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BACTERIOLOGICAL STUDIES ON SUB-CLINICAL MASTITIS IN COWS AND BUFFALOES WITH TRAILS FOR ITS TREATMENT

(With 5 Tables)

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(Received at 15/3/2011)

دراسات بكتريولوجية علي إلتهاب الضرع الخفي في الأبقار والجاموس مع محاولات لعلاجه

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أجريت هذه الدراسة لمعرفة البكتريا المسببة لإلتهاب الضرع الخفي في الأبقار البلدية والجاموس، حيث تم فحص ٩٤ بقرة بلدية و٨٠ جاموسة. كانت جميعها ملكية خاصة وفي مجموعات صغيرة وتحلب يدوياً. أسفرت نتائج الفحص باستخدام اختباري الكاليفورنيا ماستيتس(CMT) والويتسيد المعدل (MWST) عن ايجابية الفحص لعدد ٢٦(٢٧,٧٤%) و٣٠(٣١,٩%) من الأبقار البلدية وعن عدد ١٣(١٦,٣%) و١٤(١٧,٥%) من الجاموس لكلا الاختبارين علي الترتيب. وقد خضعت هذه العينات الإيجابية للاختبارات الحقلية للفحص البكتريولوجى وتم زرعها على المستنبتات البكترية العامة والخاصة لزيادة فرص العزل وتم عزل ٥٨ عترة من الأبقار البلدية و٢٧ عترة من الجاموس تمثل مجموعتين من المسببات البكترية كانت علي النحو التالي البكتريا الوبائية ١٤عترة (٢٤,١٤%) و١١عترة(٤٠,٧٤%) وبكتريا التلوث البيئي ٤٤عترة(٧٥,٨٦%) و١٦عترة (٥٩,٢٦%) من الأبقار البلدية والجاموس ، علي التوالي. وكانت العدوى المنفردة تمثل ١٩,٢٣ و١٥,٣٨% أما العدوى المختلطة كانت تمثل ٨٠,٧٧ و٨٤,٦٢% من العترات المعزولة في الأبقار البلدية والجاموس. تمثلت البكتريا الوبائية في عزل ميكروب المكور العنقودي الذهبي بنسبة ٤٠,٧٤% في الجاموس ، أما في الأبقار البلدية تم عزل المكور العنقودي الذهبي والسبحي ديسجلاكتيا ١٠,٣٥% لكل منهما والمكور السبحي أجالاكتيا ٣,٤٥%. أما بكتريا التلوث البيئي في الأبقار البلدية فقد تمثلت في عزل الميكروب القولوني ٢٠,٦٩% والمكور العنقودي سابروفيتكس ١٧,٢٤% والمكور العنقودي انترميديس ١٥,٥٢% والمكور السبحي بيوجين والمكور السبحي أوبرس ٥,١٧% لكل منهما والمكور العنقودي أبيدرميدس والكلبسيلا نومني ٣,٤٥% لكل منهما وكذلك تم عزل ميكروب أنتيروباكتر أيروجينز بنسبة ١,٧٢%. أما في الجاموس تمثلت بكتريا التلوث البيئي في عزل الميكروب القولوني والمكور العنقودي انترميديس ١١,١% لكل منهما والمكور العنقودي سابروفيتكس ١٨,٥٢% والمكور العنقودي أبيدرميدس ٧,٤١%

