Egypt. J. Chem. Environ. Health, 2 (2):516-533 (2016)
On line ISSN: 2536-9164.

Studies on bacterial infection of cow's milk with special reference to Mycopasma Bovis Recoverd from marketing and mastitic milk

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Abstract

Bacterial infection of cow's milk was studied for this aim out of 124 samples of cow's milk were collected from 38 mastitic cow, 46 subclinical mastitis, 10 bulk tank and 30 market milk, 124 these samples were subjected obtained 131 pathogens was detected and the most frequently identified microbes was *Staphylococcus aureus* 54(43.5٪) followed by *Sterptococcus agalacteae* 25(20.2٪), *Escherichia coli* 23(18.5٪), *Corynebacterium pyogenes* 16(12.9٪), *Enterococcus feacalis* 10(8.1 ٪) and *Mycoplasma Bovis* 3(2.4٪). Rate of isolation from different types of milk samples,where 27 isolates where identified from 38 mastitic cow`s milk. *S. aureus* showed the highest rate 48٪ (number=13), followed by *S.agalacteae* 26٪(n=7), *C. pyogene* 19٪(n=5) and lowest persent *MB* 7٪ (n=2).

Concerning subclinical mastitis *S. aureus* showed the highest rate of isolation 38٪ (n=20), followed by *E.coli* 28% (n=15), *S.agalacteae* 19٪ (n=10), *C. pyogene* 13٪ (n=7) and lowest persent was *MB* 2٪ (n=1). In as regards to the examined bulk milk, *E.coli* showed the highest rate of isolation 42%(n=8), followed by *S. aureus* 37٪ (n=7), *C. pyogene 2*1٪ (n=4) while *S.agalacteae* and *MB* were not detected. About the examined marketing milk, *S. aureus* showed the highest rate 44٪ (n=14), followed by *S.agalacteae* 25% (n=8) , *E.faecalis 3*1٪ (n=10) while S.agalacteae, *E.coli* and *MB* were not detected.

Three isolates were identified as *MB* (Two isolates from clinical mastitis and one isolate of subclinical mastitis) and confirmed by PCR

S.aureus isolates showed multidrug resistance ranged from 60%-100%, where 100% of isolates were resistant to tetracycline, amplicillin, cephalothin, amikacin, clindamycin and lincomycin.

S.agalactea showed multidrug resistance ranged from 60%-100%, where 100% of isolates were resistance to tetracycline, neomycin, sulfa/trimethoprim and clindamycin.

E.coli showed multidrug resistance ranged from 40%-100%, where 100% of isolates were resistance to sulfa/trimethoprim and lincomycin.

C.pyogenes showed multidrug resistance ranged from 61%-100%, where 100% of isolates showed multidrug resistance, and were resistance to tetracycline, amplicillin, neomycin, sulfa/trimethoprim, amikacin and gentamicin.

E.faecalis showed multidrug resistance ranged from 20% -100%, where 100% of isolates were resistance to gentamicin and lincomycin. The public health concern of different isolated strains was discussed.

Introduction

Bovine mastitis is a result of inflammation of the mammary gland. Depending on the severity of the inflammation, mastitis can be classified as sub-clinical, clinical and chronic. The degree of inflammation is dependent on the nature of the causative agent and on age, breed, immunological health and lactation state of the animal, a many bacteria mycoplasmas, yeasts and algae may cause mastitis in dairy cows **(Viguier *et al.* 2009).**

Subclinical mastitis in dairy cows is a big economic problem for farmers. The monitoring of subclinical mastitis is usually performed through Somatic Cell Count (SCC) in farm but there is a need for new diagnostic systems able to quickly identify cows affected by subclinical infections of the udder. The most frequent pathogen isolated was *Staphylococcus aureus* followed by coagulase negative staphylococci (CNS), *Streptococcus uberis, S. agalactiae* and others **(Bortolami *et al.*,2015)**.

