



Bacteriological Studies of Most Bovine Mastitis Pathogenes From Small Holders in Upper Egypt

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Abstract

There is limited epidemiological knowledge on udder health in Upper Egypt dairy cattle and buffalo that would aid in a pro-active approach towards mastitis prevention. The study objectives were to investigate the prevalence rate of clinical mastitis for dairy bovine animals owned by small holders, located at all Upper Egypt Governorates. Also detection of the most common mastitis pathogens that affect the level of milk production and to test whether there is a correlation between the location, animal species, and seasons of breeding with prevalence of clinical mastitis. Also to detect the drugs of choice for the treatment of bovine clinical mastitis in different Governorates of Upper Egypt .A total of 345 lactating cattle cows and 111 lactating buffalo cows, suffered from clinical mastitis, at different lactation seasons from small holders of six Governorates of Upper Egypt, were tested by general bacteriological investigations, during three cycling visits for the presence of clinical mastitis. The prevalence of clinical mastitis ranged from 7.5 % to 29.3 % for cattle cows and from 13.5 % to 27.9 % for buffalo cows based on the Governorate location and atmospheric nature. Milk samples from clinical mastitic cattle and buffalo cows were examined bacteriologically; 95.4% and 92.8 were culturally positive, meanwhile the culturally negative samples were 4.6% and 7.2% for cattle and buffalo cows' respectively. As a mean of infections in all Upper Egypt Governorates ,*Staphylococcus aureus* (*S. aureus*) was the predominant mastitis pathogen followed by *Escherichia coli* (*E.coli*), Other strept.and *Coagulase negative Staphylococcus* (CNS) 37.7%, 26.7%, 18.0% and 13.0% respectively for cattle clinical mastitis .On the other hand ,also *S.aureus* was the main cause of infection for buffalo cows clinical mastitis (46.8%) followed by CNS, *E.coli* and Other strept. infections (16.2%,15.3% and 14.4% respectively) .Animal species,Regions and seasons play an important significant role in bovine clinical mastitis affecting causes and treatment in Upper Egypt We concluded from the results of sensitivity test for clinically mastitic milk samples that Amoxicillin/clavulinic acid, Cefquinom, Ciprofloxacin and Florofenicol were the top antibiotics for the treatment of all pathogens isolated from cattle cows in all Governorates of Upper Egypt.Meanwhile Florofenicol, Gentamycin, Neomycin, Tetracyclin, Penicillin and Ampicillin were the most important antibiotics used for the treatment of clinically mastitic buffalo cows.These findings provide information about nature of clinical mastitis for improved treatment efficacy and pro-active approaches towards mastitis prevention in Upper Egypt . Sampling of clinical mastitic milk in different seasons would provide complete understanding of udder health throughout the year.

1. INTRODUCTION

Mastitis is a multietiopathogenic condition of mammary gland affecting dairy cows and remains the most economically important disease of dairy industries around the world. It is characterized by physical, chemical and microbiological changes in

the milk and pathological changes in the glandular tissues of the udder (Merk Veterinary Manual ,1998 and Patnaik et al, 2013a.)

Milk production per cow due to the clinical and subclinical prevalence of mastitis is usually recognized as the main pathway in causing the

economic losses due to the disease. Mastitis is most often transmitted by contact with the milking machine, and through contaminated hands or other materials, in housing, bedding and other equipments. (Radostits et al, 1995.) .

Large numbers of infectious agents are responsible in causing the disease in dairy animals. Bacterial agents like *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Corynebacterium spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, Mycoplasmal agents, fungal agents, viral agents are responsible for the disease (Nagal, et al., 1999), *Staphylococcus aureus* is a major pathogen in dairy cattle mastitis (Waage,et al., 1998)

Frequency of occurrence of mastitis is influenced by different managemental, environmental and genetic factors. 25% of susceptibility to infection is attributed to environmental factors, 25% to genetic factors and 50% to herd management. Therefore prevalence of mastitis can be used as a litmus test to judge the management practices and hygienic conditions of any organized dairy animals.(Klastrup ,2007)

The determination of antimicrobial susceptibility of clinical isolates is required not only for therapy but also for monitoring the spread of resistant strains throughout the populations (Patnaik,etal.2013c) . Because spontaneous recovery occurs infrequently and there is no proven way of increasing it, the introduction of antibiotic therapy was a major advance in controlling bovine mastitis. Infusing antibiotics via the teat duct into the udder is a simple way of overcoming nearly all clinical mastitis and of eliminating many infections. Spread of infection among the producing animals; subsequently increasing the economic losses particularly in small-scale farming with sublevel of sanitary measures leading to a relatively high culling rate. Consequently, reliable method for the identification of bacterial agents from mastitic milk is crucial for preventing the spread of infection and for control of udder problems (Maha et al., 2014).