والمكور السبحي أوبرس والستروباكتر دايفرسيس ٣,٧% لكل منهما. وبإجراء اختبار الحساسية لأهم هذه العترات كل على حدة ضد ١٥ من المضادات الحيوية المختلفة أسفرت النتائج عن حساسية جميع العترات المعزولة للسيبروفلوكساسين وأفلوكساسين بنسبة ١٠٠% والجنتاميسين ٩٧,٦% والكاناميسين ٩٠,٥% ودوكسي سيكيلين ٨٥,٧%، كما أنها أظهرت مقاومة لكل من كلوكسيلين وأمبيسيلين أموكسيسيلين وسيفوتكسيم. وبعلاج بعض الحيوانات المصابة بالتهاب الضرع الخفي في ثلاث مجموعات (كل مجموعة ٣ أبقار بلدية وجاموسة واحدة)؛ خضعت المجموعة الأولي والثانية لحقن الضرع باستخدام جنتاميسين وكاناميسين علي التوالي، أما المجموعة الثالثة تم حقن الضرع بمحلول ١٠% عسل الشمر المصري. بعد العلاج كانت جميع عينات اللبن في المجموعة الأولي سلبية لاختباري الكاليفورنيا ماستيتس والويتسيد المعدل وكذلك للعزل البكتيري. أما المجموعة الثانية كانت عينتان من أربع عينات من اللبن بعد العلاج في اليوم السابع والعاشر ايجابية لاختباري الكاليفورنيا ماستيتس والويتسيد المعدل وكذلك للعزل البكتيري. بينما كانت جميع عينات اللبن بعد العلاج في المجموعة الثالثة ايجابية لاختباري الكاليفورنيا ماستيتس والويتسيد المعدل وسلبية للعزل البكتيري. ويمكن الخلاصة إلي أن المسبب الرئيسي لالتهاب الضرع الخفي يرجع إلي الميكروب المكور العنقودي والميكروب القولوني؛ وللسيطرة علي هذا المرض يجب أتباع الوسائل الصحية وكذلك رفع الوعي الصحي بين مربي الحيوانات الحلابة.

SUMMARY

This study is concerned with bacterial causes of subclinical mastitis in baladi cows and buffaloes. A total of 94-baladi cow and 80-buffaloes were examined. All animals rear in a smallholder private cases and hand milked. Screening tests of the milk samples using both field tests (California Mastitis Test and Modified Whiteside Test), revealed that 26(27.74%) & 30(31.9%) baladi cows and 13(16.3%) & 14(17.5%) of buffaloes showed positive results by both tests, respectively. These positive milk samples were examined bacteriologically on general and specific enriched media. The isolated bacteria were 58 isolates from baladi cows and 27 isolates from buffaloes. These isolates resembled two categories: contagious bacteria 14 (24.14%) & 11 (40.74%) and environmental bacteria 44(75.86%) & 16(59.26%) in baladi cows and buffaloes, respectively. The single infection resembled 19.23 and 15.38%, while mixed infection was 80.77 and 84.62% in baladi cows and buffaloes, respectively. The isolated contagious strains were *Staph. aureus* and *Strept. dysgalactiae* 6(10.35%) for both species and *Strept. agalactiae* 2(3.45%) in baladi cows. In buffaloes, *Staph. aureus* were 11 isolates (40.74%). Concerning to environmental bacteria in baladi cows *E. coli* were 12(20.69%); *Staph. saprophyticus* 10(17.24%); *Staph. intermedius* 9(15.52%); *Strept. Pyogenes* and *Strept. uberis* 3(5.17%) for each, while *Staph. epidermidis* and *Klebsiella pneumoniae* 2(3.45%) for both and

Enterobacter aerogenes was isolated from a single milk sample (1.72%). In buffaloes, *E. coli* and *Staph. intermedius* resembled 3(11.1%) for both, *Staph. saprophyticus* 5(18.52%), *Staph. epidermidis* 2(7.41%); *Strept. uberis* and *Citrobacter diversus* 1(3.7%)for each. Antimicrobial susceptibility testing revealed that all isolated strains were sensitive to Ciprofloxacin and Ofloxacin with percentage 100% followed by Gentamycin 97.6%, Kanamycin 90.5% and Doxycycline 85.7%. All tested bacterial isolates showed resistance to Cloxacillin, Ampicilin, Amoxicillin and Cefotaxime. Treatment of some subclinical cases carried on three groups (each of three baladi cows and one buffalo), the first and second group treated with intramammary infusion of Gentamicin and kanamycin, respectively and the third group subjected for intramammary treatment with 10% Egyptian fennel honey solution. All milk samples in the 1st group were negative for CMT&MWST and bacterial culture post treatment, while in 2nd group two out of four milk samples at 7th &10th days post treatment were positive for CMT&MWST and bacterial culture. The third group all milk samples post treatment were positive for CMT & MWST and negative for bacterial culture. It can be concluded that high prevalence of subclinical mastitis caused by *Staph*ylococcus spp. and *E. coli*, so strict hygienic measures and effective control for pathogens should be applied. Program of teat dipping and intramammary antibiotic treatment at drying off period is thus recommended. In addition to, enhancing the awareness between dairy farmers is the main point for control of subclinical mastitis in dairy animals.

