



Bacteriological and immunological studies on some bacterial pathogens incriminated in bovine Mastitis.

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ABSTRACT

In this study a total of 1200 quarter milk samples were selected from 300 cows from different localities at El-Kaluobia governorate. The bacteriological examination revealed that a total of 125 isolates from clinical and subclinical mastitic milk samples of which 57 *S.aureus* isolates were isolated from both mastitic milk with incidence of 45.6% of total isolates, 28 *E.coli* with 22.4%, 13 *S.agalactia* with 10.4%, 9 *S. dysgalactia* with 7.2%, 7 isolates of both *S.uberis* & *coagulase negative staph* (5.6%), 3 *Klebsiella sp* with 2.4% and 1 *Pseudomonas sp* with 0.8%. Mean titer of anti *S aureus* specific IgA was higher in milk of subclinical mastitic cases (1:845) than clinical one(1:290), while mean titer in milk of clinical and subclinical mastitic cases much higher than in serum of clinical(1:54)and subclinical (1:41), while mean titer of anti *S. aureus* specific IgM and IgG was much higher in serum of clinical(1:1382 , 1:868 for IgM&1:1267 , 1:634 for IgG) than milk of clinical and subclinical mastitic cases (1:111 , 1: 53 for IgM& 1: 302 , 1: 206 for IgG). Results of sensitivity tests of *S.aureus* against different antibiotic discs revealed that 93%,91.2%,87.2% and 63.2%, were sensitive to Amoxicillin /Clavulanic acid, Gentamycin, Cloxacillin, Sulphamethoxazole /Trimethoprim while87.7%,86.0%,84.2% and 82.5%of isolates were resistant to Nalidixic acid, Streptomycin, Oxytetracycline and Penicillin-G.

Keywords: Mastitis, Cattle, Immunoglobulins, Antibacterial.

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1. INTRODUCTION

Mastitis is one of the most common diseases affecting dairy Cows. It is defined as inflammation of mammary gland accompanied with physical, chemical, microbiological and cellular changes in the milk as well as pathological changes in the udder (Merck veterinary manual, 2006). The severity of inflammation depends on the causative agents and the host response (Petzl et al., 2008). It is important to note that *S. aureus* is considered as the main contagious udder bacteria causing clinical and sub clinical mastitis. It impairs the alveolar function, reduces milk yield and has a deleterious effect on milk composition and increase the milk somatic cell count

(Leitner et al., 2000),the pathological effect of *S. aureus* infection is formation of abscesses in deep tissue and become foci for bacterial shedding in chronically infected animals and the diseased Cow become high refractory to antibiotic therapy (Alluwaimi et al., 2003).Economic losses due to mastitis have been established worldwide as \$ 35 billion (Wellenberg et al., 2002).Mastitis can be diagnose by physical methods such as redness, hotness, swelling of udder and painful on palpation, also CMT(Cow side test)and SCC test .So the aim of this work was to determine the Prevalence of mastitis with its two main forms(clinical and subclinical). Application of the traditional methods of isolation and identification for

common bacteria causing mastitis in clinically diseased and apparently healthy dairy cows with special regards to *S. aureus*, also studying the correlation between type of mastitis and the production of specific IgA, IgM and IgG antibodies in blood and milk beside studying antibiotics sensitivity test to choose the most effective antibiotic against *S. aureus* mastitis.

2. MATERIAL AND METHODS

2.1. Milk collection

A total 1200 individual quarter milk samples were collected from 300 Cows from different localities at El-Kaluobia Governorate according to National Mastitis Council (1990). By using CMT 1060 quarter milk samples were apparently normal (from 265 Cows) while the remaining 140 quarter milk samples were taken from 35 Cows showing clinical mastitis also 50 blood samples were taken from normal and mastitic cases for immunological examination.

2.2. Bacteriological examination

A loopful of each quarter milk sample streaked into nutrient agar, blood agar, MacConKey's agar, Mannitol salt agar, Baired parker agar, eosin methylene blue agar(EMB)and *Pseudomonas* agar as described by Quinn et al., 2002.All plates were incubated for 24 hours at 37°C. The developed colonies were picked up&sub culturing for purification of the isolates. The purified colonies were subjected for morphological identification by Gram stain &biochemical tests.

2.3. Immunological assay (Leitner et al., 2000)

Serum and milk whey samples were examined by enzyme linked immunosorbent assay (ELISA) for determination of immunological changes occurred to the immunoglobulins (IgA, IgM and IgG) in case of clinical and sub clinical mastitis as described.

2.4. Antibiotic sensitivity test by Leitner et al., 2000 for the isolated *S. aureus*

In-Vitro sensitivity test was done for isolated *S. aureus* strain (57 isolates) to study its antibiotic Sensitivity. The disc diffusion technique was applied according to (CLSI, 2011). The used discs were Amoxicillin Clavulanic acid 20-10ug, Cloxacillin25ug, Oxytetracycline 30ug, Gentamycin 10ug, Erythromycin15ug, Trimethoprim- Sulphamethoxazole1 25-23.75mcg, Penicillin-G 10IU, Ampicillin 10mcg, Streptomycin 10ug and Nalidixic acid 30 mcg.