In most Governorates of Upper Egypt, many dairy owners introduced foreign blood. This might result in a progeny of mixed blood cows with lowered resistance to endogenous and locally prevailing diseases such as mastitis. Clinical mstitis was diagnosed when there were visible or palpable signs of udder, inflammatory changes in milk secretions, or through bacteriological examination of milk. (Reem and Salih, 2015).

MATERIALS AND METHODS

2.1. Study area

The study was carried out in six Governorates of Upper Egypt (Fig 1) through one year, between December 2014 and December 2015. It covers an area of about 1000 km² and is situated between Giza and Aswan Governorates. The project included many different vilages which constructed on the farmers by a non-governmental organisation and randomly selected dairy animals without digesters in the region, age and number of cows. Due to the similarity of breeding practices across small holders in the district, the sample was considered fairly representative of the populations in the district. The study Governorates was visited triple during the study period to increase the sample size and determine the consistency of the results.

2.2. Animals and Samples:

Samples were collected from six Governorates of upper Egypt (Fig 1) through one year under supervision from Animal Reproduction Research Institute (ARRI) team of therapeutic campignes .Examination of each suspected mastitic cow was carried out according to the enclosed questionnaire of bovine mastitis, this questionnaire included data about area, cow species, seasons,udder and milk production. The enclosed questionnaire was to determine the roles played by dairy species, environment, hygiene, ventilation, drainage system, floor type and building of breeding as a contribution causing factors for mastitis. Polled milk samples were ciollected from quarters of 345 cattle cows and 111 buffaloe cows with clinical mastitis. Milk samples were taken under critical aseptic condition for bacteriological studies.. All samples collected were immediately placed on ice in a thermotank after collection until transported to ARRI bacteriological lab. . The farmers responded highly to the questionnaire regarding general breeding data. The main system applied was sporadic. The ventilation was satisfactory. Drainage system was poor. Housing materials were a mixture between modern and traditional. Type of floor was found ranged between earth mainly and concrete .

Table (1). Number of cattle and buffalo cows cases suffered from different types of mastitis examined by ARRI campaigns team for small holders in upper Egypt .

Type of mastitis.	CattleCows		Buffaloe cows		Total
	No	%	No	%	
Clinical	345	93.5	111	97.4	456
Chronic	19	5.1	3	2.6	12
Gangarinous	5	1.4	0	0.0	5
Total	369	76.4	114	23.6	483

Table (2): Number of cow and buffalo polled clinically mastitic milk samples collected from different Governorates of upper Egypt .

GOV.	Cow milk		Buffaloe milk		Total
	No	%	No	%	
Giza	59	17.1	19	17.1	78
Fayoum	31	9.0	11	9.9	42
Beni Swif	26	7.5	18	16.2	44
Menia	101	29.3	31	27.9	132
Sohag	52	15.1	15	13.5	67
Aswan	76	22.0	17	15.3	93
Total	345	75.7	111	24.3	456

