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**PUBLIC HEALTH HAZARD ASSOCIATED WITH
CONSUMPTION OF MILK FROM CATTLE
INFECTED WITH SUBCLINICAL MASTITIS IN
ASSIUT GOVERNORATE**
(With 6 Tables and 4 Figures)

By

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**المخاطر الصحية على الإنسان المرتبطة بتناول ألبان الماشية المصابة
بالتهاب الضرع الغير ظاهري بمحافظة أسيوط**

توفيق البسيوني ، ايناس البرنس ، آمال على عبد الحليم ، أنسى صادق

يعتبر التهاب الضرع تحت الأكلينيكي من الأمراض المؤثرة اقتصاديا وصحيا لما يسببه من نقص في إنتاج اللبن بالإضافة إلى نقل بعض الميكروبات الخطيرة للإنسان. نظرا لأهمية هذا المرض فقد تم تجميع عدد 787 عينة من ألبان الأبقار والجاموس، وتم فحصها بكتريولوجيا وميكولوجيا لعزل وتصنيف المسبب. بفحص 379 عينة من لبن الأبقار باختبارات CMT و MWST، كانت النتائج ايجابية بنسبة 29.55 و 30.87%. وبفحص 408 عينة من لبن الجاموس كانت النتائج ايجابية بنسبة 11.76 و 12.01% لكلا الاختبارين على الترتيب. وقد دل الفحص البكتريولوجي على أن النتائج كانت ايجابية بنسب 28.50 و 10.54% لكل من الأبقار والجاموس على الترتيب. أما في حالة نتائج الفحص الميكولوجي فقد كانت ايجابية بنسب 16.09 و 0.25% على الترتيب. كما تبين أن نسبة عزل البكتريا المسببة لإلتهاب الضرع تحت الأكلينيكي في الأبقار هي: 13.19، 4.49، 1.32، 2.64، 9.50، 15.57، 1.32، 1.58 و 0.26% لكل من المكور العنقودي الذهبي، المكور العنقودي السالب التلجط، المكور السبحي بيوجين، المكور السبحي اجالاكتيا، المكور السبحي ديس جالاكتيا ، الميكروب القولوني كلبسيلا نوموني، ستروباكترا داي فيرساس وبروتياس ميرابيليز. بينما في الجاموس كانت النسب كالتالي: 8.33، 1.96، 0.25، 0.25 و 1.72% لكل من المكور العنقودي الذهبي، المكور العنقودي السالب التلجط ، المكور السبحي بيوجين ، المكور السبحي ديس جالاكتيا والكورائني باكتريام بوفس على الترتيب. أما الخمائر والفطريات فكانت نسب عزلها في الأبقار كالتالي: 4.75، 1.58، 2.38، 2.90، 1.32، 1.06، 0.53، 0.79، 0.26، 0.26، 0.26، 0.26، 0.26، 0.26، 0.53، 0.26، 0.26

Candida albicans ، Candida tropicalis ، Candida krusei ، Candida sp. ، Geotrichum candidum ، Aspergillus niger ، Cladosporium

cladosporioides ، *Fusarium proliferatum* ، *Penicillium duclauxi* ، *Rhodotorula sp.*، *Stachybotrys elegans* ، near to *Pyssochlamys nivea* ، *Alternaria alternate* ، *Stemphylium botryosum* ، *Thermoascus aurantiacus* ، *Trichosporon cuteanum* and sterile mycelium على الترتيب .

بينما تم عزل *Phialophora sp.* من الجاموس بنسبه 0.25%. هذا وقد تمت مناقشة الأهمية الصحية والوبائية والاقتصادية لمرض التهاب الضرع تحت الاكلينيكي في الإنسان والحيوان والشروط الواجب إتباعها لمنع انتشاره واختباره في مزارع الألبان المختلفة لدرء خطره على الإنسان.

