

The study revealed that the subclinical mastitis occurrence among cows is 70.77%. The finding aligns with previous research conducted in Algeria, where Ouakli *et al.* (2022) stated a prevalence rate of 71.15% in the Blida province. Similarly, in Kenya, Mbindyo *et al.* (2020) noted a prevalence rate of 73.1% (Table 1).

Our study differs from previous studies conducted by Belmamoun *et al.* (2016), who reported a lower prevalence of 33.6% in the western region, Sohiddullah *et al.* (2024), who found an incidence of 28% in Bangladesh and Kumar *et al.* (2021) in India who reported prevalence rates of 36.74%.

At the quarter level, the prevalence of subclinical mastitis was found to be 35% as shown in Table 1. These findings are similar to the results of Saidi *et al.* (2013) in the central region of Algeria and Seddar-Yagoub *et al.*, (2024), who reported rates of 28.77% and 32.12%, respectively. In contrast, this prevalence is lower than that reported by Fartas *et al.* (2017), who found a rate of 61.6% and Zalani and Fougou, (2024), who found a rate of 44.8%.

According to Radostitis *et al.* (2007), the difference in mastitis occurrence across various countries and even within the same country can be linked to many factors such as management practices, the cow breed, the milking process, geographical and agro-ecological differences, screening methods, hygiene and preventive measures. In Algeria, most farms are family-run and conventionally

Table 1: Prevalence of mastitis at the quarter and cow levels, CMT and bacterial cultures.

Number of examined herds	Number of examined cows	Number of infected cows	Number of infected quarters	Number of negative quarters	Number of isolated strains	Number of negative cultures	Number of contaminated cultures	Prevalence at the cow level (%)	Prevalence at the quarter level (%)
1 st	7	3	5	23	5	0	0	42.85	1.92
2 nd	10	7	13	27	8	3	2	70	5
3 th	11	8	18	25	11	4	2	72.73	6.92
4 th	8	5	10	22	6	4	0	62.5	3.85
5 th	9	7	11	23	9	3	0	77.78	4.23
6 th	10	8	18	20	12	5	2	80	6.92
7 th	10	8	16	29	11	3	3	80	6.15
Total	65	46	91	169	62	22	9	70.77	35

managed, with many farmers lacking training and knowledge about subclinical mastitis or effective screening methods (Meskini *et al.*, 2022).

Microbiological analysis

In Table 2, the bacteriological examination of the samples revealed that out of 91 samples tested, 62 strains were isolated. Coagulase-negative Staphylococcus (CNS) was the predominant pathogen found in cases of subclinical mastitis, accounting for 54.84% of cases.

These results are similar to studies conducted by Meskini *et al.* (2021) in the North West region of Algeria and Mbindyo *et al.* (2020) in Kenya. CNS is the most commonly isolated pathogenic agent in intramammary inflammation in cattle, as noted by Breser *et al.* (2018). Santos (2010) highlighted that these microorganisms are prevalent in milking environments, on equipment and udder skin, contributing significantly to bovine mastitis. Their role as primary pathogens has gained increasing recognition in recent years.

Staphylococcus aureus was the second most isolated strain in this study with a rate of 20.97%. This result is in line with the findings of Boufaïda Asnour *et al.* (2012) in Algeria and Melesse and Minyahil, (2019) in Ethiopia. Remy (2010) stated that *Staphylococcus aureus* is the bacterium most frequently colonizing mammary tissues. It can be found in milk, as well as in various cells such as epithelial cells and white blood cells and in the extracellular environment and micro-abscesses. This allows the bacterium to effectively avoid detection and attack from both natural defences and medical treatments. Colonization occurs quickly, as *Staphylococcus aureus* breaches epithelial barriers by the third-day post-infection. It is released in milk, facilitating transmission to healthy animals, especially at the time of milking (Radostits *et al.*, 2007).

This table also shows that the predominant bacterium identified within the enterobacteria group was *Escherichia coli*, demonstrating a prevalence of 11.29%, succeeded by *Serratia liquefaciens* at a prevalence of 3.23%. *Enterobacter cloacae*, *Serratia odorifera*, *Klebsiella pneumoniae ssp ozaenae* rate a prevalence of 1.61 % for each. *Pseudomonas* spp comprised 4.84% of the total isolated bacteria.

The prevalence of *Escherichia coli* in this study is comparable to previous findings by Boufaïda Asnour *et al.* (2012) and Asmare and Kassa (2017), who reported

Table 2: Bacterial species isolated from mastitis milk.

