

Prevalence of clinical and subclinical ovine mastitis caused by *Staphylococcus aureus*

Kh. M. Hammadi* and A. A. Yousif**

*College of Veterinary Medicine/ University of DIALA

**College of Veterinary Medicine/ University of Baghdad

Abstract

The study was carried out to determine the prevalence of mastitis caused by *staphylococcus aureus* in 100 lactating Awassi ewes located around Baghdad. A total of 194 milk samples were collected, samples were subjected to physical and, chemical tests (California mastitis test (CMT) as well as Somatic cell count (SCC) and bacteriological examination, and the isolates conducted to antimicrobial sensitivity test. Six ewes showed clinical mastitis (a cute mastitis) in 12 halves, 4 milk samples from gives a positive results to *S. aureus* in percentage (33.3%) from 182 apparently normal milk samples, Fifty two samples gives positive results to CMT, 14 samples gives positive results for *S. aureus* isolation, The percent of subclinical mastitis was 26.9% according to CMT. Fifty milk samples from 182 samples have SCC positive (more than 500.000 cells /ml) and 14 samples only gives positive result to *S. aureus*. Antibiotic susceptibility result show that isolates were highly sensitive to cefotaxime (100%), Erythromycine (100%), Ciprofloxacin (100%), Amikacin (94.1%), Ceftriaxone (88.2%), Oxacillin (88.2%), Cephalexin (76.4%), Kanamycine (52.9%), while less sensitive to others like, Pencillin G, Ampicillin, Optochin, Amoxicillin, as (17.6%), (23.5%), (29.4%), (35.2%), respectively.

نسبة انتشار التهاب الضرع الضأني السريري وتحت السريري المتسبب عن المكورات

العنقودية الذهبية

خالد محمود حمادي* وعفاف عبد الرحمن يوسف**

*كلية الطب البيطري/ جامعة ديالى

**كلية الطب البيطري/ جامعة بغداد

الخلاصة

صممت هذه الدراسة لمعرفة نسبة الاصابة بالتهاب الضرع المتسبب بالمكورات العنقودية الذهبية في 100 نعجة حلوبة في مناطق حول بغداد. تم جمع 194 نموذج حليب، خضعت النماذج للاختبارات الفيزيائية والكيميائية اختبار كاليفورنيا لالتهاب الضرع (CMT) واختبار حساب الخلايا الجسمية (SCC) والفحوصات البكتريولوجية وخضعت العزلات لفحص الحساسية للمضادات الحياتية. أظهرت ستة نعاج (12 نصف) التهاب ضرع سريري حاد، وأعطت أربع نماذج نتائج موجبة للمكورات العنقودية الذهبية وبنسبة 33.3%. اثنان وخمسون نموذج من 182 نموذج حليب طبيعي ظاهريا، أعطت نتيجة موجبة لاختبار كاليفورنيا لالتهاب الضرع ومن 52 نموذج موجب للاختبار اظهر 14 نموذج، نتيجة موجبة لعزل المكورات العنقودية الذهبية وكانت نسبة التهاب الضرع تحت السريري 26.9% تبعا لاختبار كاليفورنيا. وأعطى خمسون نموذج من 182 نموذج حليب طبيعي ظاهريا أعطت نتيجة موجبة لاختبار عد الخلايا الجسمية. (أكثر من 500000 خلية / مل). وأظهرت 14 عذلة فقط نتيجة موجبة. ان معظم عزلات المكورات العنقودية الذهبية كانت حساسة للمضادات البكتيرية التالية:

cefotaxime (100%), Erythromycine (100%), Ciprofloxacin (100%), (94.1%), Amikacin (94.1%), Ceftriaxone (88.2%), Oxacillin (88.2%), Cephalexin (76.4%), Kanamycine

(52.9%), Pencillin G, Ampicillin, Optochin, Amoxicillin, as (17.6%), (23.5%), (29.4%), (35.2%) على التوالي.

Introduction

Mastitis is a significant problem in dairy sheep flocks and lead to decreased milk production, mastitis has importance for 3 perspectives: economics, hygienic and legal (1). Mastitis is characterized by physical, chemical and bacteriological change in the milk and pathological changes in the glandular tissue of the udder and affect the quality and quantity of milk (2). *Staphylococcus aureus* is a major causative agent of Intramammaryinfections(IMI) in dairy animals. In Sheep the *S. aureus*, may account 25-30% of total IMI. The presence of organisms in milk can be detected directly by microbiological analysis, and the resultant infections indirectly by various Somatic cell counting methods (3). Control mastitis consists of antibiotic treatment, culling and environmental sanitation, antibiotic treatment of ewes at weaning has been demonstrated to be effective in reducing the persistence the infection and preventing new infections (4). The objective of this study was to identify the prevalence of clinical and subclinical mastitis in ewes with *S. aureus* pathogens and to study sensitivity test to antibiotics against this bacteria.