3. RESULTS

3.1. Prevalence of clinical and sub clinical mastitis using CMT

Examination of the healthy state of the mammary glands of 300 Cows revealed that 35 Cows clinically mastitis with prevalence of 11.66 %, while the other remaining Cows 265 were apparently normal with prevalence of 88.33 % Examination of quarter milk samples of the Cows by CMT showed that the incidence of subclinical mastitic Cows among the apparently normal Cows were 80 with prevalence of 26.66%, while the clinically mastitic were 35.The total number of mastitic Cows were 115 out of 300 Cows with an incidence of 38.33% as shown in (table I).

3.2. Results of bacteriological examination

Bacteriological examination of 38 clinically mastitic quarter milk samples , 140 subclinically mastitic quarter milk samples revealed single and mixed infection in the clinically mastitic quarter milk samples with prevalence of 77.1 % and 22.9 % respectively, while single and mixed infection of the subclinically mastitic quarter milk samples with prevalence of 90.8 % and 9.2 % respectively , while 64 quarter milk samples of the subclinical mastitic samples were negative for bacterial isolation with prevalence of 45.7 % (Table2). It is clear that the total number of

isolates were 125. From the clinical mastitic cases 43 bacterial isolates with prevalence of 34.4% meanwhile 82 isolates from sub clinical mastitis cases with prevalence of 65.6%. The predominant bacterial species isolated were as follow, *S.aureus* 57 isolates with incidence of 45.6% , *E.coli* 28 with prevalence of 22.4% , *S. agalactia* 13 with prevalence of 10.4 % , *S.dysgalactia* 9 with prevalence of 7.2 % , *S.uberis* 7 with prevalence of 5.6 % , *CNS* 7 with prevalence of 5.6 % , *Klebsiella spp* 3 with prevalence of 2.4 % *P. aeruginosa* 1 with prevalence of 0.8 % (Table 3).

3.3. Results of immunological assay

The results of ELISA showed that: The high mean titer of anti *S.aureus* specific IgA was found in sub clinical mastitis milk than clinical ones (Table 4).

Also the high mean titer of anti *S.aureus* specific IgM was found in clinical mastitis milk and serum than sub clinical ones (Table 5). More over the high mean titer of anti *S.aureus* IgG was found in clinical mastitis milk and serum than sub clinical ones (Table 6).

3.4. Result of antimicrobial sensitivity

The in- vitro sensitivity tests of *S.aureus* Table (7) showed that, the isolated *S.aureus* was highly sensitive to Amoxicillin-Clavulanic acid (93%), Gentamycin (91.2%). Cloxacillin (87.2%), meanwhile they were resistant to Nalidixic acid (87.7%), Streptomycin (86.0%)and Oxytetracycline (84.2%).

Table (1) Prevalence of mastitis using the California mastitis test (CMT)

No. of Cows	Clinical mastitic cases		Subclinical mastitic cases		Total mastitic cases		Healthy Cows	
	No.	%	No.	%	No.	%	No.	%
300	35	11.7	80	26.7	115	38.3	185	61.7

Table (2) Incidence of bacteria (single & mixed) recovered from examined quarter milk samples

Health state of Cows	Number of examined quarters	Results of bacterial examination						Total infected cases	
		Negative		Single infection		Mixed infection		No.	%
		No.	%	No.	%	No.	%		
Clinically mastitis Cows Positive CMT quarters (subclinical mastiticCow)	38	3	7.9	27	77.1	8	22.9	35	92.1
Total	140	64	45.7	69	90.8	7	9.2	76	54.3
	178	67	37.6	96	86.5	15	13.5	111	62.4

Table (3) Prevalence of different bacterial isolates recovered from examined quarter milk sample

Bacterial isolates	Clinical mastitis (35)		Positive CMT (76)		Total (111)	
	No.	%	No.	%	No.	%
<i>S.aureus</i>	21	48.8	36	43.9	57	45.6
<i>E.coli</i>	8	18.6	20	24.4	28	22.4
<i>S. agalactia</i>	3	7.0	10	12.2	13	10.4
<i>S. dysgalactia</i>	4	9.3	5	6.1	9	7.2
<i>S. uberis</i>	3	7.0	4	4.9	7	5.6
<i>Klebsiella sp.</i>	1	2.3	2	2.4	3	2.4
<i>P. aeruginosa</i>	--	---	1	1.2	1	0.8
<i>CNS</i>	3	7.0	4	4.9	7	5.6
Total	43	100	82	100	125	100

Table (4) Effect of type of mastitis on anti *S. aureus* specific IgA in milk whey and serum

	-ve CMT samples	Clinical mastitis samples	Sub clinical mastitis
Milk	31	290	845
serum	20	54	41

Table (5) Effect of type of mastitis on anti *S. aureus* specific IgM in milk whey and serum

