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ORIGINAL ARTICLE

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Staphylococcal Subclinical Mastitis in Dairy Cows in Blida Region (Algeria)

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Abstract

Subclinical mastitis is an insidious disease of the mammary gland that negatively impacts the production of dairy cows. The objective of the present study is to screen subclinical mastitis in dairy cattle farms. A total of 208 lactating cows from five herds were examined and screened by the California mastitis test (CMT). Bacteriological analysis was performed on all CMF positive milk samples to characterize the presence of infection due to the genus Staphylococcus; as well as studying of the sensitivity of these isolated strains to antibiotics. The results showed that out of 208 cows screened by CMT, 71.15% were positive. Bacteriological analysis revealed that 111 samples found to be of the genus *Staphylococcus*, (75%). The characterization of the strains affirmed that the coagulase positive *staphylococci* represent 51.34% with a predominance of *Staphylococcus aureus* with a rate of 40.54% and the coagulase negative *staphylococci* represent 48.61%. The results of the antibiotic resistance revealed that the strains of coagulase positive *Staphylococci* particularly *S aureus* are resistant to the combination of amoxicillin and clavulanic acid, oxacillin and tetracycline with levels ranging from 4.4%, 44, 4% and 11.1% respectively. While coagulase negative *Staphylococci* showed resistance to the same molecules with 9.2%, 16.6% and 31.3% respectively. The obtained results demonstrated a very strong correlation between CMT and bacteriological findings. CMT seems to be an advantageous tool to expand, for systematic and regular screening against subclinical mastitis.

Keywords

Algeria, Antibiotics, Bovine, Californian Mastitis Test, *Staphylococci*, Subclinical mastitis

1. Introduction

The milk sector is of capital importance in Algerian agricultural economy, it represents one of the country's priorities with an annual consumption of 5 billion liters and a production unfortunately covers only 40% of the needs **(Benblidia, 2016).** Mastitis is considered one of the most important, frequent and costly pathologies affecting dairy cows **(Bradley, 2002; Boutet et al., 2005)** and the most penalizing for dairy farms **(Remy, 2010).** They cause considerable economic losses, due to the fall in milk production, losses in the dairy industry as well as the therapeutic and prophylactic costs of animals **(Faye et al., 1994).**

Subclinical mastitis is the most common pathology and poses many problems, due to the difficulty of its detection which makes treatment difficult **(Latereche, 2010).** According to **(Hovinen and Pyörälä, 2011)** farmers do not easily recognize it. Furthermore, **(Dieser et al., 2014)** reported that cows suffering from subclinical infections should considered as a source of new infections within herds. *S aureus* remains one of the most important organisms associated with contagious bovine subclinical mastitis, not only in Algeria, but throughout the world. Of all the bacteria that can enter the udder and cause mastitis, *Staphylococcus aureus* is not only

the most common, but also one of the most difficult germs to treat **(Maga, 2005).**
Non-compliance with hygienic conditions and the uncontrolled use of unsuitable antibiotic molecules have created problems of resistance and the persistence of mastitis, especially clinical mastitis in dairy farms; hence, the increased rate of cows cured for unhealed or recurrent mastitis.

Californian mastitis test (CMT) was used by many researchers for detection of subclinical mastitis in cattle **(Eshak 2002; Aly 2006; Joshi Gokhale 2006)** mentioned that CMT is the most sensitive (95.16%) and specific (98.02%) test for detection of subclinical mastitis. **Iqbal et al., (2006)** also confirmed the great sensitivity of CMT applied at farm or laboratory for detection of subclinical mastitis.

With this in mind, the present study conducted with the objective of screening for the subclinical form of mastitis in lactating cows through using California mastitis test (CMT) and bacteriological characterization of the genus *Staphylo-coccus.*

2. Materials and Methods

2.1. General Information

2.1.1. Breeding

This study focused on dairy cattle farms in the department of Blida (Algeria). Five herds were chosen at random, totaling 208 cows, with herd sizes varying between 29 and 55 purebred cows (Holstein black pie, Holstein red pie). Milking was carried out in farms using the milking cart morning and evening.