SUMMARY

Seven hundred and eighty seven random milk samples were collected from cows and buffaloes at different localities and farms in Assiut Governorate. These samples represented by 379 and 408 quarter milk samples from 105 cows and 105 buffaloes, respectively. Animal-wise incidence of Subclinical Mastitis (SCM) based on California Mastitis Test (CMT) and Modified Whiteside Test (MWST) were 59.05 & 60.95% positive in cows and 33.33% & 33.33% positive in buffaloes, respectively. The animal-wise incidences of SCM in cows & buffaloes by bacteriological and mycological examinations were 60.95 & 28.57% and 34.29 & 0.95% positive, respectively. The most common bacteria causing SCM in cows were *Staph. aureus*, coagulase negative staphylococci (CNS), *Str. pyogenes*, *Str. agalactiae*, *Str. dysgalactiae*, *E. coli*, *Klebsiella pneumoniae*, *Citrobacter diversus* and *Proteus mirabilis*, respectively. While, in buffaloes were *Staph. aureus*, CNS, *Str. pyogenes*, *Str. dysgalactiae* and *Corynebacterium bovis*. Additionally, the most common yeasts and molds causing SCM in cows were *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida sp.*, *Geotrichum candidum*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium proliferatum*, *Penicillium duclauxi*, *Rhodotorula sp.*, *Stachybotrys elegans*, near to *Pyssochlamys nivea*, *Alternaria alternata*, *Stemphylium botryosum*, *Thermoascus aurantiacus*, *Trichosporon cuteanum* and sterile mycelium. However, *Phialophora sp.* was only isolated from buffaloes in a percentage of 0.25%.

Key words: Subclinical mastitis; cow's, buffalo's milk; public health hazards

INTRODUCTION

Mastitis is an inflammation of udder, usually as a result of microbial infection by invading bacteria or other microorganisms including fungi and possibly viruses. Mastitis can generally be characterized as clinical, subclinical and chronic disease (Watts, 1990). The economic impact of both clinical and subclinical forms of mastitis is large in the current dairy industry. Furthermore, in dairy industry, SCM remains an important cause of reduced milk production and poor quality value products such as cheese or casein (Roux *et al.*, 1995). Losses occur from decreased milk production, treatment and labour costs, non deliverable milk and veterinary fees, reduced milk quality, reduced milk price, increased risk of subsequent mastitis and increased risk of culling or death of the animal (Harmon, 1994). Diagnosis of acute mastitis presents little difficulty as compared to subclinical by ordinary clinical tests and as a result, SCM is responsible for great losses to the dairy industry therefore, more attention has been given for the diagnosis of SCM by indirect tests (Joshi *et al.*, 1976). It is the most serious form as both infected udder and milk show no obvious clinical abnormalities, whereas, several causative organisms are discharged with the milk for long time. Regarding public health importance, mastitis is considered of quite vital importance due to its association with many zoonotic diseases in which milk act as a vehicle of infection. Of the various conditions of udder, the SCM is attracting the eye world over as this condition not only leads to suboptimal milk production but it also results in transmission of certain diseases to human beings too (Tijare *et al.*, 2000). As a results of the economic and public health importance of SCM, the objective of this work was done to detect SCM in cows and buffaloes by using screening or field tests, bacteriological examination as well as mycological examination.

MATERIALS and METHODS

Milk samples:

A total number of 787 milk samples were collected from apparently healthy cows and buffaloes at different farms in Assiut Governorate, comprising 379 quarter milk samples from 105 lactating cows and 408 quarters of milk samples from 105 lactating buffaloes.

I-Screening tests to detect SCM:

A- CMT according to Schalm *et al.* (1971).

B- MWST according to Murphy and Hanson (1941).

II- Microbiological examination of subclinical mastitic milk samples:

A- Isolation of members of Family *Enterobacteriaceae* according to Cruickshank *et al.* (1975); Finegold and Martin (1982); and Koneman *et al.* (1992).

B- Isolation of *Staph. aureus* (Bailey and Scott, 1994) .