For this case, as an environmental pathogen, produces a wide range of symptoms, going from a mild disease showing only local inflammatory changes of the mammary gland, to a severe form presenting significant systemic signs including rumen stasis, dehydration, shock, and even death (**Wenz *et al.* 2001**). The host defense of the bovine mammary gland has been shown to be efficient in controlling and eliminating *E. coli* infection (**Hill *et al.* 1979**); however, this ability has been shown to be less effective during early lactation, due to deficiencies in neutrophil function and number (**Shuster *et al.* 1996**).

S. agalactiae is a major cause of bovine mastitis, which is the dominant health disorder affecting milk production within the dairy industry and is responsible for substantial financial losses to the industry worldwide **(Richards *et al.* 2013)**.

Mycoplasma mastitis is caused by a number of species, MBis the most common cause and resulted in the most severe disease. **(*Karahan et al., 2010*)**

Mycoplasma firstly reported in Egypt by (**El-Ebeedy *et al*. 1985),** spread of mycoplasma infection was throughout the Egyptian farms and become endemic in some areas. (***Eissa et al., 2011***) concluded that all M. bovis strains isolated from cattle and buffaloes nearly the same in sequencing with insignificant difference and had similarity of 98-99% this means the same strain was spreading in the different examined dairy herds). **(Sahar *et al.*, 2014)** Egyptian M. bovis (Sah.S.M.Catt.4) which was isolated from cattle was similar to other strains of Mycoplasma bovis of different sources in the world and it was deposited on the gene bank with the accession no.(JX993354) Various types of mycoplasma were

isolated from dairy Friesian cows and buffaloes with mastitis. These mycoplasma included *M.bovis, M bovigenitalium, M.dispar, M.bovirhinis and M. arginini. Mycoplasma bovis* is most important etiologic agent of mastitis **(Nicholas *et al*., 2006).**

The present study was aimed to investigate find the most important pathogens causing bovine mastitis with special reference to *MB* and study the public health of the isolated strains in Fayoum governorate.

Material and Methods

2.1 Samples

A total of 124 cattle milk samples were collected from some dairy farms, individual farmers and markets in EL Fayoum Governorate, Egypt. 38 mastitic milk samples of cows; 46 subclinical mastitis; 10 bulk milk tank from farms and 30 market milk samples as raw fresh milk. **Table (1)**

Table (1): Type and number of collected samples

Type of samples	No.
Clinical mastitic milk	38
Subclinial mastitic milk	46
Bulk milk	10
Market milk	30
Total	124

Microbiological examination: according to **(Rysanek, *et al.* 2007)**

E. coli detection was performed by the inoculation of 0.1 ml milk sample smears on MacConkey agar. After 24h of incubation at 37°C, five lactase-positive colonies were marked and selected. These colonies were isolated by subculture on blood agar (BA). After 24 h of incubation, the cultures were tested by the OXI test (PLIVALachema, Brno, Czech Republic) for oxidase test. OXI -negative strains and controls were inoculated on Simmons citrate agar and Motility Test Medium and incubated for 24h at 37°C. After their assessment, biochemical identification was carried out.

Detection *of S. aureus* was performed by the inoculation of 0.1ml milk sample smears on Mannitol Salt Agar. After 36h of incubation at 35°C, typical colonies were subcultured on blood agar (BA) and incubated 24h at 37°C. Catalase test and staphytect test (Oxoid), were conducted. Staphytect positive strains were examined by a VP test (Voges-Proskauer test). **(Rysanek, *et al.* 2007)**

Detection of Streptococcus species was performed by the inoculation of 0.05ml milk sample on BA. After 24-48h of incubation at 37C, the β- haemolytic colonies were subcultured on BA and incubated at 37C for 24h.catalase test was

conducted, AP|I 20 Strep was carried out for identification and lancifield grouping was applied. **(Rysanek, *et al.* 2007).**

Mycoplasma was isolated from milk samples using PPLO broth and agar by traditional techniques**.** The isolation was confirmed by using PCR

Culture procedure for *Mycoplasma* from milk samples: according to **(OIE 2008)**

Mycoplasma broth and agar were prepared for the indirect culture 0.1 ml of milk was inoculated into 5 ml of PPLO broth. The inoculated media were incubated at 37^{o}C in moist CO_2 incubator for 7th days. The cultures were examined for growth every day. The final reading was made on the 7th day. Samples were accepted as negative after five transfers that did not show growth. PPLO agar plate were only incubated from the positive broths at 37^{o}C in moist CO_2 incubator for 7 days and examined under the inverted microscope to detect the characteristic (Fried egg colonies).