Keywords: *Subclinical mastitis, bacteriological examination, baladi cows, buffaloes.*

INTRODUCTION

Mastitis is incriminated as one of the critical problems of the dairy animals leads to losses during the lactation period. These losses are primary due to lower milk yield, reduced milk quality and higher costs of treatment and control (Palanivel *et al.*, 2008). The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, subclinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk *et al.*, 2003). Subclinical mastitis is the most serious form, as both infected udder and milk show no obvious clinical abnormalities, whereas, several causative organisms are discharged with milk for long time during which the causative organism acts as invisible potential source of spreading

infection in the herd without the farmer being aware of it, so the infection becomes difficult to eradicate. This may cause sever harm from the epizootiological and epidemiological as well as economic points of view (Salem *et al.*, 1993).

Mastitis pathogens of dairy animals are numerous, but the majority of udder infections caused by pathogens of two categories including, contagious bacteria *as Staphylococcus aureus, Streptococcus agalactiae* and *Streptococcus dysgalactiae,* and environmental bacteria as *E. coli, Strept. uberis* and other *Staphylococcus sp.* (Bramley *et al.*, 1996; El-Balkemy *et al.*, 1997 and Anwer *et al.*, 2003). *Staph. aureus* seemed to be the predominant organisms causing subclinical mastitis (Kader *et al.*, 2002 and Ali *et al.*, 2008). It may predispose the herd to infection by coliforms or other pathogens (Ibtisam *et al.*, 1993).

In Egypt, buffaloes are the first animal for milk yield and the majority of these buffaloes are private ownership (Metry, 1996). The greatest problem in the treatment and control of mastitis is emergence of drug resistance pathogens (Jha *et al.*, 1994). The pattern of drug resistance continues to change in a particular area depending upon various epidemiological factors and indiscriminate use of antibiotics (Choudhury and Narayan, 1984).

The intramammary fennel honey infusion was applied in subclinical mastitic cows resulted in significant decline of total bacterial count and highly significant increase in milk yield (Abd El-Hafeez *et al.*, 2005). Due to economic and public health importance of subclinical mastitis, the present study was aimed to detect subclinical mastitis and determine the predominant contagious and environmental bacteria causing it in baladi cows and buffaloes using the most available media for isolation. Determination of antibiogram of the most prevalent bacterial isolates and trials for treatment of some affected cases was the second aim.

MATERIALS and METHODS

Ninety-four baladi cows and eighty buffaloes with clinically sound udder and secreting apparently normal milk were included in this study. All animals related to small-scale herds (1-4 animals). They reared in private cases under the farmers hand and hand milked twice daily. The animals ages ranged from 4-6 years and at different stages of lactation. They housed in a barn with dust ground and their feed consists of green fodder, hay and concentrates.

Quarter milk samples (366 from baladi cows and 314 from buffaloes) were examined at once, where 10 and 6 quarters were blind non lactating, respectively, using the field tests, (California Mastitis Test, CMT, using Delaval Mastitis Test, 3804101, Poland, Schalm *et al.*, 1971 and modified Whiteside Test, MWST, Murphy and Hanson, 1941).

For bacteriological examination, ten ml of fresh milk samples from 30 baladi cows and 14 buffalos which showed sub-clinical mastitis positive reaction (individual sampling), as a pooled milk samples were of the four quarters in a sterile screw capped vials, were collected aseptically. Milk samples centrifuged at 3000 rpm for 20 minutes, then a loopfull from milk sediment streaked onto Azid Blood Agar plate and a loopfull was inoculated into each of nutrient broth (Himedia Lab. Limited, India) MacConkey broth (Biomark Lab. India) and modified EC-medium (Diffco No.7197405) with Novobiocin 2%. The previously inoculated tubes were incubated at 37°c for 24 hours. From the incubated tubes, a loopfull was streaked onto the surface of each of the nutrient agar, blood agar with 5% sheep blood, Mannitol salt agar (BBL), MacConkey agar (Biomark Lab. India) and Sorbitol MacConkey agar plates (Diffco). The inoculated plates were incubated overnight aerobically at 37°c.