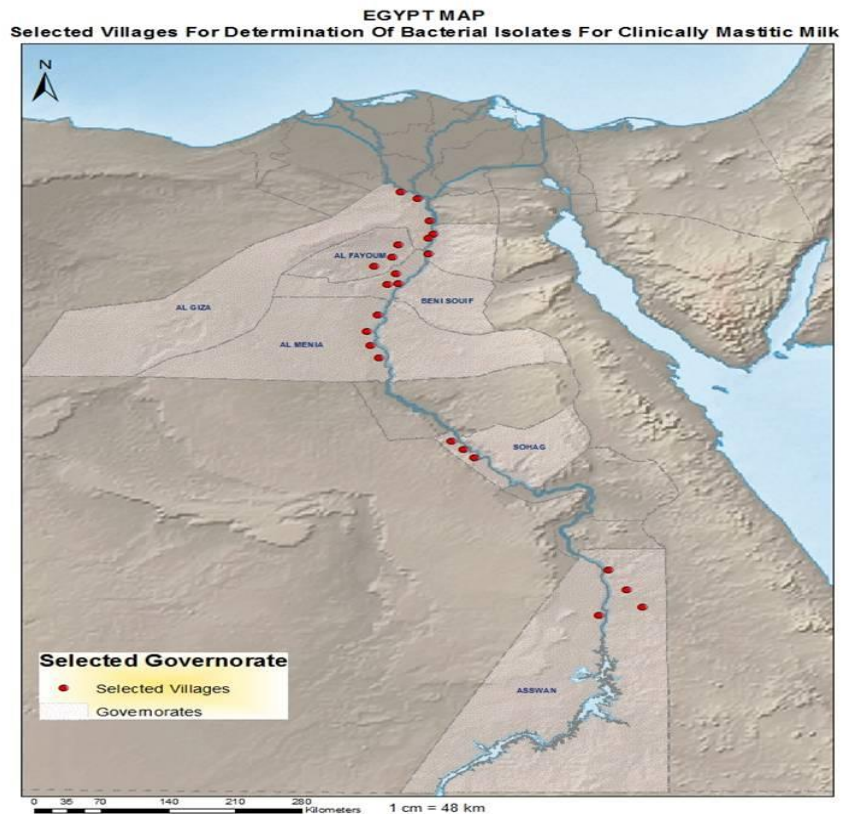


Fig 1. Selected villages for determination of bacterial isolates from clinically mastitic milk .

2.3. Isolation and Identification of Bacteria

Each milk sample was streaked onto Mannitol salt agar, Edward agar, MacConky agar, Neutrient agar and 5% sheep blood agar plates (Hi Media) and incubated at 37°C for 24 hrs. Colonies were initially assessed by their morphology and hemolysis

patterns, followed by Gram staining and motility tests. The isolates were identified according the procedures of Quinn *et al.* (2002). Biochemical tests, specifically, catalase, coagulase, oxidase, carbohydrate fermentation tests (glucose, mannitol, ribose, sorbitol, and trehalose), biochemical reaction on MacConkey agar, indole production, Methyl red

tests, urease production and citrate utilization tests, Triple sugar iron agar reactions (TSI) were performed as required. In cases where no growth was detected, plates were re-incubated at 37°C for an additional 24 h.

2.4. Antimicrobial susceptibility test (Sensitivity test):

The susceptibilities of isolates to antimicrobial agents were determined by using the disk diffusion method

according to the NCCLS Guidelines (2002). The antimicrobial disk used are :- Ciprofloxacin, Florofenicol, Amoxicillin, Amoxicillin + Claviulonic Acid, Pencillin G, Trimethoprim/sulphonamides, Cefiquinom, Neomycin, Oxytetracyclin, Gentamycin, and Streptomycin

3. RESULT AND DISCUSSION

Table (3): The antimicrobial sensitivity test used in the agar diffusion method and interpretation of their sensitivity zone.

Antimicrobial agents	Code	Disc content	Zone of inhibition		
			Resistant	Intermediate	Sensitive
Ciprofloxacin	CIP	5 µg	≤15	16-20	≥21
Florofenicol	C	30 µg	≤12	13-17	≥ 18
Ampicillin	AMP	25 µg	≤11	12-13	≥ 14
Amoxycillin+clavulanic acid	AMC	20+10 µg	≤13	14-17	≥ 18
Penicelline G	P	10 units	≤20	20-27	≥ 29
Trimethoprim/sulphonamides	SXT	1.25+23.75 µg	≤10	11-15	≥ 16
Cefiquinome	CEQ	30 µg	≤13	14-18	≥ 19
Neomycin	N	30 µg	≤12	13-16	≥ 17
Oxytetracycline	OT	30 µg	≤14	15-18	≥ 19
Gentamycine	GM	10 µg	≤12	13-14	≥ 15
Streptomycin	S	10 µg	≤11	12-14	≥ 15

Table (4): Prevalance of bacterial isolates from cattle clinically mastitic milk in different Governorates of Upper Egypt.

GOV.	Total mastitic cows	<i>Staph.aureus</i>		CNS		<i>Other Strept.</i>		<i>E.coli</i>		No growth		Total bacterial isolates	
		No	%	No	%	No	%	No	%	No	%	No	%
Giza	59	27	45.8	9	15.3	6	10.2	14	23.7	3	5.1	56	94.9
Fayoum	31	9	29.0	5	16.1	4	12.9	11	35.5	2	6.5	29	93.5
Beni Swif	26	8	30.8	3	11.5	5	19.2	7	26.9	3	11.5	23	88.5
Menia	101	40	39.6	7	6.9	20	19.8	30	29.7	4	4.0	97	96.0
Sohag	52	21	40.4	10	19.2	8	15.4	10	19.2	3	5.8	49	94.2
Aswan	76	25	32.9	11	14.5	19	25.0	20	26.3	1	1.3	75	98.7
Total	345	130	37.7	45	13.0	62	18.0	92	26.7	16	4.6	329	95.4

Table (5): Prevalance of bacterial isolates from buffaloe clinically mastitic milk in different Governorates of Upper Egypt.