Differentiation of *Mycoplasma* and *Acholeplasma* isolates:

It was made by using digitonin sensitivity test **(Erno and Stipkovitis., 1973).**

Biochemical characterization: (Erno and Stipkovits., 1973)

It was carried out by glucose fermentation, arginine deamination tests and film and spot formation.

Identification of of mycoplasma isolates by using conventional PCR:

Procedure for DNA amplification of *Mycoplasma bovis* was carried out using 16S ribosomal RNA for ruminant *Mycoplasma* according to **Alberto *et al.*, (2006**). and *MB* primer (**Yleana *et al.*, 1995), Table (2)**

Table (2): Oligonucleotide primers for identification of *MB* (Segma).

Species	Designation	Sequence	According to
Sequence of 16S common gene for *Mycoplasma* spp.	MunivF MunivR	5′- AGA CTC CTA CGG GAG GCA GCA -3′ 5′- ACT AGC GAT TCC GAC TTC ATG -3′	**Alberto *et al.*, (2006)**
MB	MboF MboR	5′- CCT TTT AGA TTGGGATAGCGGATG-3′ 5′- CCGTCAAGGTAGCGTCAT TTCCTAC-3′	**Yleana *et al* ., (1995)**

Antimicrobial susceptibility test of different bacterial isolates:

Four or five typical colonies of similar morphological appearance were transferred to a tube containing 5 ml of Muller-Hinton broth and incubated at 37ºC for 8 hours until its turbidity exceeds that of the standard McFarland 0.5 barium sulphate tube. A sterile cotton swab was dipped into the standardized bacterial suspension. The dried surface of Muller-Hinton plates were streaked by the swab in 3 different planes. The plate lids were replaced and the inoculated plates were allowed to remain on a flat and level surface undistributed for 3 to 5 min (not more than 15 min. Then the disks (Tetracycline (TE 30µg), Ampicillin (AM 10 µg),

Neomycin (N30 µg), Erythromycin (E 10µg), Nalidixic acid (NA 30µg), , Chloramphenicol (C 30µg), Sulfa/trimethoprim (SXT 25µg), Cephalothin (KF 30µg), Amikacin (KA 30µg), Clindamycin (DA 2µg), Colistin sulfate (CT 2 µg), Gentamicin (CN 10 µg), Lincomycin (L 2µg, Ernofloxacin (Er 10µg), Kanamycin (KM), Ciprofloxacin (CPFX 5µg), Cefotaxime (CTX 30µg)) were applied with a fine pointed forceps on the inoculated plates and incubated in 37ºC for 24h. Then measure the sensitivity by measuring the clear zone of inhibition around the disks and the interpretation was applied according to **CLSI (2007)**

Results and Discussion

Mastitis is a serious disease in dairy animals causing great economic losses due to reduction in milk yield as well as lowering its nutritive value. Generally mastitis occurs in two forms i.e., clinical or overt and sub-clinical or hidden (**Radostitis *et al.*, 2000).** In addition to causing colossal economic losses to farmers, the disease is important from consumers and processors' point of view. The milk from affected animals may harbour the organisms potentially pathogenic for humans **(Barbano, 1989).** Mastitis affects the milk quality in terms of decrease in protein, fat, milk, sugar (lactose) contents and increase in somatic cell count. The processing of such milk results in substandard and sub-optimal output of finished fermented products like yoghurt, cheese etc. The shelf life of processed milk is also reduced (**Urech *et al.*, 1999).**

Of contagious pathogens of the udder, *S. aureus* and *S. agalactiae* predominate in all regions of the world, causing subclinical mastitis (**Benić *et al.* 2012**), despite intensive research efforts aimed to reduce the rate of the spread.

Out of 124 samples 131 isolates was detected, Table (3) and fig. (1) showed that the most frequently identified microbes isolated from 124 cows milk were as follows *S. aureus* 54 (43.5٪) followed by *S. agalacteae* 25 (20.2٪), *E.coli* 23 (18.5 ٪), *C. pyogenes* 16 (12.9 ٪), *E. faecalis* 10 (8.1 ٪) and *MB* 3 (2.4٪).