The suspected colonies were identified: morphologically, by Gram's stain and biochemically confirmed according to Quinn *et al.* (1994), using catalase activity, coagulase test as well as Novobiocin (5 mcg) and polymixin-ß sulphate (300 U) sensitivity tests for identification of *Staphylococcus spp.* Identification of *Streptococci spp.* was done by catalase test, haemolytic activity, sodium hippurate hydrolysis, aesculin hydrolysis on blood agar with 0.1% aesculin, growth in 6.5% Nacl broth, growth on MacConkey agar, Sorbitol & lactose fermentation and Bacitracin, 0.04 unit susceptibility. Enterobacteriace identified biochemically by conventional IMVIC (Indole, Methyl red, Voges-proskauer and citrate utilization) test, motility, triple sugar iron agar (TSI) inoculation and sugar fermentation (Sorbitol, raffinose & cellobiose), according to Quinn *et al.* (1994).

Antibiogram of the recovered isolates was adapted using antimicrobial susceptibility testing by disc diffusion standard technique according to Quinn, *et al.* (1994). The isolated strains were tested against 15 antibiotics (Ampicilin 10 µg, Amoxicillin 25 µg, Cefotaxime 30 µg, Cephalexin 30 µg, Ciprofloxacin 5 µg, Cloxacillin 1 µg, Doxycycline 30 µg, Gentamicin 10 µg, kanamycin 30 µg, Neomycin 30 µg, Novobiocin 30 µg, Ofloxacin 5 µg, Oxytetracycline 30 µg, Trimethoprim 5 µg, Spiramycin 100 µg), (Bioanalyse-Turkey).

Treatment trials: Selected baladi cows and buffaloes with subclinical mastitis were allocated into three groups (each of three cows and one buffalo). The first group administered Gentamicin 100 mg/ quarter (B.V.Co., France), the second was administered Kanamycin 100000 I.U./ quarter (Univet, Ireland). All drugs were administered twice daily after milking for three consecutive days and the third group was subjected for intramammary treatment with Egyptian fennel honey. Unprocessed honey diluted with sterile normal saline solution to achieve 10% honey solution then, filtered under complete aseptic conditions using sterilized filter papers to remove any debris, wax, or large particles, Al-Waili (2003). Ten ml of 10% honey solution intramammary/ quarter infused daily for three successive doses guarded with intra-muscular administration of antihistaminic (Abd El-Hafeez *et al.*, 2005). The milk samples were collected before treatment and at the 4^{th} & 7^{th} and 10^{th} days post treatment. Each samples was subjected to CMT & MWST and for bacterial culture.

RESULTS

Detailed obtained results were illustrated in Tables (1-5).

In this study, the outcome of treatment in first group all milk samples after treatment were negative for CMT & MWST and for bacterial culture. In the second group all milk samples at 4^{th} day post treatment were negative for CMT & MWST and for bacterial culture, but two out of four milk samples were positive CMT & MWST and also for bacterial culture at 7^{th} &10^{th} days post treatment. In the third group, all milk samples after treatment were positive for CMT & MWST and negative for bacterial culture.

Table 1: Incidence of subclinical mastitis in examined baladi cows and buffaloes by both tests.

Types of animals	No. of animals	California mastitis test				Modified Whiteside test				Positive for bacterial culture	
		Positive		Negative		Positive		Negative		No.	%
		No.	%.	No.	%	No.	%	No.	%		
Cows	94	26	27.7	68	72.3	30	31.9	64	68.1	26	27.7
Buffaloes	80	13	16.3	67	83.3	14	17.5	66	82.5	13	16.3

Table 2: Incidence of subclinical mastitis quarters in the examined baladi cows and buffaloes

Types of animals	Examined quarter	One quarter		Two quarters		Three quarters		four quarters		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
Cows	366	10	2.73	7	1.91	7	1.91	6	1.64	30	8.20
Buffaloes	314	7	2.23	4	1.27	3	0.96	0	0	14	4.46

Table 3: The frequency percentage of the single and mixed infection in positive milk samples of the examined baladi cows and buffaloes by using bacteriological examination.

Types of animals	Single Infection		Double infection		Triple infection		Total	
	No.	%	No.	%	No.	%	No.	%
Cows	5/26	19.23	10/26	38.46	11/26	42.31	26/26	100
Buffaloes	2/13	15.38	8/13	61.54	3/13	23.08	13/13	100

Table 4: Frequency distribution of bacterial species recovered from subclinical mastitic milk samples of baladi cows and buffaloes.

