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Bacteriological Evaluation of Present Situation of Mastitis in Dairy Cows

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Abstract: The purpose of study was to surveying and identifying the bacterial causes of bovine mastitis. One hundred and twelve bovine milk samples from clinical, sub-clinical and apparently healthy cases were collected and were tested by CMT and SCC The percentage of subclinical mastitis was 56.3% while that of clinical mastitis was 13.3%, these +ve samples were used for bacteriological culture to isolate the bacterial agents causing mastitis on different types of media. And then were identified using different API systems. The causative agents were either single pathogens like *E. coli* (25.5%), *S. aureus* (14.8%), CNS (12.7%) *St. agalactiae* (12.7%), *St. pyogens* (10.6%) *K. pneumoniae* (8.5%), *Salmonella* spp. (4.2%), *Proteus species*. (4.2%), *Ps. aerguinosa* (4.2%) and *C. albicans* (2.1%) or mixed infection were including S. aureus plus *E. coli* had the highest prevalent rate (17.8%) followed by E. coli plus *K. pneumoniae*, *S. aureus* plus *K. pneumoniae* and CNS plus *E. coli*, gave14.2%. then followed by *S. aureus* plus *St. agalactiae* plus *E. coli*, *S. aureus* plus *E. coli* plus *K. pneumoniae*, *St. agalactiae* plus *E. coli*, *St. pyogens* plus *E. Coli* and CNS plus *K. pneumoniae* gave 7.1%. The lowest prevalent mixied infection was *Proteus* spp. plus *S. aureus* displayed 3.5%.

Key words: Mastitis · California Mastitis Test And Somatic Cell Count

INTRODUCTION

Mastitis means inflammation of the mammary gland and is characterized by physical, chemical, microbiological and cellular changes in milk as well as pathological changes in the udder [1].

Mastitis includes clinical and subclinical forms. Subclinical-mastitis is that form of mastitis where infection and inflammation occurred in the udder without observable or palpable external changes in the udder or in the secreted milk [2]. In clinical mastitis, however, abnormalities of the udder and milk are to be observed as changes in the milk such as flanks, clots and watery appearance as well as cardinal signs of inflammation on the udder or even atrophy of the tissue [3].

From the economic point of view, mastitis especially the-subclinical form causes extensive economic losses that include reduction of milk yield, changes in the milk composition and reduction in milk as well as shortens the productive life span of the affected animals. Also costs of drugs-and veterinary services [4, 5]. In addition to economic impact and the bacterial contamination of the milk may render it unsuitable for human consumption [6].

Bovine mastitis mostlyoccurs due to bacterial, rickettsial and fungal invasion and infection as well as physical injury of the udder during the act of milking process. So, [7] recorded that machine milked cows show high incidence of mastitis than that of hand milked cows.

The microbial causes of mastitis have been categorized according to [2]to either contagious pathogens (e.g., *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*) or environmental pathogens (e.g., *E. coli* and *Streptococcus uberis*). Regardless the fact that more than 135 different microbial species have been reported as a cause of bovine mastitis, the majority of infections are caused by staphylococci, streptococci and Gram negative bacilli.

More attention has been given to the diagnosis of clinical and subclinical mastitis using indirect tests, which depends upon the cellular interaction between reagent and certain protein factor in mastitis milk. These tests includes somatic cell count (SCC) [8],and California mastitis test (CMT) [9].

Several methods have been reported for detection of mastitis as isolation of the causative microorganism(s) which is the most accurate one. But, it is expensive and

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time consuming. So, the need for a simple quite sensitive, rapid and reliable test sufficient to be applied on large scale of animals is therefore required [10]. The aim of our work to determined the bacteriological evaluation of Present Situation of Mastitis in Dairy Cows in Egypt that help to treatment and control of bovine mastitis.

MATERIALS AND METHODS

Milk Samples: A total number of 112 milk samples was collected from dairy cattle aseptically for bacteriological examination. The samples were collected from cases either atrophied quarter, dried or suffering from severe mastitis according to the procedures of Put the authors with each Ref. separate [10, 11].