C- Isolation and identification of *Streptococci* (Cruickshank *et al.*, 1975 and Quinn *et al.*, 1994).

D- Isolation and identification of yeasts and molds

Fungal cultures were kindly identified by staff members of the Assiut University Mycological Centre (AUMC), Assiut, Egypt, using the following references: Kwon-Chung and Bennett (1992), Moubasher (1993), Odds and Bernaerts (1994) and Hoog *et al.* (2000)

RESULTS

Table 1: Quarter-wise incidence of SCM in cow's and buffalo's milk samples based on the results of CMT and MWST.

Type of animal	No. of quarters	CMT				MWST			
		Positive		negative		positive		negative	
		No.	%	No.	%	No.	%	No.	%
Cows	379	112	29.55	267	70.45	117	30.87	262	69.13
Buffalos	408	48	11.76	360	88.24	49	12.01	359	87.99

Table 2: Animal-wise incidence of SCM in cow's and buffalo's milk samples based on the results of CMT and MWST.

Type of animal	No. of animals	CMT				MWST			
		Positive		negative		positive		negative	
		No.	%	No.	%	No.	%	No.	%
Cows	105	62	59.05	43	40.95	64	60.95	41	39.05
Buffalos	105	35	33.33	70	66.67	35	33.33	70	66.67

Table 3: Quarter-wise incidence of SCM in cow's and buffalo's milk samples based on the results of bacteriological and mycological examination.

Type of animal	No. of quarters	Bacteriologically				Mycologically			
		Positive		negative		positive		negative	
		No.	%	No.	%	No.	%	No.	%
Cows	379	108	28.50	271	71.50	61	16.09	318	83.91
Buffalos	408	43	10.54	365	89.46	1	0.25	407	99.75

Table 4: Animal-wise incidence of SCM in cow's and buffalo's milk samples based on the results of bacteriological and mycological examination.

Type of animal	No. of animal	Bacteriologically				Mycologically			
		Positive		negative		positive		negative	
		No.	%	No.	%	No.	%	No.	%
Cows	105	64	60.95	41	39.05	36	34.29	69	65.71
Buffalos	105	30	28.57	75	71.43	1	0.95	104	99.05

Table 5: Incidence of the isolated bacteria causing SCM in the examined cow's and buffalo's milk samples.

Isolated species	Cows		Buffaloes	
	No./379	%	No./408	%
<i>Staph. aureus</i>	59	15.57	34	8.33
CNS	36	9.50	8	1.96
<i>Strept. pyogenes</i>	10	2.64	1	0.25
<i>Strept. agalactiae</i>	5	1.32	0	0.00
<i>Strept. dysgalactiae</i>	17	4.49	1	0.25
<i>E. coli</i>	50	13.19	0	0.00
<i>Klebsiella pneumoniae</i>	5	1.32	0	0.00
<i>Citrobacter diversus</i>	6	1.58	0	0.00
<i>Proteus mirabilis</i>	1	0.26	0	0.00
<i>Corynebacterium bovis</i>	0	0.00	7	1.72
Total	189	49.87	51	12.50

Table 6: Incidence of the isolated yeasts and molds causing SCM in cow's and buffalo's milk samples.