	-ve CMT samples	Clinical mastitis samples	Sub clinical mastitis
Milk	16	111	53
serum	161	1382	868

Table (6) Effect of type of mastitis on anti *S. aureus* specific IgG in milk whey and serum

	-ve CMT samples	Clinical mastitis samples	Sub clinical mastitis
Milk	61	302	206
serum	103	1267	634

Table (7) In-vitro anti-microbial sensitivity test of isolated *S. aureus*

Antibiotics	highly sensitive	intermediate sensitive	Resistant
Amoxicillin/Clavulanic acid	93	1.7	5.3
Gentamycin	91.2	1.8	7.0
Cloxacillin	87.2	3.3	9.5
Trimethoprim/ Sulphamethoxazole	63.2	7.5	29.3
Ampicillin	38.6	10.5	50.9
Erythromycin	31.6	19.3	49.1
Penicillin G	12	5.5	82.5
Oxytetracycline	11	4.8	84.2
Streptomycin	10.5	3.5	86.0
Nalidixic acid	9.1	3.2	87.7

4. DISCUSSION

The prevalence of subclinical mastitis was higher than clinical mastitis where it was 26.7%. This result was correlate with that of El-Sayed, (2009)27.45%, but disagree with that reported by Karimuribo et al, (2008) 75.9%. Concerning bacteriological examination a total number of (178) quarter milk sample (QMS) collected from (140) QMS of subclinical cases and (38) QMS of clinical mastitis were examined Bacteriologically and as shown in table (1), it revealed that 111 QMS (62.4%) were

Bacteriologically positive while 67 QMS (37.6%) were negative .In this respect lower results were reported by El-seedy et al., (2010) who found that the bacteriologically positive QMS was 44.3% and higher estimates was recorded by Lakew et al., (2009) who concluded that bacteriologically positive QMS was 92.36%. more over single and mixed infection were detected in (77.1%) and (22.9%) respectively. while in subclinically mastitic the mixed infection was (9.2%) only while single infection was (90.8%) as in table (1), also 64 (45.7%) samples were culturally negative among subclinical

mastitis quarters. As shown in table (2) *S.aureus* was the predominant bacteria 45.6% of total isolates. Nearly rephrase results were recorded by Parmar, et al., (2006) who found that 34% from subclinical mastitic cases caused by *S.aureus*, however these results were lowered than that observed by Risvanl and Kalkan, (2002) who reported 51.4%. these results were nearly similar to that obtained by EL –Seedy et al., (2010) who reported that bacteriologically positive QMS was 44.3%, but it was differed with results of Lakew et al., (2009) who reported that bacteriologically positive samples was 92.36%. Concerning the results of *S.aureus* mastitis in clinical cases these results were 48.8% and in subclinical mastitis was 43.9%. These results were nearly agreed with that of Miranda –Morales et al (2008) 30% while it was slightly higher than that obtained by Dubal et al., (2010) 42.14%. In the same time, the results were much higher than that reported by Nam et al., (2010) who found that *S.aureus* isolated at percentage of 12.2%. Referring to antibiotic and sensitivity a total number of (57) *S. aureus* strains isolated from clinical and subclinical mastitic cases were subjected to antibiotic sensitivity tests against different antibiotics. The results showed that *S.aureus* was highly sensitive to Amoxicillin / Clavulanic acid (93%) but resist Nalidixic acid (87.7%) , Streptomycin (86.0%) .These results were mostly agreed with that reported by El Sayed, (2010) who found that *S.aureus* sensitive to Amoxicillin / Clavulanic acid by 89.2% and El-Seedy et al., (2010) who found that the examined *S.aureus* isolates in Cows were sensitive to Gentamycin (90.6%) and Amoxicillin / Clavulanic acid (84.9%) while resistant to Penicillin (64.2%) .In this respect Andrade et al., (2001) revealed that 90.72% of *S.aureus* was sensitive to Gentamycin. Ayman et al., (2012) mentioned that Penicillin resistance was of 85.7% Also Ikiz et al., (2013) who reported that Gentamycin sensitivity was (91.66%), Streptomycin resistance was (83.4%). Concerning the

effect of mastitis type on immunoglobulins level and as in table (4) there was a higher mean titer of anti *S.aureus* specific IgA in subclinical mastitic milk than that of clinical and normal milk samples. These results were in agreement with that reported by Leitner et al., (2000) who reported that IgA antibody was the major immunoglobulin isotype in most of the infected quarters with subclinical chronic mastitis but not in non-infected ones. Table (5) showed that there was a higher mean titer of anti *S.aureus* specific IgM in clinically mastitic milk and in serum than that of subclinical cases, also the level in subclinical milk was higher than normal milk samples. Table (6) illustrated higher mean titer of anti *S.aureus* specific IgG in clinical mastitic milk and serum than that of subclinical mastitic milk and serum. The mean titer in subclinical mastitic cases was higher than that recorded in normal milk samples and the mean titer of anti *S.aureus* specific IgG in sera was higher than that in milk ones. These results were in agreement with that reported by Leitner et al., (2000).

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