2.1.2. Samples

148 milk samples were collected from 208 lactating cows distributed over the five herds. A three-month follow-up was carried out for each farm (with a visit once a month) to detect new cases. Visits took place in the morning after milking.

2.1.3. Antibiogram

For the study of the sensitivity of the strains, a range of 03 antibiotics packaged in the form of discs with a diameter of 9mm (OxoidR) were used with the loads recommended by the Algerian Network for the Surveillance of Bacteria Resistance to Antibiotics. **(Rahal et al., 2017).** Regarding the use of antibiotics at the farm level to treat mastitis, an information sheet was used.

2.2. Udder examination

A visual examination to assess the physical characteristics of the udder and palpation of the udder for the presence of lesion, pain, heat and edema were systematically performed on all lactating cows.

2.3. California Mastitis Test (CMT)

CMT performed on lactating cows showing no clinical signs according to the procedures described by **(Quinn et al., 1994)** to detect subclinical mastitis. The reaction was numbered from 0 to 4 depending on the level of infection **(Gambo and Etchike 2001; Allain 2011).**

2.4. Sample Collection

The milk mixture from the affected quarters for each cow was collected in a sterile bottle after washing with water and disinfection of the teats with 70-degree alcohol and elimination of the first streams **(National Mastitis Council, 1999).** All these samples were identified stored in a cooler and sent to the bacteriology laboratory of the veterinary institute (Blida 1 University).

2.5. Bacteriological analyzes of milk

Samples were inoculated on Chapman agar and incubated at 37°C for 48 hours. Characteristic colonies were preliminarily identified with Gram stain and catalase assay. Biochemical identification was carried out by Api Staph galleries **(Bio Mérieux, France®).**

2.6. Antibiogram

The antibiogram was performed by the disc diffusion method on Mueller Hinton agar after incubation at 37 ° C for 24 hours. The plates were examined by measuring the diameters of the zones of inhibition and the results obtained were compared with the critical values recommended by the Algerian Network for the Surveillance of Bacteria Resistance to Antibiotics **(Rahal et al., 2017).**

2.7. Statistical analysis

The data collected were analyzed using XLSTAT (version 2021). Two types of analysis were performed the first; is a descriptive analysis estimated as a percentage calculated for all the examinations sought (CMT, bacteriological analysis, identification of strains, antibiogram). While the second concerned with the analysis of variance of the farm based on the results of the CMT.

3. Results

3.1. Farm visit

3.1.1. Udder Exam and Californian Mastitis Test

Clinical examination by inspection and palpation of the glands showed the absence of clinical mastitis. Examination by the CMT test of all lactating cows in the herds during the three months of follow-up revealed the results reported in **Table (1).**

Table 1: Interpretation of the Californian Mastitis Test

Farm	Number of cow	CMT positive		R^2	P
		N (cows)	%	0.80	< 0,0001
1	50	39	78		
2	55	41	74.54		
3	40	25	62.5		
4	34	25	73.52		
5	29	18	62.06		
Total	208	148	71.15		

Total of 208 cows tested by the California mastitis test revealed that subclinical mastitis was present in 148 samples, with a rate of 71.15%. According to the general model, the analysis of the variance of the results of the CMT according to the farm showed that there is a significant difference (0.0001) from one farm to another concerning the results of the CMT and variability estimated at 80%, thus explained by the type of farms.

4.1.2. Bacteriological analyzes of milk

The results of inoculation of samples on Chapman agar for the detection of *staphylococci* are reported in **Table (2).**

The 148 samples which were subjected to bacteriological analysis, 111 were found to be of the genus Staphylococcus (75%) **(Table, 2).**

4.1.2.1. Identification of strains

The results of the biochemical identification of the 111 strains of the genus *Staphylococcus* are illustrated in **Table (3).**

The results showed that coagulase positive staphylococci represent 51.34% with a predominance of *Staphylococcus aureus* (40.54%). While coagulase negative staphylococci represented 48.61% with predominance of *Staphylococcus epidermidis* (13.51%) **(Table 3).**

4.1.2.2. Antibiogram

The analysis of the information collected is illustrated in **Table (4).**

Analysis of the results concerning the use of antibiotics by the intra mammary route showed very variable rates depending on the antibiotics used and physiological stage. Cephalosporins and penicillins represented the most commonly used intra-mammary antibiotics with a rate of 32% (cefalexins) and 28% (penicillin) respectively. It should also note that penicillin (28%) and amoxillin (20%) used the most. While cefalexin and rifaximin are much more used outside lactation 32% and 22% respectively **(Table 4).**

Table 2: Bacteriological analysis of CMT positive milk.