The obtained results presented in Table (4) and Fig. (2) showed the rate of different strains isolated from different types of milk samples,where 27 isolates where identified from 38 mastitic cows milk. *S. aureus* showed the highest rate 48٪ (n=13), followed by *S.agalacteae* 26٪ (n=7), *C. pyogene* 19٪ (n=5) and lowest persent *MB* 7٪ (n=2).

In Concerning the subclinical mastitis *S. aureus* showed the highest rate of isolation 38٪ (n=20), followed by *E.coli* 28% (n=15), *S.agalacteae* 19٪ (n=10), *C. pyogene* 13٪ (n=7) and lowest persent *MB* 2٪ (n=1)

While, In bulk milk, *E.coli* showed the highest rate 42%(n=8), followed by *S. aureus* 37٪ (n=7), *C. pyogene 2*1٪ (n=4) while *S.agalacteae* and *Myco. bovis* were not detected

In market milk, *S. aureus* showed the highest rate 44٪ (n=14), followed by *S.agalacteae* 25% (n=8) , *E.faecalis* 31٪ (n=10) while S.agalacteae, *E.coli* and *MB* were not detected.

These results nearly agree with **Mihaela (2010)** who found isolates from clinical mastitis cases accounted only 36.1% of all strains of microorganisms. From this cases the strains belonging to the genera *Staphylococcus* and *Streptococcus* were isolated with equal frequency, 34.6% and the highest percentage was represented by the staphylococcal strains (53.6%) from subclinical mastitis. Also, **Elhaig and Selim (2015)** studied the prevalence of subclinical mastitis (SCM) in smallholder dairy farms in Ismailia, Egypt. A total of 340 milking cows and buffaloes were sampled from 60 farms. Bacteriological analysis showed that the most frequently identified bacteria were *S.aureus* (38.3 %) and *S. agalactiae* (20 %). Subclinical mastitis due to *S. aureus* and *S.agalactiae* is endemic in smallholder dairy herds in Ismailia**.**

The rate of *C.pyogenes* in mastitic milk was relatively near the result obtained by **Charaya *et al.* (2014**) who isolated *C. pyogenes* 29 (7.88%) from mastitc milk The isolated strains of MB was confirmed by PCR, Many authors developed a simplified polymerase chain reaction (PCR) assay for fast and easy screening of Mycoplasma mastitis in dairy cattle as **Hirose *et al.* (2001**),**Yassin *et al.* (2004)**,**Ghadersohi *et al.* (2005), McDonald *et al.* (2009)** and **Hidetoshi *et al.* (2011).**

Two isolates were identified as *Mycoplasma bovis* from mastitic milk and one isolate from subclinical mastitic milk using PCR. **(Fig. 3)**

MB in the present stud from mastitis and subclinical mastitis cases by 7٪ and 2٪ respectively. *MB* in dairy cattle by using isolation and biochemical characterization has been reviewed by **EL-Morsy (2001) and Osman *et al.* (2008)** and **Hassan *et al.* (2011)** who reported *MB* in cattle with the incidences of 50%, 70.83%, 14.37%, 24%, 71.43%, 18.52% and (32%) respectively.

MB is widely found as a normal inhabtion bovine respiratory tract of apparently normal cows, transfer from the lungs to the mammary gland by hematogenous or other routes has been postulated (**Jasper, 1982**). Once an udder infection is established, rapid spread within a herd can occur by more routine methods for spreading mastitis. Hematogenous spread of *MB* was demonstrated when the organism was recovered from viable fetuses and calves of cows with mastitis (**Pfutzner and Schimmel, 1985**).