Isolated fungi	Cows		Buffaloes	
	No./379	%	No./408	%
Yeasts				
<i>Candida albicans</i> (Robin) Berkhout	18	4.75	0	0.00
<i>Candida tropicalis</i> (Castellani) Berkhout	6	1.58	0	0.00
<i>Candida krusei</i> (Castellani) Berkhout	9	2.38	0	0.00
<i>Candida sp.</i>	11	2.90	0	0.00
<i>Geotrichum candidum</i> Link	5	1.32	0	0.00
Molds				
<i>Aspergillus niger</i> van Tieghem	4	1.06	0	0.00
<i>Cladosporium cladosporioides</i> (Fresenius) de Vries	2	0.53	0	0.00
<i>Fusarium proliferatum</i> (Matsushima) Nirenberg	3	0.79	0	0.00
<i>Penicillium duclauxi</i> Delacroix	1	0.26	0	0.00
<i>Rhodotorula sp.</i>	1	0.26	0	0.00
<i>Stachybotrys elegans</i> (Pidopl.) Gams	2	0.53	0	0.00
near to <i>Pyssochlamys nivea</i>	1	0.26	0	0.00
<i>Alternaria alternata</i> (Fries) Keissier	1	0.26	0	0.00
<i>Stemphylium botryosum</i> Wallroth	1	0.26	0	0.00
<i>Thermoascus aurantiacus</i> Miehe	1	0.26	0	0.00
<i>Trichosporon cutedanum</i> (de Beurm., Goug. & Vauch.) Ota	1	0.26	0	0.00
Sterile mycelium	0	0.00	1	0.25
<i>Phialophora sp.</i>				
Total	68	17.94	1	0.25

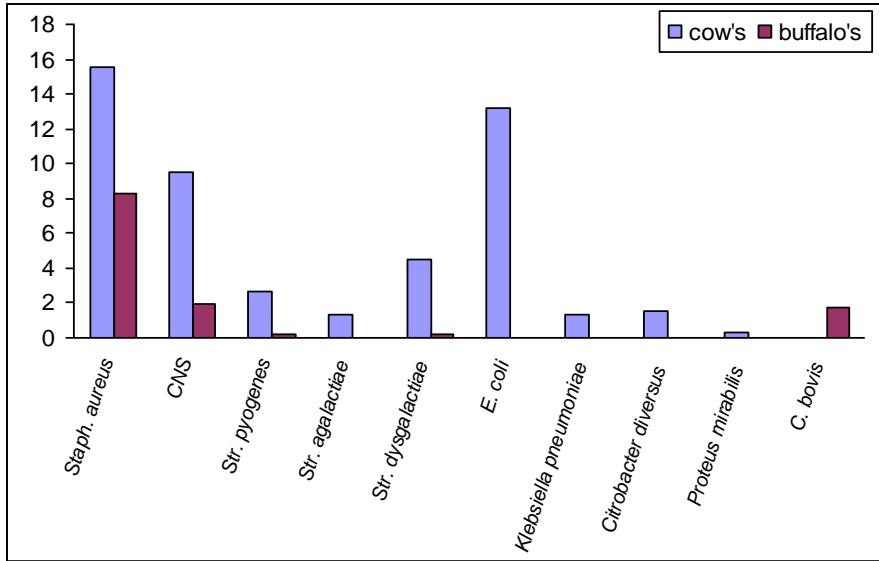


Fig. 1: Incidence of the isolated bacteria causing SCM in the examined cow's and buffalo's milk samples.