CMT positive	Positive Culture on Chapman		Negative culture on Chapman	
n	n	%	n	%
148	111	75 %	37	25 %

n : number of sample

Table 3: Bacterial species isolated

		Strains (n=111)		
Coagulase type	Isolated species	N	%	
Coagulase positive (SCP)	*S. aureus*	45	40,54 %	51,34
	S. hyicus	7	6,30 %	
	S. intermedius	5	4,50 %	
Coagulase negative (SCN)	*S. epidermidis*	15	13,51 %	48,61
	S. hominis	8	7,20 %	
	S. warneri	8	7,20 %	
	S. capitis	6	5,40 %	
	S. xylosus	6	5,40 %	
	S. chromogenes	5	4,50 %	
	S. simulans	4	3,60 %	
	S. haemolyticus	2	1,80 %	

Table 4: The most commonly used intra-mammary antibiotics

		Antibiotics used intra-mammary	
Physiological stage	Antibiotics	N	%
Lactating	Penicillin	14	28
	Amoxicillin	10	20
	Ampicillin	09	18
	Tetracyclin	07	14
	Oxytétracyclin	05	10
Excluding lactation	Cefalexin	16	32
	Rifaximin	11	22
	Cloxacillin	09	18
	Penicillin	07	14
	Tetracyclin	07	14

The results obtained showed that beta lactams and tetracyclines are the molecules most frequently used in dairy cattle breeding, the antibiogram will only take into account the antibiotics belonging to these two families, namely: amoxicillin plus clavulanic acid, oxacillin and tetracyclines. The results of the antibiogram are shown in **Table (5).**

Table 5: The results of the study of the sensitivity of the 111 strains of the genus Staphylococcus.

Strains (n=111)	Antibiotics											
	Amoxicillin+Acid clavulanic				Oxacillin				Tetracyclins			
	R		S		R		S		R		S	
Isolated species	n	%	n	%	n	%	n	%	n	%	n	%
S. aureus (n=45)	2	4,4	43	95,5	20	44,4	25	55,5	5	11,1	40	88,8
S. hyicus (n=7)	0	0	7	100	0	0	7	100	1	14,3	6	85,7
S. intermedius (n=5)	0	0	5	100	0	0	5	100	0	0	5	100
SCN (n=54)	5	9,2	49	90,7	9	16,6	45	83,3	17	31,3	37	68,5

R: Resistance, S: sensitivity, n: number, S: Staphylococcus, SCN: Staphylococcus coagulase negative

The results showed that SCP strains particularly *S. aureus* are resistant to the combination of amoxicillin and clavulanic acid, oxacillin and tetracycline with levels of 4.4%, 44.4% and 11.1% respectively. While SCNs showed resistance to the same molecules with 9.2%, 16.6% and 31.3% respectively.

4. Discussion

The prevalence of subclinical mastitis obtained by the CMT test was of the order of 71.15%. This value is located at a very high level than those found in other studies carried out in Algeria (25% and 57%) respectively **(Bouaziz 2005, Saidi et al., 2010).** This high rate can be explained on the one hand by a bad litter causing the multiplication of bacteria by increasing the risk of contamination, the inattention of the milker caused by a lack of washing and wiping of the teats before milking thus promoting the penetration of bacteria remained on the skin of the teats **(Faroult and Arzul 2005).**

On the other hand, the poor hygiene of the breeding premises, milking hygiene favoring the transfer of contamination from one cow to another (milker's hands, single mop), a lack of soaking of the teats after milking, cracked teats, a lack of screening for clinical mastitis, poorly conducted curative treatments (lactation, dry-off) and insufficient culling in cows with persistent infection favor mastitis caused by contagious bacteria **(Milhaud, 1985; Bosquet and al., 2013).**

Regarding the effect of exploitation on the positivity of the CMT test, after the analysis of variance, the general model showed that there is a significant difference (0.0001) **(Table, 1)** from one farm to the next. The other concerns was the results of the CMT and an estimated variability of 80%, thus explained by the type of farms.