There is no treatment for cows that develop mycoplasma mastitis. Antibiotics are totally ineffective for this organism **(Jasper, 1979** and **Bushnell, 1984).** Cows that are infected with mycoplasma should always be considered as infectious, regardless of their production level, appearance of their milk or subsequent negative milk culture. In most cases, infected cows should be promptly culled. The only exception to this rule is when a culling is financially

unacceptable because a large proportion of a herd is infected. In this case a herd specific strict segregation plan should be developed. (**González, and Sears, 1994 and González, *et al.* 1995)**

In bulk milk, *E.coli* showed the highest rate 42%(n=8), followed by *S. aureus* 37% (n=7), *C. pyogene* 21% (n=4) while *S.agalacteae* and *MB* were not detected, but **Elias *et al.* (2012)** isolated *S.agalacteae* from bulk milk samples in a rate of 39.7%.

Culture of bulk-tank milk is easy, economical, and an important aid in monitoring bacterial counts in milk. However, this does not replace an individual cow culture. Bulk-tank cultures can be used to monitor the status within a herd. For example, in a herd with no history of contagious mastitis, a positive culture or series of cultures would warn the producer to examine individual cows **Petersson-Wolfe *et al.* (2010)**. However, microbiological identification of *S.aureus* in milk samples from bulk tanks is an auxiliary method to control contagious mastitis.

Also, the high proportion of *S. aureus* and S.*agalactiae* among the investigated samples concurs with that of previous studies (**Gianneechini *et al.* 2002; Mdegela *et al.* 2009; Amin *et al*. 2011).**

Katholm and Rattenborg (2009) found that 21 of 33 dairy farms screened positive for *S. agalactiae*, although control measures were managed in these farms. It was reported that the herd level prevalence of *S. agalactiae* increased steadily from 2000 to 2008 in Denmark. On the other hand, **Petersson-Wolfe *et al.* (2010)** reported *Staphylococcus aureus* causes one of the most common types of chronic mastitis. Though some cows may flare up with clinical mastitis (especially after calving) the infection is usually subclinical, causing elevated somatic cell counts (SCC) but no detectable changes in milk or the udder. The bacteria persist in mammary glands, teat canals, and teat lesions of infected cows and are contagious. The infection is spread at milking time when *S. aureus*-contaminated milk from an infected gland comes in contact with an uninfected gland, and the bacteria penetrate the teat canal.

It has been hypothesized that cows are infected with *Escherichia coli* from their environment, as feces and straw (**Lipman *et al.* 1995).** It is well known that bacterial, hosts and environmental factors are interdependent and influence susceptibility to mastitis.

In market milk, *S. aureus* showed the highest rate 44% (n=14), which seems to be similar to the findings of **Santana (2010) and Zakary (2011)**, When compare with present our findings higher level of incidence of *S. aureus* have been reported by **Thaker, *et al.* (2012)**. The high occurrence of *S. aureus* in market milk could be due to environmental contamination with infected animal wastes or unsanitary food production and storage practices. This could be also due to the use of unpasteurized milk because the shedding of bacteria from the infected mammary glands of dairy animals is most likely the primary source of *S.*

aureus contamination of milk and dairy products. While commercials products are produced with pasteurized milk under sanitary condition.

S.agalacteae 25% (n=8) was islated from market milk , *E.faecalis* 31% (n=10) while S.agalacteae, *E.coli* and *Myco. bovis* were not detected. **Sumathi *et al.* (2008)** where they tested 60 milk samples and found that 40% was Staphylococcus, 16 % Streptococcus, 20% *Escherichia coli.* Also **Gwida and EL-Gohary (2013)** recorded that out of 150 examined market milk (55 out 150) 36.66% and (85 out 150) 56.66% harboring *E. coli* and S. aureus respectively.

Lesley-Anne *et al.* (2004) reported that *Escherichia coli* remains a public health concern worldwide as an organism that causes diarrhea and its reservoir in raw milk may play an important role in the survival and transport of pathogenic strains.

S.aureus showed multidrug resistance ranged from 60%-100%, where 100% of isolates were resistance to tetracycline, amplicillin, cephalothin, amikacin, clindamycin and lincomycin.

S.agalactea showed multidrug resistance ranged from 60%-100%, where 100% of isolates were resistance to tetracycline, neomycin, sulfa/trimethoprim and clindamycin.

E.coli showed multidrug resistance ranged from 40%-100%, where 100% of isolates were resistance to sulfa/trimethoprim and lincomycin.