Bacteria strains	Numbers	Frequency
SCN	34	54.84
<i>Staphylococcus aureus</i>	13	20.97
<i>Pseudomonas</i> spp	3	4.84
<i>Escherichia coli</i>	7	11.29
<i>Serratia liquefaciens</i>	2	3.23
<i>Enterobacter cloacae</i>	1	1.61
<i>Serratia odorifera</i>	1	1.61
<i>Klebsiella pneumoniae ssp ozaenae</i>	1	1.61

CNS= Coagulase-negative staphylococci.

proportions of 14.5% and 13.6%, respectively. *Escherichia coli* is the second most common environmental causative agent responsible for mastitis, following *Streptococcus uberis* (Blowey and Edmondson, 2010). Its presence in faecal matter increases infection rates, especially in confined cattle housed in humid and unsanitary conditions. During lactation, *E. coli* can enter the teat canal due to dirty teats, poor milking practices, or trauma to the teat ends. Additionally, open teat canals after milking contribute to the risk of infection. *Serratia* species and *Pseudomonas* often cause chronic infections (Oliver *et al.*, 2011).

In vitro susceptibility

Antibiotic susceptibility testing showed significant multidrug resistance, affecting 81.48% of cases; 44 bacterial strains displayed resistance to two or more antibiotics.

Escherichia coli exhibited a high level of resistance towards Ampicillin, Tetracycline and Trimethoprim + Sulfamethoxazole with a rate of 71.43% for each. Conversely, the bacterium displayed a high level of sensitivity to Kanamycin (100%) and Colistin (71.43%), while showing a moderate level of sensitivity to Amoxicillin+Clavulanic acid and Spiramycin (57.14%) as shown in Table 3. These findings align with the results of Taher *et al.* (2020) in the Bordj Bou Arreridj province, which reported significant resistance of *Escherichia coli* to Tetracycline (75%) and Amoxicillin-Clavulanic acid (59.6%). In contrast, resistance to Trimethoprim-Sulfamethoxazole was lower at 36.5%, while all tested strains demonstrated complete sensitivity to Colistin (100%). Similarly, Ghallache *et al.*, (2021) noted in their study conducted in Algiers province that *Escherichia coli* was more sensitive to Colistin, Amoxicillin/Clavulanate and Kanamycin while displaying higher resistance to tetracycline (52%) and Ampicillin (57.7%). However, Trimethoprim-sulfamethoxazole exhibited high sensitivity, with only 3.1% of the strains found to be resistant. Other studies have shown high resistance to specific antibiotics in different countries. In India, a study by Singh *et al.*, (2018) revealed that *Escherichia coli* exhibited high resistance to various antibiotics, including Kanamycin (85.18%), Trimethoprim (55.55%), Clavulanic acid (55.55%), Amoxicillin (51.85%), Tetracycline (37.03%) and Ampicillin (33.33%).

Tahar *et al.* (2020) indicate that the prevalence of resistance in *Escherichia coli* strains to Tetracycline and B-Lactams within Algeria is primarily linked to the widespread use of these antibiotics in veterinary practices, where they are affordably priced. Furthermore, the authors highlight that antibiotics are utilized not just for treating illnesses, but also for preventing diseases and promoting growth. Additionally, in the veterinary sector, these antibiotics are often available for purchase without a prescription.

On the contrary, CNS exhibited a significant susceptibility to most of the antimicrobial agents employed in this study, notably kanamycin, spiramycin, Lincomycin and Trimethoprim + sulfamethoxazole with rates of 94.12%, 82.35%, 70.59%, 64.71% respectively. Nevertheless, it displayed a lower susceptibility to erythromycin (55.88%), a high resistance to streptomycin (73.53) and a moderate level of resistance to Penicillin and Tetracycline (55.88%), as shown in Table 4. The *Staphylococcus aureus* showed a significant degree of resistance to Penicillin G and Tetracycline, with rates of 76.92 % and 84.62% respectively. In addition, *S. aureus* presented a significant susceptibility to Kanamycin (92.31%), Spiramycin (76.92%), Lincomycin (69.23%) and Trimethoprim +sulfamethoxazole (84.62%), as well as a moderate sensitivity to Streptomycin (46.15%) and Erythromycin (38.46%).

Our research is consistent with the findings of Belmamoun *et al.* (2016), who reported high resistance to Penicillin G (80.95%, 61.02 %) and tetracycline (71.43%, 74.58%) in the sidi bel abbes province, along with a high sensitivity to trimethoprim-sulfamethoxazole (100%, 91.61%) for *staphylococcus aureus* and CNS group respectively. Additionally, Saidi *et al.* (2019) in Algeria reported a (100%, 71.42%) resistance level to Penicillin and a (40%, 34.28%) resistance level to Tetracycline, while also indicating a high sensitivity to trimethoprim-sulfamethoxazole (90%, 91.42%) for *staphylococcus aureus* and SNC group respectively.