Materials and Methods

- **Ewes:** Onehundred lactatingewes located in Baghdad (Al-Taji, Al- Shulla, and Al-Tarimia) were used in this study, these ewes examined clinically to confirm infection with clinical mastitis or apparently normal, The study was carried out over a six month period starting from September 2011 to February 2012. All eweswerein the mid lactation stage and milked by hand, with no milking hygiene, dry-sheep therapy dipping or teat dipping procedures in these ewes.
- **Collection of Samples:** Milk samples were collected aseptically, from each mammary gland after washed with water and cleaned the teat end with cotton soaked in alcohol 70% solution. One hundred ninety four Milk samples were collected from 100 ewes, According to (5), the milk samples were collected from individual halves separately in sterile test tubes, under aseptic precaution after discarding the fore milk and transported immediately to the laboratory of college of Veterinary medicine/ department of internal and preventive medicine in Baghdad by cooling box.
- **Examination of milk samples:** In the laboratory, the milk samples were conducted to:
 - **Physical Examination.** Which include: colour, odor and consistency of milk and appearance.
 - **Chemical tests:** two tests were used for examination of normal apparent normal milk samples:
 1. **Somatic cell count (SCC):** this test was done according to (6).
 2. **California Mastitis Test (CMT):**the test was done according to (7).
 3. **Culturing:** Isolation and identification of bacteria from milk samples are performed according to (8), All milk sampleswere centrifuged at 3000 rpm/15 minute ,and the precipitate was cultured on blood agar and nutrient agar, incubated at 37 C° for 24 hrs. Diagnosis depend on morphological character (shape, color and size of colony) and hemolytic pattern by *staphylococcus* are observe on sheep blood agar, alpha and beta hemolytic colonies was subjected to Gram stain, then suspected isolates subcultured on Staph- 110 agar and mannitol salt agar, and biochemical tests (catalase, oxidase, Gelatin liquefaction, urease, O/F test and tube coagulase test). and Api® Staph kit were used for identification of *Staphylococcus* isolates.

4. **Sensitivity test:** Sensitivity to antimicrobial agent was determined for isolated bacteria by the disk diffusion method on Mueller-Hinton agar according to National Committee for Clinical Laboratory Standards Guidelines (9). All isolates of *S. aureus* were tested with 12 different antibiotics; Amikacin (30 mcg), Amoxicillin (30 mcg), Ampicillin (10 mcg), Cefotaxime (5 mcg), Erythromycin (15 mcg), Kanamycin (30 mcg), Oxacillin (1 mcg), Penicillin (10 IU), Optochin (5 mcg), Cephalexin (30 mcg), Ceftriaxone (30 mcg) and Ciprofloxacin (5 mcg).

Results

- **Prevalence of clinical mastitis:** Out of 100 ewes examined physically and bacteriologically for mastitis, 6 ewes (12 halves) showed clinical mastitis (a cute mastitis) after physical examination. *S. aureus* was isolated from Four milk samples (33.3%) from 2 ewes (Table 1).

Table (1) Prevalence of clinical mastitis

No.	No. examined	Ewes with clinical mastitis	+ve results for <i>S. aureus</i>	%
Ewes	100	6	2	
Milk samples	194	12	4	33.3%
%		6.18%		

These results was compatible with those recorded by (10) who found that the clinical mastitis appeared in percentage of 5% while sub clinical mastitis showed 30% also with Al-mendelawy (11) who record that clinical mastitis gives 5.5% and (12) record that clinical mastitis appeared in 5% and subclinical mastitis 3-37% of mammary gland.

- **Prevalence of subclinical mastitis:**

1. **The percentage of positive CMT in apparently normal milk samples:** The results of CMT in 182 apparently normal milk samples collected from 94 ewes were shown in table (2). Positive results for CMT were detected in 52 samples 28.57%.

Table (2) The percentage of positive CMT in apparent normal milk samples

No of milk samples/ No of ewes examined	No of milk samples/ No. of apparent normal ewes		CMT -ve No of CMT -ve samples (%)		No of CMT +ve samples (%)	
194 / 100	182 samples from 94 ewes		130 (71.43%) from 66 ewes		52 (28.57%) From 28 ewes	
	2 halves	1 halves	2 halves	1 halves	2 halves	1 halves
	176 From 88 ewes	6 from 6 ewes	128 From 64 ewes	2 From 2 ewes	48 From 24 ewes	4 From 4 ewes

2. **Relation between CMT and isolation of *S. aureus*:** From 52 CMT positive samples, 14 samples gives +ve results for *S. aureus* isolation, in same time these 14 were positive to CMT (Table 3). Table (4) showed the distribution of *S. aureus* isolates at different degree of CMT {-+, +1, +2, +3} as 2; 4; 6, 2 respectively. The CMT +2 had the highest percentage.