Bacterial species	Cows		Buffaloes	
	No.	Frequency %	No.	Frequency%
Contagious organisms	N.= 14	24.14	No. 11	40.74
- *Staph. aureus*	6	10.35	11	40.74
- *Strept. dysgalactiae*	6	10.35	0	0
- *Strept. agalactiae*	2	3.45	0	0
Environmental organisms	N.= 44	75.86	N.=16	59.26
- *E .coli*	12	20.69	3	11.11
- *Staph. saprophyticus*	10	17.24	5	18.52
- *Staph .intermedius*	9	15.52	3	11.11
- *Strept. Pyogenes*	3	5.17	0	0
- *Strept. uberis*	3	5.17	1	3.70
- *Staph. epidermidis*	2	3.45	2	7.41
- *Klebsiella pneumoniae*	2	3.45	0	0
- *Enterobacter aerogenes*	1	1.72	1	3.70
- *Hafnia alvei*	1	1.72	0	0
- *Serratia marcescens*	1	1.72	0	0
- *Citrobacter diversus*	0	0	1	3.70
Total	58	100	27	100

Table 5: The percentage of *in vitro* antimicrobial susceptibility pattern of the most frequent isolates against different antibiotics.

Isolated microorganisms	No. of the tested isolates	Ampicilin(10µg)	Amoxicillin (25µg)	Cefotaxime (3µg)	Cephalexin (30µg)	Ciprofloxacin (5µg)	Cloxacillin (15µg)	Doxycycline (30µg)	Gentamycin (10µg)	Kanamycine (30µg)	Neomycin (30µg)	Novobiocin (30µg)	Ofloxacin (5µg)	Oxytetracycline (30µg)	Trimethoprim (5µg)	Spiramycin (1005µg)
		Number and percentage of sensitive strains														
Staph. aureus	16	1/16 (6.3%)	5/16 (31.3%)	0/16 (0%)	4/16 (25%)	16/16 (100%)	2/16 (12.5%)	15/16 (93.8%)	15/16 (93.8%)	14/16 (87.5%)	5/16 (31.3%)	9/16 (56.3%)	16/16 (100%)	8/16 (50%)	10/16 (62.5%)	8/16 (50%)
Strept. Spp.	12	2/12 (16.7%)	5/12 (41.7%)	3/12 (25%)	1/12 (8.3%)	12/12 (100%)	1/12 (8.3%)	12/12 (100%)	12/12 (100%)	11/12 (91.7%)	10/12 (83.3%)	12/12 (100%)	12/12 (100%)	6/12 (50%)	9/12 (75%)	10/12 (83.3%)
E. coil	14	1/14 (7.1%)	4/14 (28.6%)	13/14 (92.9%)	13/14 (92.9%)	14/14 (100%)	0/14 (0%)	9/14 (64.3%)	14/14 (100%)	13/14 (92.9%)	12/14 (85.7%)	9/14 (64.3%)	14/14 (100%)	8/14 (57.1%)	11/14 (78.6%)	0/14 (0%)
Total	42	4/42 (9.5%)	14/42 (33.3%)	16/42 (38.1%)	18/42 (42.9%)	42/42 (100%)	3/42 (7.1%)	36/42 (85.7%)	41/42 (97.6%)	38/42 (90.5%)	27/42 (64.3%)	30/42 (71.4%)	42/42 (100%)	22/42 (52.4%)	30/42 (71.4%)	18/42 (42.9%)

DISCUSSION

Subclinical mastitis means that, although there are no visible or palpable external changes, the infection is present and the inflammation occurs in the udder and apparently healthy milk, but subclinical mastitis leads to undesirable effect on milk constituents and its nutritive value. Economical losses are due loss in milk production, discarding abnormal milk and milk withheld from animals treated with antibiotics, degrading of milk due to higher bacterial or somatic cell count, cost of drugs, veterinary services and increased labor cost. In addition, the problems related to antibiotic residues in milk and its products (Bramely *et al.*, 1996).