GOV.	Total mastitic cows	<i>Staph.aureus</i>		CNS		<i>Other Strept.</i>		<i>E.coli</i>		No growth		Total bacterial isolates	
		No	%	No	%	No	%	No	%	No	%	No	%
Giza	19	7	36.8	3	15.8	3	15.8	4	21.1	2	10.5	17	89.5
Fayoum	11	5	45.6	1	9.1	2	18.2	2	18.2	1	9.1	10	90.9
Beni Swif	18	8	44.4	3	16.7	2	11.1	5	27.8	0	0.0	18	100.0
Menia	31	15	48.4	6	19.4	5	16.1	2	6.5	3	9.7	28	90.3
Sohag	15	8	53.3	3	20.0	2	13.3	2	13.3	0	0.0	15	100.0
Aswan	17	9	53.0	2	11.8	2	11.8	2	11.8	2	11.8	15	88.2
Total	111	52	46.8	18	16.2	16	14.4	17	15.3	8	7.2	103	92.8

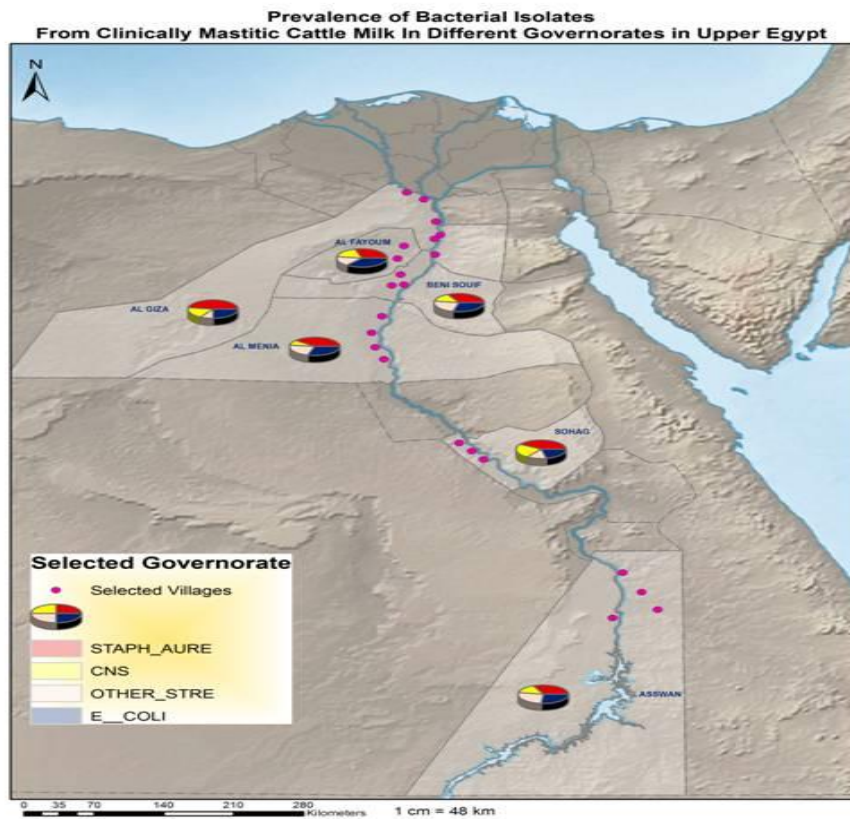


Fig 2. Prevalence of bacterial isolates from clinically mastitic cattle milk in different Governorates of upper Egypt

Table (6): Drugs of choice for cattle clinical mastitis treatment for isolated bacteria in different Governorates of upper Egypt .

Governorates	Bacterial spp.isolated from dairy animals (Cattle cows)			
	<i>Staph.aureus</i>	<i>CNS</i>	<i>Other strept..</i>	<i>E.coli</i>
Giza	AMC-CFQ	N-T	N-S	AMC-FF
Fayoum	FF-CIP	T-CFQ	G-CFQ	CFQ-CIP
Bani suif	N-AMC	FF-AMC	N-G	CFQ-G
Menia	CFQ-G	P-T	S-N	FF-T
Sohag	CIP-N	CFQ-N	N-CFQ	CIP-AMC
Aswan	AMC-CIP	AMC-CIP	CFQ-CIP	FF-N

Table (7): Drugs of choice for buffalo clinical mastitis treatment for isolated bacteria in different Governorates of upper Egypt .