Variable rates of subclinical mastitis reported by studies in Algeria ranged from 25% to 57% **(Saidi et al., 2010, Niar et al., 2000, Bouzid et al., 2011, Boufaida Asnoune et al., 2012).** Other studies at the African level showed fluctuation in rates. In Niger, **(Bada-Alambedji et al., 2005)** reported a rate of 44%; in Tanzania, **(Karimuribo et al., 2006)** showed that the rate ranged from 46 to 76% and in Senegal, **Shyaka et al., (2010)** found a rate of 68.75%. This variation in the values of the prevalence of subclinical mastitis obtained by the different studies can be explained by the variability of the definition of the infection **(Eberhart, 1986),** the use of different diagnostic methods (CMT, somatic cell count), the breed effect through genetic selection to obtain high-producing cows, thus leading to a predisposition of the latter to be affected by pathogens, and particularly mastitis (stress linked to milk production) **(Faye et al., 1994).**

Bacteriological analysis of samples tested positive for CMT showed that 75% tested positive for the genus *Staphylococcus*. The characterization of the strains showed that the SCP represented 51.34% with a predominance of *Staphylococcus aureus* (40.54%). While SCN represented 48.61% with predominance of *Staphylococcus epidermidis* (13.51%). Similar studies on staphylococci have shown the importance of the *Staphylococcus aureus* species. According to the study carried out by **(Boufaida Asnoune et al., 2012)** in eastern Algeria showed that the genus SCP dominated isolates with *Staphylococcus aureus* (30%) and for SCN with the predominance of *Staphylococcus chromogenic* followed by *Staphylococcus epidermidis*. In Senegal, **Shyaka et al., (2010)** reported a frequency of *Staphylococcus aureus* isolated in subclinical mastitis quarters of 13% and 27% for SCN. In Niger, **(Bada-Alambedji et al., 2005)** showed that the frequency of isolated *Staphylococcus aureus* was 36.7%, with a high proportion of SCN of 22%. **(Nagahata et al., 2007)** reported that *Staphylococcus aureus* was detected with a frequency of 41.2% in 54 lactating cows in Japan.

Staphylococcus aureus is classified among the major pathogens; it is present in large numbers in raw milk. Its main reservoir is the contaminated and infected mammary glands but also by the cutaneous carriage of healthy animals. In fact, mastitis due to *Staphylococcus aureus* is considered a major disease in dairy cows, the diagnosis and control of which is very difficult due to the subclinical and chronic forms it causes. They are responsible for considerable economic losses due to the reduction in milk production quantitatively and qualitatively **(Wallemacq et al., 2010).** The high prevalence of *Staphylococcus aureus* in our study can be explained by the poor conditions and hygienic measures of milking **(Boutet et al., 2005).**The presence of lesions on the teats is also an important reservoir for this germ, as well as the hands of the milker which constitute a risk of transmission during milking **(Durel et al., 2011).** With regard to SCN, they have long been considered to be pathogens weakly

responsible for the development of subclinical mastitis **(Fabre et al., 1991).** However, in recent years, several studies undertaken have shown the importance and direct implication of SCN in the development of clinical and subclinical mastitis in cattle, thus classifying them as a major agent of mastitis (**Bes et al., 2000, Ben Hassen et al., 2003, Taponen, 2008).** According to the work of **(Bareille et al., 2005; Poutrel 2005;Taponen 2005)** who pointed out that SCN are involved in subclinical mastitis and their isolation in large numbers in milk, still poses the problem of their attribution in mastitis in dairy cows. For **(Pyorala and Taponen, 2009)** SCN are the pathogens most frequently isolated from the mammary gland and particularly in primiparous dairy cows. In addition, SCN appears to be opportunistic and infect the teat duct and mammary gland from sores in the skin or the environment (Oliver and al.2005). Regarding the use of antibiotic therapy in dairy cattle breeding against mastitis showed a rate of 38%. The survey conducted by **Cazeau et al., (2010)** in France showed that the use of antibiotics was linked to udder problems in 36% of cases. While **Mensah et al., (2014)** in Benin reported a rate of 27%.