A sample of 15-20 ml of milk was drown in a clean sterile screw capped bottle then labeled. From each quarter, two milk samples were taken in a sterile tube. The milk samples were kept in an ice container till delivered to the laboratory. One of the two samples was examined for somatic cell count (SCC) (this sample was kept on formalin 10% if it will not examined for the somatic cell count at the same day). The second sample was subjected for bacteriological examination by incubating the sample in an incubator for 24 hours [10] then subjected for the bacteriological examination.

California Mastitis Test (CMT): It is a rapid, accurate, cow-side field test recommended by the American Public Health Association [12].

The CMT reagent reacts with DNA of epithelial and inflammatory cells present in the milk sample. CMT results were read immediately and were scored for each quarter depending on the thickness and amount of the gel formed, the CMT scores of '0' andtrace (±) were taken as negative or normal whereas, CMT scoresof 1+ (weak positive), 2+ (distinct positive) and 3+ (strong positive)were considered as indicators of subclinical mastitis. In thepresent study, a subclinical mastitis case was defined as an animalwith at least one of the quarters with a CMT score of ≥1+.

Somatic Cell Count (SCC): The milk samples were examined for somatic cell count automatically using *Bently Soma Count 150 (Bently, USA)* according to Zecconi*et al*.[8]to detect any possible variation as well as bacteriological examination to correlate and investigate the possible associated causal agent, even the samples showed negative CMT were studied as a control.

Bacteriological Examination: Milk samples showed strong positive reactions in SCC and CMT were taken for bacteriological culture. Isolation and identification of bacterial agents causing mastitis was carried out [2, 13, 14].

Isolation: The milk samples were pre-incubated at 37°C for 18-24 hours, then a loopful from the samples was streaked onto 5% sheep blood agar, mannitol salt agar and MacConkey's agar plates, then incubated aerobically at 37°C for 24-48 hours. Suspected colonies were picked up,sub-cultured onto nutrient agar slants then incubated aerobically at 37°C for 24 hours.

Identification of the Bacterial Isolates: According to Quinn *et al*. [13], suspected colonies were examined for their gross appearance (morphological characteristics) including the colony size, shape, surface texture (rough or smooth), color of the colonies or the pigment production, the consistency (mucoid or non-mucoid), the hemolytic activity on the blood agar, type of hemolysis (α or ß or γ type of hemolysis) and the metabolic activity on MacConkey agar((lactose fermenter (LF) or non-lactose fermenter (NLF).

Suspected colonies were examined microscopically using Gram stained films before transferred onto slope agar for further biochemical identification using different API systems.

For *Enterobacteriaceae and Pseudomonas aerguinosa* API 20E reagent kit (Biomerieux –France cat# 20-100), for *Staphylococcus* species and CNSAPI-Staph Kit (bioMe´rieux SA, l'Etoile, France), for *streptococcus* speciesAPI 20 STREP (bioMe´rieux SA, l'Etoile,France) and for *Candida* species PI 20C AUX (bioMe´rieux SA, l'Etoile,France) were used.

RESULTS

One hundred and twelve milk samples from dairy cows were examined clinically for presence of mastitis and by using California mastitis test. They were grouped into; healthy, subclinically and clinically mastitic dairy cows.

According to CMT the prevalence of healthy, subclinical mastitis and clinical mastitis was 30.3, 56.3 and 13.3% respectively as shown in Table (1).

Seventy eight milk samples positive with CMT were examined by SCC and bacteriological examination.