Governorates	Bacterial spp.isolated from dairy animals (Buffaloe cows)			
	<i>Staph.aureus</i>	<i>CNS</i>	<i>Other strept.</i>	<i>E.coli</i>
Giza	FF-P	G-N	N-FF	AMP-FF
Fayoum	FF-N	G-S	T-P	T-CFQ
Bani suif	CFQ-CIP	N-AMC	FF-G	FF-P
Menia	P-N	S-CFQ	AMP-P	G-FF
Sohag	N-CFQ	AMC-G	G-T	CFQ-CIP
Aswan	CIP-FF	CFQ-N	AMP-N	P-T

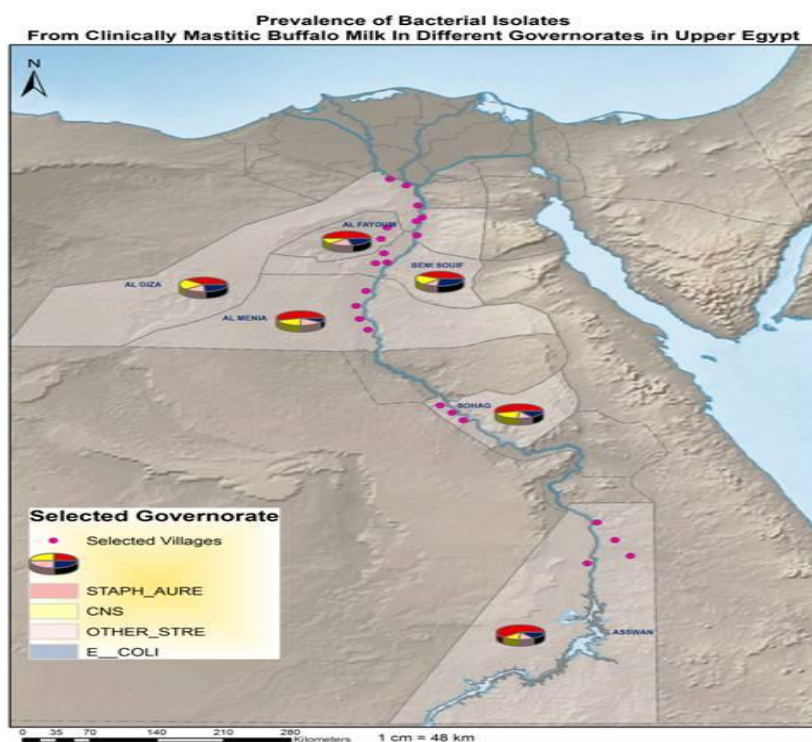


Fig. 3. Prevalence of bacterial isolates from clinically mastitic buffalo milk in different Governorates of upper Egypt

Table (8). Odds Ratio, 95% Confidence Intervals (CI) and Probability Value (P) for different bacterial isolates computed from logistic regression coefficient for variable significantly associated in clinical bovine mastitis .

Variable	Staph.aureus			CNS			Other strept.			E.coli		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
A)Species 1-Cattle 2-Buffaloe	1.5	0.8,11.0	0.032	0.70	0.8,3.0	0.60	1.30	0.56,7.0	0.034	2.01	1.0,17.0	0.015
B)Regions	1.45	1.0,17.0	0.03	0.40	0.7,2.0	0.50	2.80	2.0,18.0	0.01	4.2	1.0,22.0	0.003
C)seasons 1-summer and spring 2-winter and autumn	1.6	1.0,12.0	0.02	1.70	1.50,13.0	0.01	0.90	0.50,3.0	0.08	1.5	0.80,14.0	0.03

Mastitis is considered the main disease in dairy herds . It is the inflammation of the mammary gland and mammary tissues, (Kaneen and Bandhard, 1990). However, the udder disease of major concern is that associated with microbial infection ,the most common major pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Coliforms* and *Other streptococci* ,while *Corynebacterium bovis* were considered to be minor pathogens(Sandholm et al., 1995) .

Most common types of mastitis bacteria originate in the udders of infected cows and in sores on

teats.These pathogens multiply in teat sores and are spread during milking.

In the present study the greatest type of mastitis from small holders was clinical mastitis, 345 cows (93.5%), followed by chronic cases of mastitis, 19 cows (5.1%) and gangrenous cases of mastitis, 5cows (1.4%)for cattle .Meanwhile it was 111 cows (97.4%), 3 cows (2.6%) and no cows (0%) for buffalo respectively (Table 1). Chronic and gangrenous cases of mastitis were due to Poor treatment success and limited use of dry cow therapy would also contribute to chronic infections that would act as a reservoir (Radostits 2001). Minimal

use of post-milking teat dip by the farmers was also likely contributing to the spread specially of the contagious pathogens.