Table (3) Relation between CMT and Bacteriology

No. of apparent normal ewes	No of Milk samples examined	CMT -ve CMT -ve	Samples +ve for <i>S. aureus</i> from -ve CMT	CMT +ve	Samples +ve for <i>S. aureus</i> from all +ve CMT	+ve <i>S. aureus</i> and +ve for CMT
94	182	130	0/130	52	14/52	14/14
(88 with 2 halves and 6 with 1 halves)	94%	8.5%	0	38.2%	26.9%	100%

Table (4) Relation between CMT scores and *S. aureus* isolation

CMT scores	No of samples +ve to CMT	<i>S. aureus</i> mastitis sample
0	130	-
±	10	2
+	12	4
++	20	6
+++	10	2
	182	14

California mastitis test (CMT) indirectly detect increased number of leucocytes in mammary secretion therefore, it can be considered as a good screening test and more accurate diagnostic technique for detection of subclinical mastitis (7). CMT scores values in our result was compatible with those obtained by other authors (3, 13) and according to these studies the predictive value of positive result is mainly influenced by the prevalence of mammary infections in the flocks. Also our result revealed that scores +2 of CMT had the highest diagnostic accuracy. This agrees with (13) who recorded that score +2 of CMT was appropriate threshold value for detection of subclinical mastitis. Intramammary infections caused by *S. aureus* warrant special attention because this bacterium is the responsible for both acute clinical mastitis and subclinical mastitis as recorded by (14).

3. **Percentage of positive SCC in apparent normal milk samples:** Fifty milk samples 27.47% from 182 milk samples apparently normal, 28 ewes (24 ewes with 2 halves affected and 4 ewes with 1 halve affected) have SCC positive (more than 500,000 cells/ml) (Table 5).

Table (5) Percentage of positive SCC in apparent normal milk samples

Total No of milk samples/ total No of ewes examined	No of milk samples/ No. of apparent normal ewes		CMT -ve SCC -ve samples		SCC +ve samples	
194 / 100	182 samples from 94 ewes		132(72.53%) from 66 ewes		50 (27.47%) From 27 ewes	
	2 halves	1 halves	2 halves	1 halves	2 halves	1 halves
	176 From 88 ewes	6 from 6 ewes	130 From 64 ewes	2 From 2 ewes	46 From 23 ewes	4 From 4 ewes

4. **Relation between SCC and isolation of *S. aureus*:** The relationship between SCC and isolation of *S. aureus* showed that all +ve samples 14 to this bacteria was +ve for SCC table (6). Table (7) showed the results of SCC in relation to *S. aureus* isolation, and all these 14 +ve detected as positive for SCC (Number of SCC more than 500,000).

Table (6) Relation between SCC and isolation of *S. aureus*.

No. of apparent normal ewes	No of Milk samples examined	Samples SCC +v	Samples +ve for <i>S. aureus</i> from -ve SCC	Samples +ve for <i>S. aureus</i> from +ve SCC	No. of <i>S. aureus</i> Isolates From +ve SCC
94 (88 with two halves and 6 with 1 halves)	182	50	0/132	14/50	14/14
		(27.47%)	(1.15%)	(28%)	(100%)

Table (7) Relation between SCC No. and *S. aureus* isolation

SCC No.	No of samples	<i>S. aureus</i> isolation
100.000 – 500.00(-ve)	130	0
500.000 – 1.000.000	12	3
1.000.000 – 1.500.000	20	5
1.500.000 – 2.000.000	18	4
More than 2.000.000	2	2
Total	182	14

Our results showed that somatic cell count (SCC) is considered the best indirect method for detecting subclinical mastitis and the threshold level of which were used for differentiation between infected and non-infected values in our result was (500.000) cells/ ml. This results agreed with (15) who record that SCC in milk and CMT can be used as reliable methods for detecting subclinical mastitis in Syrian Awassi ewes. But some authors recorded that SCC threshold values has difficult established for ewes. This value is affected by different factors, such as the breed, lactation period or organisms producing Intramammary infections (16). The increase in SCC was reliable index of ongoing mastitis (17). Our result recorded isolated of bacteria from milk sample with SCC lower than 500.000 cells/ml, this agreement with result of some authors (18) who had isolated mastitis pathogens from milk sample with very low SCC. Our results showed that the subclinical mastitis according to CMT and SCC and its relation to isolation of bacteria was very important due to the high percentage of occurrence. This incompatible with (19) whose found that subclinical mastitis give a percent of (24.8%). Also (20) in the north of Palestine insure that the milk samples from animals with bacterial infection of the mammary gland showed significantly higher somatic cell count. The variation between our result and others may attributed to variation in sheep management and geographic area.