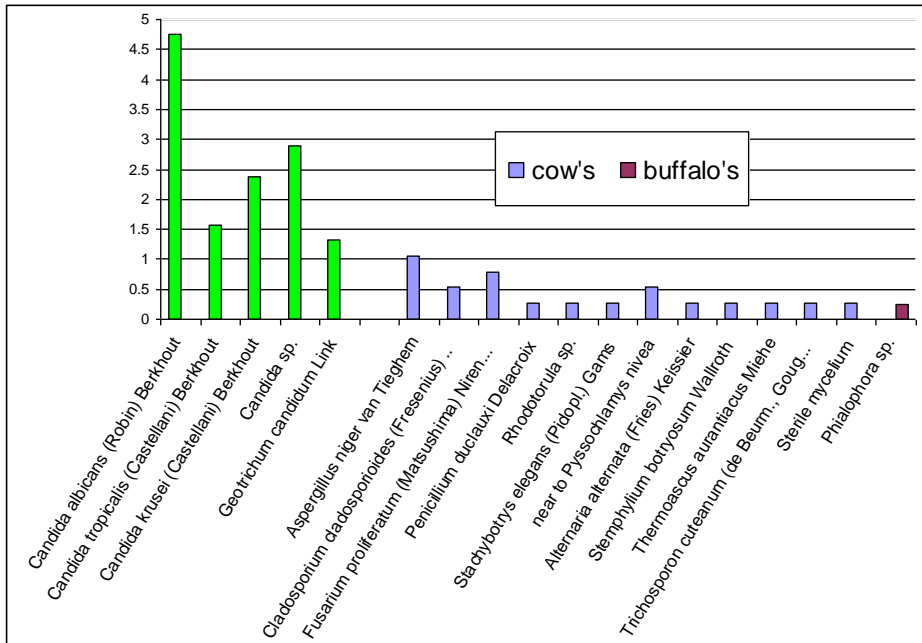


Fig. 2: Incidence of the isolated yeasts and molds causing SCM in cow's and buffalo's milk samples.

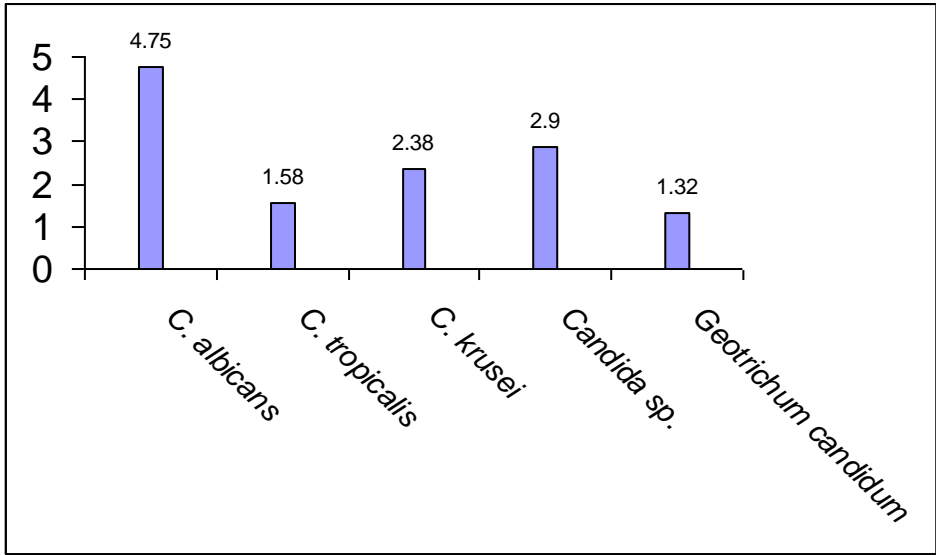


Fig. 3: Incidence of the isolated yeasts causing subclinical mastitis in cow's milk samples