Bulk numbers of examined cows were in Menia Governorate ,it was 101 cattle cows (29.3 %) from total investigated number in all Upper Egypt Governorates,also it was the highest count for buffaloe cows 31(27.9%). The little count of cattle cows were in Bani suif , 26 (7.5%) and for buffaloe cows were in Fayoum Governorates 11(9.9%) ,(Table 2) . These results may be returned to absence of animals market in Menia, middle location of Upper Egypt and the farmers their always bring new blood animals from up and down Governorates. Meanwhile Fayoum and Bani suif Governorates with small animals market and also their locations are near from Giza and Cairo Governorates wheres no remarkable markets of farm animals.

A total of 329/345 clinically mastitic cattle cow's milk samples gave a positive culture results (95.4%) ,Table (4) and 103/111 buffaloe cow's milk samples were bacteriologically positive (92.8%), Table(5) .These results were higher than results reported by Ahmed and Mohammed,(2010) which could be attributed to infected zone as well as bad management and poor hygiene for small farmers, as no enough facilities were available .Correspondingly in the same results tables 4&5 ,negative samples, revealed no growth of any pathogenic microorganismes on ordinary media of general bacteriology, were 16 samples (4.6%) of cattle cows mastitic milk and 8 samples (7.2%) of buffaloe cows mastitic milk respectively. The results of no growth were disagree with Dawn et al,(2017) ,who said that Pathogens were isolated from 71 percent of the glands, with milk from the remaining 29 percent having no bacterial growth.This attributed to the big differences in sanitation,hyagine and system of breeding between USA and North of Egypt. And also may be due to a big differences between specific media used for isolation .

The isolated bacteria from cattle cows, clinically mastitic milk samples were as follows: *Staphylococcus aureus* (37.7%), *CNS* (13%) ,*Other strept.* (18%) and *E.coli* (26.7%) from cattle cows milk samples, on the other hand they were (46.8%) ,(16.2%),(14.4%) and (15.3%) for the same microorganisms isolated from buffaloe cows mastitic milk samples respectevilly (Table 4 &5) (Fig 2&Fig 3).These results were incoorporation with Lafi et al, (1994), they reported that the organisms from cows with clinical and subclinical cases of mastitis were bacteriologically tested. Out of all tested cows (34%) were *coagulase positive Staphylococci*, (16%), *coagulase negative*

Staphylococci and (17.1%) *Other strept* but our results were disagree with their result of gram negative bacteria,*E.coli* (7.0%),and this result was clear with small holder breeding on earth with minimum degree of cleaning and managment .Also in the same manner with Jirata and Telila(2016) ,they commented that in positive samples, *Staphylococcus* accounts for 43.54%, *Streptococcus* 28.89%, *Coliform* 19.35% respectively, of the total bacteria isolated. In addition to Frandos and spurgeon, (1992) who historically found a significantly higher prevalence rate in housed animals, this has substantiated by an easy transmission of organism from infected to healthy ones. The result obtained from microbiological analysis of the samples revealed that the predominant organism isolated were *staphylococcus aureus* which accounted for 43.54 %.

S.aureus isolated from our study was the king of infection in both cattle and buffaloe milk samples of clinical mastitis cases (37.7%&46.8%), this could be associated with absence of post milking teat dipping, lack of culling of chronically infected cows, absence of dry cow therapy and the invariable hand milking practice among the dairy cows.The same idea was indicated by Rahmeto et al(2016),they confirmed that *S. aureus* and other contagious microorganisms are usually found on the udder or teat surface of infected cows and are the primary source of infection between uninfected and infected udder quarters, usually during milking .

Table (6) showed the drugs of choice and the best antibiotics used for the treatment of clinically mastitic cattle cows in different Governorates of Upper Egypt . The results showed that in Giza Governorate, Amoxicillin/clavulanic acid and Cefiquinom are the best antibiotics used for the treatment of *Staph.aureus*, Neomycin and Tetracycline were the drug of choice used for treatment of *CNS* infection in addition to Streptomycin and Florofenicol for the treatment of *Other strept.* and *E.coli* infection . In Fayoum Governorate Florofenicol and Ciprofloxacin were the best antibiotics used for the treatment of *S.aureus* ,Cefiquinom was the best drug for nearly all bacterial infections , in addition to tetracycline for *CNS* and Gentamycin for *Other strept.*.The results in Bani suif Governorate showed that, Amoxicillin/clavulanic acid was the drug of choice for the treatment of *S.aureus* and *CNS* infection; meanwhile Neomycin and Gentamycin were the best for *other strept.* and coliform treatment . Also the study resulted that Cefiquinom and Gentamycin, Penicillin G and Tetracyclin, Streptomycin and