Many studies conducted in various countries indicate significant resistance to Penicillin G, Tetracycline and other antibiotics among staphylococci. In India, Solanki *et al.* (2023) noted that *Staphylococcus aureus* strains in India exhibited high resistance rates to Penicillin G (88.9%), Tetracycline (83.3%) and Erythromycin (76.9%).

Table 3: Antibiotics susceptibility test of *Escherichia coli*.

Strain	Antimicrobial	Break point	R		I		S	
			N	%	N	%	N	%
<i>Escherichia Coli</i> N=7	AUG	< 14-21≥	2	28.57	1	14.29	4	57.14
	AMP	≤ 13-17≥	5	71.43	1	14.29	1	14.29
	K	< 15-17≥	0	0	0	0	7	100
	S	< 13-15≥	3	42.86	0	0	4	57.14
	TE	< 17-19≥	5	71.43	0	0	2	28.57
	CL	< 15-18≥	2	28.57	0	0	5	71.43
	SXT	< 10-16≥	5	71.43	1	14.29	1	14.29

AUG= Amoxicillin+ Clavulanic Acid, AMP= Ampicillin, K= Kanamycin, S= Streptomycin ; TE= Tetracycline, CL= Colistin, SXT=Trimethoprim +Sulfamethoxazole.

Table 4: Antibiotics susceptibility test of staphylococci strains.

Strain	Antimicrobial	Break point	R		I		S	
			N	%	N	%	N	%
SNCN=34	P	< 29≥	19	55.88	0	0	15	44.12
	K	< 15-17≥	2	5.88	0	0	32	94.12
	S	< 13-15≥	25	73.53	0	0	9	26.47
	E	< 17-22≥	13	38.24	2	5.88	19	55.88
	SP	< 20≥	6	17.65	0	0	28	82.35
	TE	< 17-19≥	19	55.88	1	2.94	14	41.18
	L	< 17-21≥	8	23.53	2	5.88	24	70.59
	SXT	< 10-16≥	11	32.35	1	2.94	22	64.71
<i>Staphylococcus aureus</i> N=13	P	< 29≥	10	76.92	0	0	3	23.08
	K	< 15-17≥	0	0	1	7.69	12	92.31
	S	< 13-15≥	5	38.46	2	15.38	6	46.15
	E	< 17-22≥	5	38.46	3	23.08	5	38.46
	SP	< 20≥	3	23.08	0	0	10	76.92
	TE	< 17-19≥	11	84.62	0	0	2	15.38
	L	< 17-21≥	3	23.08	1	7.69	9	69.23
	SXT	< 10-16≥	2	15.38	0	0	11	84.62

P=Penicillin G, K= Kanamycin, S= Streptomycin, E=Erythromycin, SP= Spiramycin, TE= Tetracycline, CL= Colistin, SXT=Trimethoprim +Sulfamethoxazole.

Kenar *et al.* (2012) revealed that strains of coagulase-negative Staphylococci (CNS) in Turkey showed significant resistance to multiple drugs: 76.2% to Trimethoprim-Sulfamethoxazole, 73.2% to Erythromycin, 58.3% to Penicillin and 52.3% to Tetracycline. According to Saidi *et al.* (2015), the resistance seen in staphylococci may be attributed to the presence of antibiotic-resistant strains in specific areas. This resistance could arise from the frequent and ongoing use of certain antimicrobials, such as penicillin-G, streptomycin, ampicillin, amoxicillin and tetracycline.

Various factors contribute to this resistance. Veterinarians frequently prescribe broad-spectrum antibiotics without performing tests to assess antibiotic susceptibility and herd health management is often inadequate. Furthermore, farmers usually use antibiotics without consulting a veterinarian and tend to overlook the recommended dosages and treatment durations, primarily to prevent financial losses related to unsold milk.

CONCLUSION

The research revealed that mastitis is highly prevalent, affecting 70.77% of cows, with multidrug resistance observed in 81.48% of tested strains, particularly against Beta-Lactams, Tetracycline and Streptomycin. This scenario can be ascribed to multiple variables, notably farmers' insufficient awareness of subclinical mastitis and its diagnosis, inadequate management and hygiene practices on farms and the improper use of antibiotics for both prevention and treatment. This situation underscores the need for strict hygiene during milking and in the overall management of dairy herds. Consistent cleaning and sanitization of milking apparatus and barns are crucial to

mitigate the danger of illness. Additionally, ongoing education and training for farm staff on mastitis management, preventive practices and the significance of responsible antibiotic use are crucial. Research indicates that kanamycin is the most effective antibiotic for treating mastitis in this region due to its high sensitivity.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare no conflict of interest.

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