The 63 subclinical mastitis were examined by SCC and the given numbers were 8, 25 and 30 in ($< 10^5$), ($>10^5$ – $3x10^5$) and ($>3x10^5$ – $5x10^5$) ranges respectively. On the

Table 1: Prevalence of mastitis by using CMT and SCC in the examined dairy cow milk samples

	Milk samples			
	CMT	SCC		
The test	no	%	Ranges	no
Healthy	34	30.3	-	34
Subclinical mastitis	63	56.3	$< 10^5$	8
			$>10^5 – 3x10^5$	25
			$>3x10^5 - 5x10^5$	30
Clinical mastitis	15	13.3	$>5x10^5 - 10^6$	13
			$>10^6$	2
Total	112			100

Table 2: Results of bacteriological examination of the positive CMT dairy cow milk samples

	Bacteriologically positive samples						Bacteriologically negative samples	
	Single infection		Mixed infection		Total			
Total no. of bacteriologically examined milk samples	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
78	47	60.2	28	35.8	75	96	3	4

Table 3: Biochemical identification of isolated causative agents using API system

	Gram negative bacteria							Gram positive bacteria						Yeast	
	Enterobacteriaceae					*Psendomonas spp.*		*Staph. spp*			*Strept. spp*			*Candida spp*	
API types	API 20E					API 20E		API-Staph Kit			API 20 STREP			API 20C AUX	
Bacterial isolates	Test list	*E.coli*	*K. pneumoniae*	*Salmonella spp*	*Proteus spp*	Test list	*Ps. aeruginosa*	*Test list*	*S.aureus*	*CNS*	Test list	*S. agalactiae*	*S. pyogens*	Test list	*Candida albicans*
Biochemical reactions	ONPG	+	-	--	-	ONPG	-	0	-		VP	+	-	0	-
	ADH	-	+	+	-	ADH	+	GLU	+	+	HIP	+	-	GLU	+
	LDC	+	+	+	-	LDC	-	FRU	+	+	ESC	-	-	GLY	-
	ODC	+	-	+	+	ODC	-	MNE	+	+	PYRA	-	+	2KG	+
	CIT	-	+	+	-	CIT	+	MAL	+	+	_GAL	-	-	ARA	-
	H_2S	-	-	+	+	H_2S	-	LAC	+	+	GUR	+	-	XYL	+
	URE	-	+	-	+	URE	+	TRE	+	-	GAL	-	-	ADO	+
	IDA	-	-	-	+	IDA	-	MAN	+	-	PAL	+	+	XLT	+
	IND	-	-	-	-	IND	-	XLT	-	-	LAP	+	+	GAL	+
	VP	-	+	-	-	VP	-	MEL	-	-	ADH	+	+	INO	-
	GEL	-	-	-	+	GEL	+	NIT	+	+	RIB	+	-	SOR	+
	GLU	+	+	+	+	GLU	+	PAL	+	+	ARA	-	-	MDG	+
	MAN	+	+	+	-	MAN	-	VP	+	+	MAN	-	-	NAG	+
	INO	-	+	+	-	INO	-	RAF	-	-	SOR	-	-	CEL	-
	SOR	-	+	+	-	SOR	-	XYL	-	-	LAC	+	+	LAC	-
	RHA	+	+	+	-	RHA	-	SAC	+	+	TRE	+	+	MAL	+
	SAC	-	+	-	-	SAC	-	MDG	-	-	INU	-	-	SAC	+
	MAL	+	+	+	-	MAL	-	NAG	+	-	RAF	-	+	TRE	+
	AMY	-	+	-	-	AMY	-	ADH	+	+	AMD	-	-	MLZ	-
	ARA	+	+	+	-	ARA	-	URE	+	+	GLYG	+	+	RAF	-
	OXY	-	-	-	-	OXY	-							HYPH	+

API 20E: (ONPG)2-nitrophenyl-BDgalactopyranoside, (ADH) L-arginine, (LDC) L-lysine, (ODC) L-ornithine, (CIT) TRisodium citrate, (H2S)Sodium thiosulfate, (URE) urea, (TDA) L-tryptophane, (VP) sodium pyruvate, (GEL) Gelatin, (GLU) D-glucose, (MAN) D-mannitol, (INO) inositol, (SOR) D-sorbitol, (RHA)L-rthamnose, (SAC) D-sucrose, (MAL) D-melibiose, (AMY) amygdain, (ARA) L-arabinose, (OX) oxidase test and (NO2) NO2 production.