Neomycin in addition to Florofenicol and Tetracyclin were the best twins of antibiotics used for the treatment of *S.aureus*, *CNS*, *Other strept.* and *E.coli* infections in Menia Governorate . On the other hand, Sohag and Aswan Governorates were nearly the same antibiotics gave a good results for treatment of all types of bacterial infections and these were mainly Amoxicillin/clavulanic acid , Cefiquinom and Ciprofloxacin . We concluded from these results of sensitivity test for clinically infected cattle cows mastitic milk samples that Amoxicillin/clavulanic acid , Cefiquinom ,Ciprofloxacin and Florofenicol were the top antibiotics for the treatment of all types of clinical udder infection in all Governorates of Upper Egypt , where the treatment advised for farmers was gave a good results for controlling of clinical cattle mastitis. These results were agree with the idea of Dawn et al, (2017),where their conclusion of antibiotics treatment resulted that when antibiotics were not administered, more cows developed clinical mastitis in new quarters than did those given antibiotics. In addition to having more clinical mastitis events, cows not receiving antibiotics developed more severe disease.

Also the sensitivity results of cattle clinically mastitic milk were as same as indicated by MOHAMMED SALIH ,(2015),who results that the best antibiotics in overall of his study were Chloramphenicol (CH) and Ciprofloxacin (CP) because the percentage of the effectiveness was 100% that mean all isolated organisms are susceptible to these two drugs. This study was conducted in Om durman and Khartoum North because these towns are considered as the largest milk producing and marketing.

As illusterated befor for cattle cows sensitivity results, in the same pattern, buffaloe cows mastitic milk samples showed in Table (7) that: in Giza Governorate Florafenicole antibiotic was the most suitable one for the treatment of clinically mastitic buffaloe cows for nearly all bacterial infection. Meanwhile Neomycin, Gentamycin, Tetracyclin and Cefiquinom were the primes antibiotics gave results for the treatment of *S.aureus.*, *CNS*, *Other strept.* and *E.coli* respectively in Fayoum Governorate . Cefiquinom and Ciprofloxacin, Neomycin and Amoxicillin/clavulanic , Florofenicol and Gentamycin with Florofenicol and Penicillin were the best twins of antibiotics for the treatment of *S.aureus.*, *CNS*, *Other strept.* and *E.coli* infection respectively in Bani suif Governorate .Penicillin,Streptomycin,Ampicillin , Gentamycin ,Ciprofloxacin and Cefiquinom were the drugs of choice for the treatment of buffaloe cows suffered from clinical mastitis in Governorates of Menia

,Sohag and Aswan . We concluded from these results of sensitivity test for clinically infected buffaloe cows mastitic milk samples that Florofenicol,Gentamycin,Neomycin,Tetracyclin,Penicillin and Ampicillin were the most important antibiotics used for the treatment of clinically mastitic buffaloe cows.By judgement on this result we were easily found that completely difference between drugs of choice for treatment of clinical mastitis for cattle and buffaloe cows ,and this probably due to rare using of antibiotics for the treatment of mastitis in buffaloes comparing to cattle one . In addition to the fact which confirm the version media of udder tissues for buffaloe cows which can easily affected by these old generation of antibiotics , at the same time these drugs not gave good results for cattle cows whose treated too much by different and new generation of recent available antibiotics . These results discussed in a narrow area by Reem et al,(2015), and they were agree with a limit with our drugs of choice for treatment ,because the selected group of antibiotics used in sensitivity test were different between the two studies .

Table (8) showed the effect of some risk factors which play an important role in control and treatment of bovine clinical mastitis .Same idea was discussed by (Almaw et al., 2008) who stated that prevalence of mastitis was found to be influenced by the stage of lactation, anatomical abnormality of the udder, and some management aspects such as nutrition in addition to breeds, location and seasons.