C.pyogenes showed multidrug resistance ranged from 60.9%-100%, where 100% of isolates were resistance to showed multidrug resistance, where 100% of isolates were resistance to tetracycline, amplicillin, neomycin, sulfa/trimethoprim, amikacin and gentamicin.

E.faecalis showed multidrug resistance ranged from 20% -100%, where 100% of isolates were resistance to showed multidrug resistance, where 100% of isolates were resistance to Gentamicin and Lincomycin. **(Table 5)**

In the present study, multidrug resistance of different isolates was observed which revealed the misused of antimicrobial agents among different farms.

S. aureus strains are known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier and because of their location within microabscesses, which limit the action of drugs (**Gündogan *et al.*, 2006**).

In Brazil, **Langoni *et al.* (2000)** reported a discrete level of resistance to tetracycline (13.0%) and ampicillin (12.0%) among *E. coli* isolates from bovine mastitis, while **Amaral *et al.* (1996**) also reported high levels of resistance to ampicillin.

The present study indicated considerable prevalence of the disease and pathogens from clinical mastitis in Fayoum governorate. Appropriate treatment and control strategies should be formulated to eradicate or reduce major

pathogens *S. aureus*, *S. agalactiae* and *E. coli*. where a practical mastitis control strategy in the herd and national approach is needed.

The control of mastitis in any herd in which mastitis has become a problem is best attained by adopting a control program that includes an accurate diagnosis, adequate sanitary and management practices, proper treatment, and close cooperation between the dairy man and veterinarian.

Results clearly suggested a possibility of potential public health threat of different isolates specially *S.aureus* and *E.coli* resulting from contamination of milk with pathogenic bacteria is mainly due to unhygienic processing, handling and unhygienic environment.

Negligence of hygienic condition such as improper cleaning of bulk tank, dirty udder, milking equipments, milk handling technique and improper storage will increase the proportion of Gram-positive and Gram- negative bacteria in the bulk tank milk.

Mycoplasma-infected cows must be segregated and milked last or with a separate milking unit from those used on uninfected cows to minimize the risk of infection for other cows.

Antibiotic resistance development among the bacteria posses a problem of concern. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

A strong control of antimicrobial drugs commercialization and access to data related to resistance to antimicrobial drugs presented by the pathogens responsible for bovine mastitis would first be necessary before a conclusive answer about this matter is given.

The results of the present study clearly indicated that microbial quality and safety of raw milk was unsatisfactory. The presences of fecal indicator organisms not only indicate poor hygiene but also itself may be pathogenic.

The pathogenic bacteria such as *S. aureus* and *E. coli* may pass to the milk; this suggests that raw milk should be considered as a vehicle for the transmission of potentially pathogenic bacteria. Since a lot of people still drink raw milk, especially in rural areas, this emphasis's the need for educational efforts to improve dairy farmers' awareness of milk borne zoonoses, how these pathogens transmitted to milk, risk factors associated with milk borne pathogens and how to obtain fresh clean milk. It is of utmost importance to examine the stool specimens of apparently healthy dairy handlers (non diarrhoeic stool samples) to clarify their role in shedding bacterial pathogenic agents.

Egypt. J. Chem. Environ. Health, 2 (2):516-533 (2016)
On line ISSN: 2536-9164.

Table (3): Rate of different bacteria among all milk samples

Type of isolates	Total No. of milk samples	No. of isolates	**%**
S.aureus	124	54	43.5
S.agalacteae		25	20.2
E.coli		23	18.5
C.pyogenes		16	12.9
E.faecalis		10	8.1
Mycoplasma		3	2.4
Total		131	105.6
Negative samples		12	9.7