API STAPH: (GLU) D-glucose,(FRU) D-fructose, (MNE) D-mannose, (MAL) D-maltose, (LAC) D-lactose, (TRE) D-tréhalose, (MAN) D-mannitol, (XLT) xylitol,(MEL) D-melibiose, (NIT) nitrate potassium, (PA) L ß-naphtyl phosphate,(VP) sodium pyruvate, (RAF) D-raffinose, (XYL) D-xylose, (SAC) D-saccharose, (MDG) methyl-Dglucopyranoside, (NAG) N-acetyl-glucosamine, (ADH) L-arginine and (URE) urea.

API 20 STREP: (VP) Pyruvate, (HIP) Hippurate, (ESC) Esculin, (PYRA) Pyrrolidonyl-2-naphthyamide, (GAL) 6-Bromo-2-Naphthyl-D-glucuronte, (GUR)NaphtholAS-Bi-D-Glucuronate, (GAL) 2-naphthyl-D-galactopyranoside, (PAL) 2-naphthyl phosphate, (LAP) L-leucine-2-naphthylamide, (ADH) Arginine, (RIB) Ribose, (AEA)L-Arabinose, (MAN) MAnnitol, (SOR) Sorbitol, (LAC) Lactose, (TRE) trehalose), (INU) Inulin, (RAF) Raffinose, (AMD) Starch and (GLYG) Glycogen.

API 20C AUX: (0) None, (GLU) D-Glucose, (GLY) Glycerol, (2KG) calcium 2-keto-gluconate, (ARA) L-arabinose, (XYL) D-xylose, (ADO) Adonitol, (XLT) Xylitol, (GAL) D-Galactose, (INO) Inositol, (SOR) D-sorbitol, (MDG) Methyl-D-Glucopyranoside, (NAG) N-Acetyl-Glucosamine, (CEL) D-Cellobiose, (LAC) D-Lactose, (MAL) D-maLtose, (SAC) sucrose,(TRE) D-Trehalose,(MLZ) D-MeLezitose, (RAF) D-raffinose and (HYPH) Hyphae

Table 4: incidance of bacterial agents from the total examined CMT positive samples

	Single bacterial infection				Mixed bacterial infection		
No. of milk samples	Bacterial species	No. of isolates	%	No. of milk samples	Bacterial species	No. of isolate	%
67	*E.coli*	12	25.5%	26	*S. aureus & E. coli*	5	17.8%
	S.aureus	7	14.8%		*S. aureus,S. agalactiae& E. coli*	2	7.1%
	Coagulase negative Staphylococci (CNS)	6	12.7%		*S. aureus, E. Coli & K. pneumoniae*	2	7.1%
	St. agalactiae	6	12.7%		*E. coli& K. pneumoniae*	4	14.2%
	St. pyogens	5	10.6%		*S. aureus & K. pneumoniae*	4	14.2%
	Klebsiella pneumonia	4	8.5%		*CNS & E. coli*	4	14.2%
	Salmonella spp	2	4.2%		*St. agalactiae & E. coli*	2	7.1%
	Proteus spp	2	4.2%		*S. pyogens& E. coli*	2	7.1%
	Pseudomonas aeruginosa	2	4.2%		*CNS & K. pneumoniae*	2	7.1%
	Candida albicans	1	2.1%		*Proteus spp& S. aureus*	1	3.5%
Total		47		total		28	

other hand, the 15 clinical mastitis milk samples gave13 and 2 samples in ($>5x10^5$ – 10^6) and ($>10^6$) ranges respectively Table (1).

Bacteriological examination of the positive CMT samples revealed that the negative milk samples were4%, while the percentage of positive samples were 96% that were divided into single bacterial infection (60.2%) and mixed bacterial infection (35.8%)(Table 2).