Our results also showed that betalactamins would remain the most used in lactation and non-lactation by intra mammary route followed by tetracycline and oxytetracycline. According to **(Cazeau et al., 2010),** the use of tetracycline is 48.9% in the occurrence of mastitis, aminoglycosides in 53.7% of cases and polypeptides in 52.8%. According to another study conducted by **(Mensah et al., 2014),** tetracycline, penicillins, macrolides and sulfonamides are the families of the substances most used by audited breeders with respective rates of 89%, 34%, 12% and 6%. Methaphylaxis, which consists of an offensive prophylaxis approach combining, before the lot is affected, treatment (animals already sick or infected) and prevention (animals exposed), remains the most common practice carried out by 64% of farmers in mastitis cases in cattle only **(Mensah et al., 2014).**

Our results have also shown that betalactamins and tetracycline are the molecules most frequently used in dairy cattle breeding. Therefore, the antibiogram will only take into account the antibiotics belonging to these two families (amoxicillin + clavulanic acid, oxacillin and tetracycline). The study of the sensitivity of the isolated strains showed resistance of SCP strains particularly *Staphylococcus aureus* especially to oxacillin and tetracycline with varying levels, as well as SCN for the same antibiotic molecules. This resistance of staphylococci of mammary origin to different antibiotics was reported by the study of **(Shyaka, 2007)** in Senegal where the analysis of the sensitivity of germs to antibiotics showed a good sensitivity of *Staphylococcus aureus* to cefalexin, gentamicin, spiramycin and trimethoprim sulfamethoxazole (87.50%). On the other hand, resistance noted of 37.5% to ampicillin and tetracycline. For SCN, the analysis revealed good sensitivity to spiramycin (88.24%), cefalexin and gentamycin (82.35%) and finally an acceptable sensitivity to neomycin (76.47%). In contrast, SCN revealed resistance of 29.41% to tetracycline and a low resistance of 11.76% to ampicillin, gentamycin, and spiramycin.

Another study carried out by **(Boufaida Asnoune et al., 2016)** pointed out that the analysis of the sensitivity of germs to antibiotics showed a good response of *Staphylococcus aureus* to ampicillin+clavulanic acid, cefoxitin, ceftiofur, gentamicin, kanamycin, neomycin, spiramycin, erthromycin, spiramycin and trimethoprim sulfamethoxazole. Furthermore, resistance rates were recorded for penicillin G (35%), ampicillin (26%) and tetracycline (29%) for all the bacteria isolated, namely mammary reservoir bacteria (*Staphylococcus aureus*, SCN, *Streptococcus agalactia*, *Streptococcus dysgalactia*) and environmental bacteria (*Escherichia coli*, *Klebsiella* and *Streptococcus uberis*).

The work of **(Bouchot et al., (1985)** has shown that aminoglycosides and macrolides remain effective molecules in the treatment of bovine mastitis. Moreover, good sensitivity of SCN was noted to spiramycin, gentamycin and cefalexin by the work of **(Boutet et al., 2005; Kadja et al., 2006).**

The observed resistance of germs to the antibiotics studied prompts us to seek the cause of this resistance, which may be the overuse of antibiotics with non-compliance with doses, or a lack of pharmacokinetics of the antibiotic. Indeed, and by way of example, most strains of *Staphylococcus aureus* have an acquired β lactamase. The latter is responsible for resistance to aminoglycosides and betalactamins by the mechanism of enzymatic modification or inactivation **(Bosquet et al., 2013).**

5. Conclusion

Subclinical mastitis is a barrier to the expansion of milk production. These conditions represent a major disorder in dairy cows, located at the crossroads of milk quality and animal welfare. In the absence of clinical signs, CMT is a technique for identifying affected cows. However, knowledge of pathogens and their sensitivity allow a control program established in order to improve the health status of the udder.

6. Conflict of Interest

The authors declare no conflict of interest

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