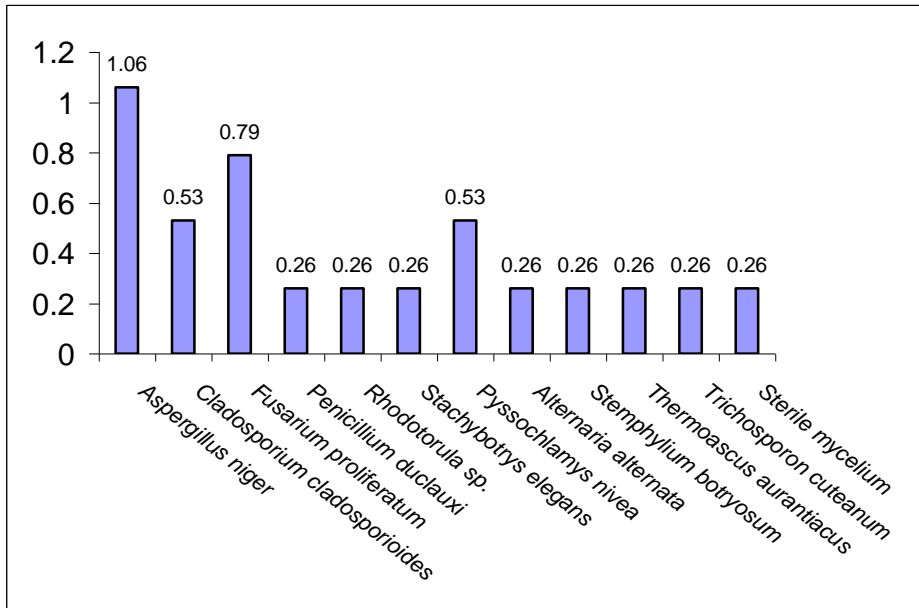


Fig. 4: Incidence of the isolated molds causing subclinical mastitis in cow's milk samples

DISCUSSION

Data presented in Table 1, showed the quarter-wise incidence of SCM in cow's and buffalo's milk samples based on the results of both CMT and MWST. Out of 379 quarters cow's milk, 112 (29.55%) samples were positive for CMT. Somewhat similar results were recorded by Hatem *et al.*, (1984) (28.28%). Lower findings were estimated by Singh *et al.*, (1982) (12.33%) and Saini *et al.*, (1994) (4.87%), however, higher incidence was detected by Mohamed *et al.*, (1993) (46%). Out of 408 quarters buffalo's milk examined by CMT, 48 (11.76%) were positive (Table 1). Nearly similar results were recorded by Singh *et al.*, (1982) (9.54%) and Naiknaware *et al.*, (1998) (9.87%). On the contrary, a lower level was recorded by Saini *et al.*, (1994) (2.59%), however, relatively higher results were reported by El-Balkemy *et al.*, (1997) (26.34%) and Salama, (2004) (43.13%).

Regarding quarter-wise incidence of SCM in cow's and buffalo's milk samples based on the result of MWST, 117 samples (30.87%) of cow's milk were positive to MWST. This incidence was in accordance with that estimated by Tijare *et al.* (1999) (28.69%). Singh *et al.* (1982) and El-Balkemy *et al.* (1997) recorded lower findings of 10.73 and 17.47%, respectively. In contrast, higher result was incriminated by Wahba *et al.* (2005) (93.8%). Concerning buffalo's quarter milk, 49 samples (12.01%) were positive to MWST (Table 1). Lower result was indicated by Singh *et al.* (1982) (7.14%), however, El-Balkemy *et al.* (1997) detected relatively higher incidence of 30.10%.

Table 2, estimated the animal-wise incidence of SCM in cow's and buffalo's milk samples based on the results of both CMT and MWST. Out of 105 cows examined, 62 animals (59.05%) gave positive result, which in harmony with that estimated by Tijare *et al.* (1999) (57.98%), while, lower percentages were recorded by Saini *et al.* (1994) (17.33%), Singh *et al.* (1994) (49.29%), El-Balkemy *et al.* (1997) (31.98%), and Mukherjee and Dash (2003) (52.32%). However, higher finding was recorded by Prasad *et al.* (2001) (61.32%). Concerning buffaloes, 35 animals (33.33%) were positive to CMT which go parallel with that estimated by Farah and Kaldes (1999) (36.6%). Saini *et al.* (1994) and Naiknaware *et al.* (1998) recorded lower incidences (9.59 and 28.63%, respectively), however, El-Balkemy *et al.* (1997) showed higher percentages of 42.55%.

The animal-wise incidences based on the results of MWST, showed that out of 105 cows, 64 animals (60.95%) were positive (Table 2). Lower results were estimated by El-Balkemy *et al.* (1997) (34.26%), however somewhat higher result was detected by Tijare *et al.* (1999) (70.59%). In case of buffalo's milk samples, 35 animals (33.33%) were positive. Higher incidences were estimated by El-Balkemy *et al.* (1997) (48.93%), and Farah and Kaldes (1999) (63.3%).