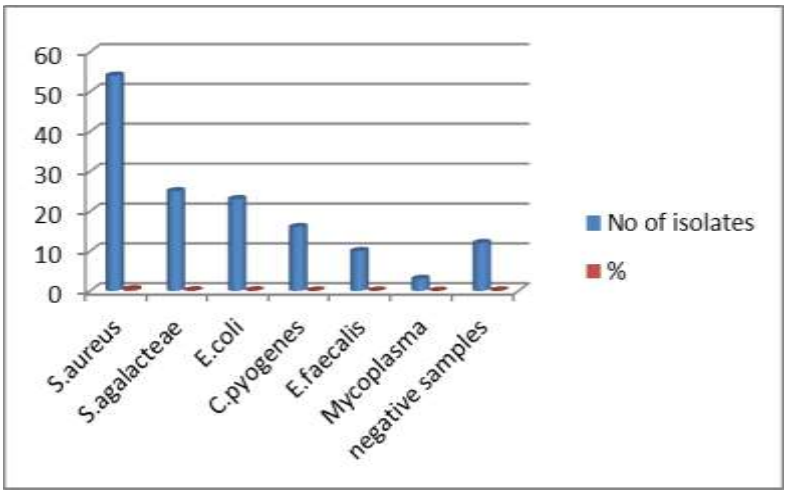

Fig. (1): Rate of different isolates among 124 milk samples

Egypt. J. Chem. Environ. Health, 2 (2):516-533 (2016)
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Table (4): Types and rate of bacterial strain isolated from milk samples.

Type of milk	Total No. of samples	Total No. of isolates	Bacterial isolates		
			Type of bacteria	N0.positive	%
Clinical Mastitic milk	38	27	*S.aureus*	13	48.1
			S. agalacteae	7	26
			C.pyogenes	5	19
			Myco. bovis	2	7
Subclinical mastitic milk	46	53	*S.aureus*	20	38
			E.coli	15	28
			S.agalacteae	10	19
			C.pyogenes	7	13
			Myco. bovis	1	2
Bulk milk	10	19	*S.aureus*	7	37
			E.coli	8	42
			C.pyogenes	4	21
Market milk	30	32	*S.aureus*	14	44
			S.agalacteae	8	25
			E.faecalis	10	31
Total	124	131	Total	131	-

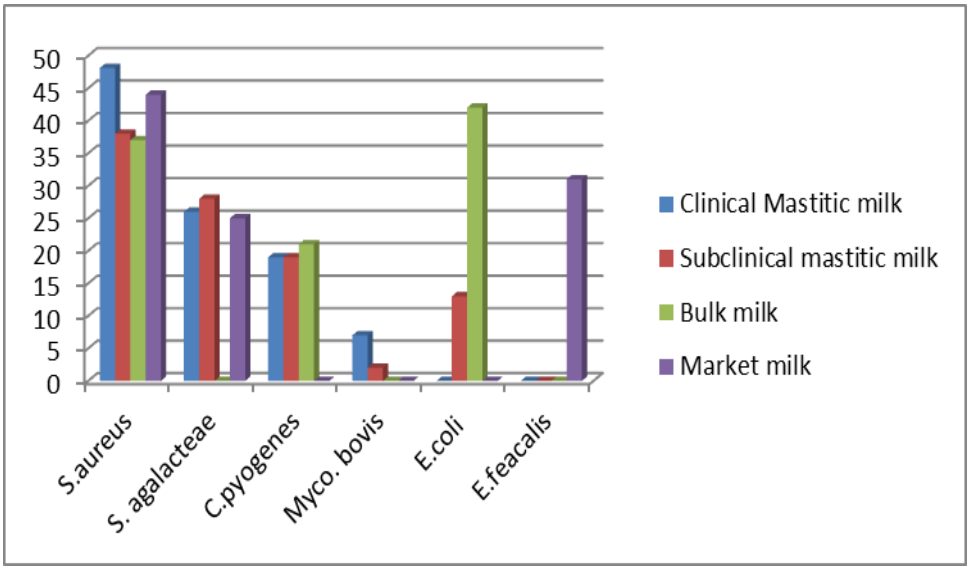

Fig. (2): Rate of different isolates indifferent types of samples among the total number of isolates

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Agaros gel electropherasis of MB isolated from mastitic milk and subclinical mastitis cow's milk