5. Percentage of *S. aureus* isolation according to type of mastitis: Out of 12 milk samples from 6 ewes affected with clinical mastitis, 4 milk samples gives a positive results to *S. aureus* 33.3% from 2 ewes only and from the 188 apparently normal milk samples, 50 samples give positive results for subclinical mastitis according to SCC and from these 50 samples only 14 samples give positive result to *S. aureus* from 9 ewes (2 quarters affected in 5 ewes and 1 quarter in 4 ewes) (Table 8).

Table (8) Percentage of *S. aureus* isolation according to type of mastitis

Type of mastitis		No of ewes affected with <i>S. aureus</i>	Infection with <i>S. aureus</i> according to halves in ewes		No. of milk samples positive to <i>S. aureus</i>	% of positive <i>S. aureus</i> according to milk samples
			2 halves	1 halves		
Clinical mastitis	6 (12 samples)	2	2(4)	-	4/12	33.3%
subclinical mastitis	27 ewes (SCC)	9	5(10)	4(4)	14/50(SCC)	28%
Total		11	7	4	18 /66	27.27%

Our result of clinical and subclinical mastitis was agreement with that recorded by other researchers, (21), who mentioned that clinical *S. aureus* mastitis ratio was 34.14%, (22) show that subclinical mastitis caused by *S. aureus* was 25.3%, (23) recorded that the *S. aureus* percentage was 25-30% of total intramammary infection, while (24) recorded the *S. aureus* percentage of 36.6% of clinical mastitis whereas (25) found that *S. aureus* ratio was 25% on the other hand our result may appear higher than the result of (26) who recorded that clinical IMI *S. aureus* was 13.17%. Our results of isolated bacteria may appear lower than results of other researchers, (27, 28, 29), recorded a percentage 65.3, 90, 57.60% respectively. The difference between our result and result of others may attributed to some factors such as breed difference, different hygiene and management practices followed in each farm, age and parity of the animal and type of the milking.

- **Antibiotic susceptibility test:** Table (10) showed that Some *S. aureus* isolates were highly sensitive to cefotaxime 100%, Erythromycine 100%, Ciproflaxacin 100%, Amikacin 94.1%, Ceftriaxone 88.2%, Oxacillin 88.2% and Cephalixin 76.4%, while the remainder isolates were moderate sensitive to kanamycine 52.9%, whereas less

sensitive to others like, Pencillin G, Ampicillin, Optochin, Amoxicillin, as 17.6%, 23.5%, 29.4%, 35.2%, respectively.

Table (9) Antibacterial sensitivity test

Antimicrobial agent	Disc (μ g) content (mcg)	No. of result.		
		Resistant (%)	Intermediate (%)	Susceptible (%)
Amikacin	30	1 (5.5%)	0 (0%)	17(94.4%)
Amoxicillin	30	5 (23.5%)	6 (29.4%)	7(35.2%)
Ampicillin	10	12 (66.6)	1 (5.5%)	5(23.5%)
Cefotaxime	5	0 (0%)	0 (0%)	18(100%)
Erythromycin	15	0 (0%)	0 (0%)	18(100%)
Kanamycin	30	5 (23.5%)	3 (16.6)	10(52.9%)
Ciprofloxacin	5	0 (0%)	0 (0%)	18(100%)
Oxacillin	1	0 (0%)	2 (11.1%)	16(88.2%)
Penicillin	10	14(76.4%)	0 (0%)	4(17.6%)
Optochin	5	6 (29.4%)	6 (29.4%)	6 (29.4%)
Cephalexin	30	1 (5.5%)	3 (16.6)	14(76.4%)
Ceftriaxone	30	0 (0%)	2 (11.1%)	16(88.2%)

Some of these result, were agree with result of (30) who found that *S.aureus* was more resistance to Ampicillin 53.4%, penicillin 45.3%, (31) demonstrated that *S.aureus* isolate were resistance to antibiotic, (26) demonstrated that *S.aureus* were sensitive more than 90% to Cephalexin, and more than 80% to erythromycin and cloxacillin. In our study the higher resistance of most *S.aureus* isolate to Penicillin and Ampicillin may attributed to continuous using of these antibiotics in systemic and local treatment, this may lead to development of resistance due to production of Pencillinase enzymes from *S.aureus* bacteria (8). On the other hand the little use of the other antibacterial like Ciprofloxacin and Erythromycin, in systemic and local treatment may lead to decrease the resistance of the *S.aureus* isolates to these drugs. The results presented are similar to previous studies in which Gram-positive bacteria were susceptible to Oxacillin, Erythromycin, Cephalexin and ciprofloxacin (32). These drugs are no longer used in veterinary medicine in many countries including Iraq, which may account for the results reported here.

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