The seventy five CMT positive milk samples were bacteriological examined and biochemically identified of bacterial isolates by using of different API systems as where the causative agents of mastitis were identified as shown in Table (3)

The identified bacterial agents related to single infection in milk samples were *E. coli* (25.5%), *S. aureus* (14.8%), CNS (12.7%), *St. agalactiae* (12.7%), *St. pyogens* (10.6%), *K. pneumoniae* (8.5%), *Salmonella* species. (4.2%), *Proteus sp*. (4.2%), *Ps. Aerguinosa* (4.2%) and *C. albicans* (2.1%). Meanwhile in case of the mixed infection the bacterial agents were *S. aureus* plus *E. coli* (17.8%), *E. coli* plus *K. pneumoniae,*(14.2%), *S. aureus* plus *K. pneumoniae,*(14.2%), *CNS* plus*E. coli,*(14.2%), *S. aureus*plus *St. agalactiae*plus *E. coli,*(7.1%), *S. aureus* plus*E. coli* plus *K. pneumoniae,*(7.1%), *St. agalactiae* plus *E. coli,*(7.1%), *St. pyogens* plus *E. coli, (*7.1%), *CNS* plus *K. pneumoniae,* (7.1%) and *Proteus spp* plus *S. aureus (*3.5%) as shown in Table (4).

DISCUSSION

Mastitis is one of the most important destructive infectious diseases of dairy cattle industry[9]and it is considered of quite vital importance to the public health as it is associated with many zoonotic diseases in which milk acts as a vehicle for the infectious agents [4].

In the present study, a total number of 112 milk samples was examined by using **CMT** for determination of mastitis prevalence in the selected dairy farms. The prevalence of subclinical Mastitisand clinical mastits were 56.3 and 13.3% respectively as shown in Table (1).

These findings are nearly similar to that recorded by EL-Rashidy *et al.,* [15]who reported that the prevalence of subclinical mastitis was 62.08% amongst the microbiologically examined Friesian cows. Awad [16] recorded that, the prevalence of subclinical mastitis was 69.4%. Meanwhilehigher prevalence was recorded by Karimuribo [17] (75.9%). and Kivaria *et al.* [18] (78%) between the dairy cow farm.

On the contrary, these findings disagree with that reported by Abou-Zaid and Bahout [19] who reported that the prevalence of subclinical mastitis per animal was 20.6 and 21.2%; also Lakew [20] stated that 32.7% of dairy cows have subclinical mastitis.

Also finding in the present work are the same as [21] who reported that there is clear correlation between infection and number of SCC.

Regarding the prevelance of clinical mastitis, findings of this study revealed that that the percentage of cases was13.3%.

These results nearly agree with Ahmed [22] who stated that the percentage of clinical mastitis was 9.66%.. Also, Asfour [23] reported that the percentage of clinical mastitis was 3.6%.andPetrovski *et al*.[24] who reported that the percentage of clinical mastitis was 10% in dairy cows. On the other hand, our findings are not supported by those of Tarek [25]. Lakew *et al*. [20] who reported that the clinical mastitis prevalence was 15%,

Concerning the bacteriological examination of the suspected isolates, the present study revealed that 96% was positive cases mean while Lakew *et al*. and

Seleim *et al*., [20, 26] reported that the bacteriological positive samples were 93 and 98 respectively. Also Char *et al*. [27] reported that bacteriologically positive samples were 86.7% and the bacteriologically negative samples were 13.3%.On the other hand Malinowski *et al*., [28] found that the bacteriologically negative samples were 32.7%. In this study, the prevalence of different bacteria isolated from the quarter milk samples and related to single infection were recorded, as *E. coli*(25.5%) representing the highest prevalence rate. Vaarst and Enevaldsen [29] reported that the prevalence of *E. coli* was 36%. Also, Akram *et al.,* [14] found that *E. coli* was the common pathogen which can be isolated from the milk samples as an environmental pathogen. Concerning *S. aureus* and *Ps. aeruginosa*, they were isolated at a rate of 14.8 and 4.2% which is correlated with EL-Rashidy *et al.,* Almaw and Gizat and Sampimon *et al.,* [16, 30, 31] findings that *S. aureus* prevalence was (17.03%) and *P. aeruoginosa* (3.66%). Meanwhilen Tufani *et al.,* [32]. found that prevalence of Staphylococcus spp. was (66.67%) and *E. coli* (15.87%).

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