The quarter-wise incidence of SCM in cow's and buffalo's milk samples based on the results of bacteriological and mycological examination was illustrated in Table 3. Out of 379 quarters cows milk samples examined, 108 (28.50%) were positive. The results were in agreement with that evaluated by Pardo *et al.* (1998) (26.5%). In the contrary, Saini *et al.* (1994) recorded relatively higher incidence of 76.13%. Moreover, in case of buffalo's milk samples, 43 (10.54%) were positive and 365 (89.46%) were negative. Higher percentages were estimated by Tijare *et al.* (1999) (26.77%). Regarding the results of mycological examination, 61 (16.09%) and one (0.25%) milk samples of cow's and buffalo's were positive, respectively (Table 3).

The obtained results in Table 4 revealed that, the animal-wise incidence of SCM in cows was 60.95% bacteriologically positive. Extremely lower results were stated by Singh *et al.* (1982) (18.4%) and Petrović *et al.* (1997) (51.0%), whereas, Abdel-Ghani (2005) recorded higher result (67.5%). In buffalo's milk, 30 (28.57%) samples were positive. Singh *et al.* (1982) revealed a lower incidence of 13.2%, while, Tijare *et al.* (1999) estimated extremely higher incidence of 67.22%. Furthermore, mycologically 34.29 and 0.95% were positive for cows and buffaloes, respectively.

The incidences of isolated bacteria causing SCM in the examined cow's milk samples were showed in Table 5 and Fig. 1. 59 isolates (15.57%) of *Staph. aureus* were recovered from the examined samples. This result was in accordance with that obtained by Kotb (2006) (14%). Lower results were obtained by Petrović *et al.* (1997) (4.69%), Pardo *et al.* (1998) (8.52%), Wahba *et al.* (2005) (10%) and Abdel-Hameed (2006) (9.28%). However, higher incidences were recorded by Singh *et al.* (1994) (35.38%), Mokhbatly *et al.* (2001) (40.90%), Al-Hawary *et al.* (2003) (29.2%) and Abdel-Ghani (2005) (25.6%). Also, 36 isolates (9.50%) of CNS were obtained which relatively similar to the result recorded by Singh *et al.* (1994) (10%) however, lower incidence (5.4%) was stated by Abdel-Hameed (2006). Extremely higher percentages were obtained by Pardo *et al.* (1998) (64.2%), Abdel-Ghani (2005) (34.1%)

and Wahba *et al.* (2005) (66%). In addition, 10 isolates (2.64%) of *Strept. pyogenes* were described in Table 5 and Fig. 1. Five isolates (1.32%) of *Strept. agalactiae* were also isolated which in accordance with that estimated by Al-Hawary *et al.* (2003) (1.5%), while, lower results were reported by Petrović *et al.* (1997) (0.34%) and Abdel-Hameed (2006) (0.7%). On the other hand, Singh *et al.* (1994) and Mokhbatly *et al.* (2001) recorded higher incidences of 10 and 11.36%, respectively. Concerning *Strept. dysgalactiae*, 17 isolates (4.49%) were recovered from the examined samples which were in harmony with that estimated by Mokhbatly *et al.* (2001) (4.55%). Singh *et al.* (1994) and Abdel-Hameed (2006) stated slightly lower incidences of 3.08 and 3.88%, respectively. Moreover, 50 isolates (13.19%) of *E. coli* were recorded and this result was nearly similar to that postulated by Abdel-Ghani (2005) (12.8%). In contrary, lower results were incriminated by Singh *et al.* (1994) (5.38%) and Al-Hawary *et al.* (2003) (6.2%). While, extremely lower incidences were obtained by Pardo *et al.* (1998) (2.84%), and Abdel-Hameed (2006) (1.66%). However, Mokhbatly *et al.* (2001) and Kotb (2006) stated higher incidences of 18.20 and 15.0%, respectively. With regard to *Klebsiella pneumoniae* 5 isolates (1.32%) were obtained which was somewhat lower than that estimated by Mokhbatly *et al.* (2001) (2.27%) and Kotb (2006) (3%). Six isolates (1.58%) of *Citrobacter diversus* were obtained, while, only one isolate (0.26%) of *Proteus mirabilis* was recovered from tested samples and Abdel-Ghani (2005) reported a higher incidence of 0.9%.