In the present study (Table 1), results revealed that subclinical mastitis in baladi cows was 27.7% by C.M.T and 31.9% by M.W.S.T. Among cows, subclinical mastitis ranged from 5.5% (Zahid, 2004), 30.69% (Ghosh *et al.*, 2004); 31.98% (El-Balkemy *et al.*, 1997); 59.05% (Sadek, 2008); up to 69.2% (Awad and Abeer, 2003) and 67% in dairy Friesian cows (Nahed Wahba *et al.*, 2005). In case of buffalo's milk samples the incidences of subclinical mastitis was 16.3% and 17.5% by C. M. T. and M. W.S.T., respectively. Similar result 18.59% was obtained by Ghosh *et al.* (2004). The lower incidences 6.95, 9.59% were obtained by Ahmad *et al.* (1991) and Saini *et al.* (1994), respectively, while, Shrirame *et al.* (1997); El- Balkemy *et al.* (1997) and Sadek (2008) recorded higher incidences of 20.71%, 42.55 and 33.3%, respectively.

The obtained results showed that the incidence of subclinical mastitis in baladi cows, which reared in small holder private cases, handy milking and obtained good management, lower than the incidence in large scale farms and machinery milking which enhance infection transmission. The sub-clinical mastitis incidence varied widely due to changing in management conditions and different diagnostic tests used (Radostits *et al.*, 2000). The high rates of subclinical mastitis in the different areas were mainly due to poor management and unhygienic milking practices (Shem *et al.*, 2001). The lower incidence of subclinical mastitis in buffaloes may be due to their thick streak canal and perfect closure mechanism of teat sphincter (Ghosh *et al.*, 2004). Since the C.M.T. field test is dependable and reliable perfect test in good agreement with bacteriological results (El-Balkemy *et al.*, 1997). It appeared to agree 100% with bacteriological isolation in the present study and proved its superiority than Modified Whiteside test, which detected false positive results. False positive results of Whiteside test is documented (Nahed Wahba *et al.*, 2005).

The results obtained in Table (2), revealed that, the incidences of subclinical mastitis in the examined baladi cows and buffaloes according

to the affected quarter, the one-quarter infection was more than two, three and four quarters infections. These results were similar to that obtained by Saini *et al.* (1994) and Sadek (2008).

As shown in Table (3), the incidence of mixed and single infection were 80.77and 19.23% in positive baladi cow's milk samples for subclinical mastitis, respectively, while buffalo's milk samples showed mixed infection incidence higher than single infection (84.62 and 15.38%, respectively). These findings reflect an idea about level of environmental bacterial contamination (Sayed and Abd El-Hafeez, 2009). In addition *Staph. aureus* may predispose the animals to infection by coliforms or other pathogens (Ibtisam *et al.*, 1993). Meanwhile, El-Khodery and Hoedemaker (2005) and Magda Essa (2007) reported that the mixed bacterial infection was lower than single bacterial infection in subclinical mastitis cases.

The results obtained in Table (4) revealed that the frequency percentage of environmental bacteria in baladi cows and buffaloes was 75.86 and 59.26%, respectively.

The environmental bacteria, which may cause mastitis usually originate from the surrounding environment including air, soil, water, bedding material, faecal matter, milking man and milking utensils (Anwer *et al.*, 2003). The portal of entry into mammary gland for Gram-negative bacteria is the teat canal. Once in the gland, bacteria must utilize available substrates in the mammary secretion to replicate and evade host defenses (El-Mahronki *et al.*, 2006). The obtained results indicated exposure of teat end to the environmental bacteria.

The highest incidence of environmental bacteria in the present study was 20.69 and 11.11% for *E. coli* in both cows and buffaloes, respectively. *E. coli* was widely reported to be subclinical mastitis bacteria in cows and buffaloes (Ahmad *et al.*, 1991; Ahmed and Azza, 2001; Kader *et al.*, 2002; Anwer *et al.*, 2003; Awad and Abeer, 2003; Magda Essa, 2007 and Sadek, 2008). Its persistence within the mammary environment was of the recurrent quarter *E. coli* mastitis and its spread among other quarters and cows during the milking process (Bradley and Green, 2001).

Staph. saprophyticus, *Staph. intermedius*, *Strept. Pyogenes*, *Strept. Uberis*, *Staph. epidermidis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Hafnia alvei*, *Serratia marcescens* and *Citrobacter diversus* were identified to be as environmental subclinical mastitis pathogens in baladi cows and buffaloes (Mokhbatly *et al.*, 2001 and Kotb, 2006).