Animal species as a risk factor for clinical mastitis in dairy bovine were significantly associated with *S.aureus* infection (OR 1.5, 95% CI 0.8,11.0 and P 0.032) , also with *Other strept.* infection (OR 1.30 , 95%CI 0.56,7.0 ,and P0.034) and with *E.coli* infection (OR 2.01 , 95%CI 1.0,17.0 and P 0.015) . But it was non significant for *CNS* infection (OR 0.70 , 95%CI 0.80,3.0 and P 0.60). Regions as a risk factor between six Governorates of Upper Egypt were significantly associated with *S.aureus* ,*Other strept.* and *E.coli* infections causing clinical mastitis between small holders (OR 1.45 , 95% CI 1.0,17.0 and P 0.03) , (OR 2.80, 95% CI 2.0,18.0 and P 0.01) and (OR 4.2 , 95% CI 1.0,22.0 and P 0.003) respectively , except it was non significant for *CNS* infection(OR 0.4 , 95% CI 0.70, 2.0 and P 0.50) .On the other hand seasons differences as a risk factor was significant for *S.aureus* ,*CNS* and *E.coli* infections (OR 1.6 , 95% CI 1.0,12.0 and P 0.02) , (OR 1.7 , 95% CI 1.50,13.0 and P 0.01) and (OR 1.5 , 95% CI 0.80,14.0 and P 0.03) ,meanwhile it

was non significant for *Other strept* infection (OR 0.9 , 95% CI 0.50, 3.0 and P .08).

Our study by these recorded past results showed that Odds ratio for *S.aureus* infection referred to the infection of udder tissues and mammary glands ,where it was nearly one and half time between cattle and buffalo species . These results were agree with Hussain et al.,(1984);and Sharma,(2003) ,they confirmed that cows are more liable to mastitis than buffaloes. The comparative high resistance of buffaloes to intramammary infections is may be possibly owing to their udder teat orifice is tightly closed due to presence of well developed circular muscles, stratified squamous keratinized epithelium of streak canal lining is thicker which provides an extra resistance against penetration of pathogens through epithelium and the stratum granulosum contained higher amount of keratohyalin granules in buffaloes than in cows. These keratohyaline granules may probably contribute in formation of large amount of keratin in lumen of streak canal. For region risk factor, the study put a high light on more infection levels in Giza and Sohag Governorates than other Governorates of Upper Egypt .While for *CNS* infection the odds ratio for region factor reflecte that it was no significant difference between different Governorates of Upper Egypt. On the other hand buffalo *CNS* infection were also not affected in different Governorates under study. Seasons factor showed the same infection level as *S.aureus* infection, nearly one and half time of summer and spring than winter and autumn seasons as recorded by Ahmed and Mohammed (2010). They noticed that the prevalence of clinical mastitis in hot weather as during summer and during spring was higher than in cold weather as during winter and during autumn .These observations were explained from the complains of the farmers, in which, poor feeding, unbalanced rations and bad hygiene become more pronounced during the hot weather as the green fodders become deficient, in addition to, increasing the insect population. That led to decrease the immunity and consequently the mastitis increased. While during the cold weather, clover becomes somewhat enough and insect population becomes limited to spread infection.

Clinically mastitic infection by *other strept.* bacteria resulted that odds ratio was clearly represented in region differences specially in Aswan ,Menia and bani suif Governorates with high level of infection .It was to limitte clear between cattle and buffalo spp. , meanwhile it was no differences between seasons ,as no effect of temperature on *other strept.*infection. Endly *E.coli* clinical mastitis was

clearly difference between cattle ,with high risk infection ,and buffalo with low risk infection . Menia and fayoum were slightly high coliform infection for cattle cows comparing with other Governorates, in contast with Menia, Sohag and Aswan were low risk of coliform infection for buffalo cows. These results may be due to nature and cultural behavior of each Governorate ,for example Menia of high risk of coliform infection depend on grazing for a long distances in addition to most probably polluted under ground water used for udders cleaning ,hand washing and so on .Also in menia ,animal production in deseart areas peripheral places ,far from river Nile .But in case of Fayoum Governorate ,it collectes different species and different ages of different located animals from surrounded animal markets of other Governorates which give a chance for transferring of different bacterial infections specially *E.coli* . Seasons were highly significant risk factor of *E.coli* infection specially in summer where the weather is too hot , with elevation in hummidity , shortage in green foods and the insects transimitted diseases are so much in this season .

4. CONCLUSION

In this study from different bacteriological investigations the total number of samples collected during three visits concluded that, *S. aureus* was the most prevalent organism isolated, followed by *E.coli*, *other Streptococci* and *CNS* while around 5% of samples had no growth.

The animal health service need to focus on regular screening of dairy cows for clinical mastitis in Upper Egypt and treating of the cases both in lactation and dry period and provision of advice to cull chronically infected animals.

Finally we advise the small holders to apply as can as possible, a good hyagin and sanitation during manual milking and management .As well as it is important to collect all bacterial strains from all separat Upper Egypt Governorates and make alocal vaccines for most important epidemic micro-organismes causig local clinical bovine mastitis

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