In case of buffalo's milk, it is clear that, 34 isolates (8.33%) of *Staph aureus* were recovered from the examined samples. Lower results were estimated by Tijare *et al.* (1999) (2.52%), however, higher incidences with a great extent were stated by El-Balkemy *et al.* (1997) (35.41%) and Mokhbatly *et al.* (2001) (26.67%). Also, 8 isolates (1.96) of CNS were obtained which is extremely lower than that stated by Tijare *et al.* (1999) (36.48%). In addition, one isolate (0.25%) of each *Strept. pyogenes* and *Strept. dysgalactiae* was isolated. El-Balkemy *et al.* (1997) (2.08%) and Mokhbatly *et al.* (2001) (4.44%) recorded higher incidences of a great extent. Concerning *Corynebacterium bovis*, 7 isolates (1.72%) were recovered which is lower than the results stated by Tijare *et al.* (1999) (6.29%) and Mokhbatly *et al.* (2001) (2.22%).

It is evident that, *Staph. aureus* was the highest isolated microorganism from both cow's and buffalo's milk. This may be due to its ubiquitous nature and due to indiscriminate using of antibiotics resulting in the emergence of resistant strains (Singh *et al.*, 1994). Also,

skin of milkers hand may play a role in its transmission from teat to teat and from animal to animal. Moreover, it is evident that, *Strept. agalactiae* couldn't be isolated from buffalo's milk.

The incidences of the isolated yeasts and molds causing SCM in cow's milk samples were shown in Table 6 and Fig. 3 & 4. 18 isolates (4.75%) of *Candida albicans* were recovered from the examined samples, Ahmed (1981) stated higher incidence of 60%. Six strains (1.58%) of *Candida tropicalis* were isolated and this finding is lower with a great extent than that recorded by Ahmed (1981) (22.5%) in Friesian cattle. Also, 9 isolates (2.38%) of *Candida krusei* were obtained which is lower than that estimated by Ahmed (1981) (5%). In addition, 11 isolates (2.90%) of other *Candida sp.* were recovered from the examined samples. With regard to *Geotrichum candidum*, 5 isolates (1.32%) were obtained which is lower than that reported by Ahmed (1981) (5%). Concerning molds, 4 isolates (1.06%) of *Aspergillus niger* were isolated. Higher results were estimated by Ahmed (1981) (6.67%) and Abdel-Ghani (2005) (5.9%). Also, *Cladosporium cladosporioides*, *Fusarium proliferatum*, *Penicillium duclauxi*, *Rhodotorula sp.*, *Stachybotrys elegans*, near to *Pyssochlamys nivea*, *Alternaria alternata*, *Stemphylium botryosum*, *Thermoascus aurantiacus*, *Trichosporon cutaneum* and sterile mycelium were isolated in percentages of 0.53, 0.79, 0.26, 0.26, 0.53, 0.26, 0.26, 0.26, 0.26 and 0.26%, respectively.

Only one isolate (0.25%) of *Phialophora sp.* was isolated from buffalo's milk samples (Table 6). It is clear that, no yeasts or other molds could be isolated from buffalo's milk and this could be attributed to the structure of buffalo's teat canal or may be due to the genetic character of buffaloes which render it more resistant to mycotic infection or other factors which need further investigations.

Under the condition of this investigation, it can be concluded that, CMT and MWST were highly sensitive for detection of SCM and their application lead to earlier detection of infected animal and its isolation either for culling or therapy. Buffaloes were less susceptible to SCM as compared to cow. *Staphylococci* either *Staph. aureus* or *CNS* were the most causative agents causing SCM followed by *E. coli*. In cows, yeasts were higher than molds for causing SCM however, buffaloes somewhat resist to mycotic SCM.

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