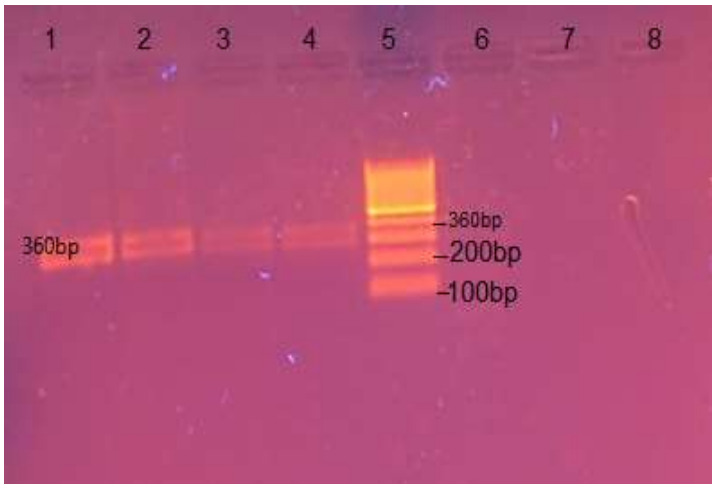

Fig. (3): lane 1: control positive *Myco. Bovis* Lanes 2-4: positve samples for *Myco. Bovis* andLane 5: 100bp DNA marker lane 6:control negative. Lane7,8 negative samples.

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Table (5): Antimicrobial susceptibility of different bacterial isolates against different antimicrobial agents

Antibiotic disc	Bacterial isolates									
	***S.aureus* (20) N0. & (%)**		***S.agalacteae* (20)**		***E.coli* (15)**		***C.pyogenes* (16)**		***E faecalis* (10)**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Tetracycline (TE30µg)	20 **(100)**	0	20 **(100)**	0	13 (86.7)	2 **(13.3)**	16 (100)	0	7 (70)	3 (30)
Amplicillin (AM 10 µg)	20 **(100)**	0	19 **(95)**	1 **(5%)**	12 **(80)**	3 **(20)**	16 (100)	0	8 (80)	2
Neomycin (N 30 µg)	19 **(95)**	1 **(5)**	20 **(100)**	0	12 **(80)**	3 **(20)**	16 **(100)**	0	7 (70)	3 (30)
Erythromycin (E 10µg)	17 **(85)**	3 **(15)**	18 **(90)**	2 **(10)**	10 **(66.7)**	5 **(33.3)**	15 **(93.8)**	1	6 (60)	4 (40)
Sulfa/trimethoprim SXT 25µg	19 **(95%)**	1 **(5)**	20 **(100)**	0	15 **(100)**	0	16 **(100)**	0	9 (90)	1(10)
Cephalothin KF 30µg	16 **(80)**	4 **(20)**	14 **(70)**	6 **(30)**	13 **(86.7)**	2 **(13.3)**	14 **(87.5)**	2 (12.5)	8 (80)	2 (20)
Amikacin KA 30µg	20 **(100)**	0	19 **(95)**	1 **(5)**	14 **(93.3)**	1 **(6.7)**	16 **(100)**	0	10 **(100)**	0
Clindamycin DA 2µg	20 **(100)**	0	20 **(100)**	0	13 **(86.7)**	2 **(13.3)**	14 (87.5	2 12.5)	10 **(100)**	0
Gentamicin CN 10 µg)	17 **(85)**	3 **(15)**	12 **(60)**	8 **(40)**	12 **(80)**	3 **(20)**	16 **(100)**	0	10 **(100)**	0
Lincomycin L 2µg	20 **(100)**	0	19 **(95)**	1**(5)**	15 **(100)**	0	14 **(87.5)**	2	10 **(100)**	0
Ernofloxacin (Er 10µg)	13 **(65)**	7 **(35)**	10 **(50)**	10 **(50)**	6 **(40)**	9 **(60)**	12 (60.9)	4 (39.1)	3 (30)	7 (70)
Ciprofloxacin (CPFX)	12 **(60)**	8 **(40)**	9 **(45)**	11 **(55)**	10 **(66.7)**	5 **(33.3)**	15 (81.3)	1 (18.7)	2 (20)	8 (80)
Cefotaxime (CTX)	15 **(75)**	5 **(25)**	10 **(50)**	10 **(50)**	11 **(77.3)**	4 **(22.7)**	14 (87.5)	2 (12.5)	5 (50)	5 (50)

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