In the present study, as shown in Table (4), the frequency percentage of contagious bacteria causing subclinical mastitis in baladi cows and buffaloes was 24.14 and 40.47%, respectively (*Staph. aureus* 10.35%; *Strept. agalactiae* 3.45% and *Strept. dysgalactiae* 10.34% in cows and

Staph. aureus 40.74% in buffaloes). The contagious bacteria are well adopted to survive in the udder and usually establish mild subclinical infection for long duration (El-Khodery and Hoedemaker, 2005 and Abdel-Khalek and El-Sherbini, 2005) and can spread from infected quarters to other quarters (Bramley *et al.,* 1996 and El-Balkemy *et al.,* 1997). Through previous studies (Ahmad *et al.,* 1991; Ahmed and Azza, 2001; Awad and Abeer, 2003 and Sadek, 2008) the highest incidence of subclinical mastitis in cows and buffaloes was due *Staph*ylococci *spp.* followed by *Streptococci spp. Staph. aureus* commonly produce long-lasting infections as it developed sophisticated system to evade phagocytosis and intra-cellular killing by neutrophils or macrophages (Vanfurth and Van Zwet, 1986).

The differences in the distribution of isolated microorganisms for cows from a farm to another may be attributed to differences in the management, housing, and species of animals in addition to an inadequate cleansed milk machine and teat trauma(El-Balkemy *et al.,* 1997).

Bacteriological examination of milk is needed not only for confirmatory process but also for drug sensitivity. Identification of the causative organism and sensitivity testing besides culling of untreatable cows are very important for control of sub-clinical mastitis. In the present study, Table (5) shows the prevalent bacteria isolates tested for antibacterial sensitivity pattern. The obtained results revealed the most effective antimicrobial agent all over the study was Ciprofloxacin, Ofloxacin followed by Gentamycin; Kanamycin and Doxycycline with susceptibility 100, 100, 97.6, 90.5 and 85.7%, respectively. While all tested strains resisted Cloxacillin, Ampicilin, Amoxicillin, Cefotaxime. The obtained results coincided to large extent with that of Abd El-Hafeez (2002); Kader *et al.* (2002); Abdel-Khalek and El-Sherbini (2005); Magda Essa (2007).

Through the present study, treatment of some subclinical cases with antibiotics selected on the basis of in *vitro* sensitivity test revealed that, in the first group treated with intramammary infusion of Gentamicin, the milk samples were negative for CMT & MWST and negative for bacterial culture post-treatment. In the second group, treated with intramammary infusion of Kanamycin, two milk samples were positive CMT & MWST and also for bacterial culture after 7^{th} &10^{th} days post treatment. These results indicated that application of screening tests leads to earlier detection of subclinically infected quarter and aid in the selection of dairy animals for either production or therapy (Sadek, 2008). The third group was subjected for intramammary treatment with 10 ml of 10% solution of Egyptian fennel honey revealed that all milk samples after treatment were positive for CMT & MWST and negative for bacterial culture. The posi-

tive reaction in CMT & MWST depends on the concentration of somatic cell count in the milk (Ahmad *et al.*, 1991). Extremely positive reaction of CMT was recorded post intramammary honey infusion, as leukocytes especially lymphocytes were significantly increased in milk post intramammary honey infusion (Abd El-Hafeez *et al.*, 2005). Hydrogen peroxide activity of honey and phytochemical antibacterial component unique to honey are effective inhibiting the growth of mastitis causing species of bacteria at quite low concentrations (Allen and Molan, 1997).

Treatment of subclinical cases using antibiotics for long time may develop bacterial resistance rather than milk withdrawal period, existence of problems associated with yogurt or cheese processing. In addition the failure to treat subclinical mastitis may allow these animals to be reservoir of infection and increase the potential exposure of uninfected animals to contagious pathogens (Ruegg *et al.*, 2008).

It can be concluded that high prevalence of subclinical mastitis caused by *Staph*ylococcus spp. and *E. coli*, so strict hygienic measures and effective control for pathogens should be applied program of teat dipping and intramammary antibiotic treatment at drying off period. In addition to, enhancing the awareness between dairy farmers is the main point for control of subclinical mastitis in dairy animals. The use of honey in treatment of subclinical mastitis would obviate for all producers the withholding of milk after therapy and any residue that did not end up in the milk would be more acceptable to consumers.

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