Bacteriological and Biochemical Studies on Recurrent Mastitis in Buffaloes

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Abstract
Staphylococcus is the most common contagious bacterial cause of recurrent mastitis. The results showed that 41 strains were identified morphologically and biochemically as *S. aureus* in addition to 14 strains of *S. citrus* and *E.coli* were also identified morphologically and biochemically. 38 out of tested strains proved to be *S. aureus* by latex agglutination test. Mortality in mice in correlation to latex agglutination test was discussed and results showed that all positive latex agglutination strains proved to pathogenic to mice with 100% mortality rate. Antibiotic sensitivity test was carried out using disc diffusion method. The results showed that the isolated *S.aureus* was highly sensitive to cefoperazone, cefotaxeme, ciprofloxacin, tobramycin and ofloxacine. Genomic DNA of *S. aureous* strain was analysed by RAPD PCR, showing the result that *S. aureus* had DNA bands of 4molecular weight. Biochemical changes in milk obtained from recurrent mastitic buffaloes revealed a reduction in fat, potassium, calcium, phosphorus, iron, copper and zinc content. Meanwhile, increase in total protein, albumin, sodium and chloride levels were detected as compared to milk obtained from apparently healthy buffaloes.

Key words: *E.coli , S. aureus, S. citrus, antibiotic sensitivity, RAPD PCR, mastitis, buffaloes.*

Introduction

Bovine mastitis is one of the most problematic diseases and continues to have major economic impact on dairy industry throughout the world. It is a global problem characterized by physical, chemical and microbiological changes in the milk and pathological changes in the glandular tissue of the udder **(Bachaya *et al*., 2011)**.

Bacterial infections are considered the primary cause of mastitis in domestic animals. Diseases of the mammary gland are regarded as the most important economic factor in milk production. It results in substantial economic losses to dairy producers where milk yield generally drop and never recovered **(Abdel Gadir *et al*., 2006).**

Bovine mastitis not only decreases milk yield, but also alters its composition **(Shuster *et al*., 1991)**. Changes in milk composition are brought about by impairment of normal synthesis of milk ingredient and also infiltration of some blood constituents into milk as a result of abnormal permeability of blood capillaries in inflamed udders **(Sandholm *et al*., 1995, Rashed *et al*., 2002** and **Rashed and El-Shafey, 2008).**

Much of these losses could be prevented by the application of control measures that were developed and proved some effectiveness within the last few years. Lack of good control of mastitis among dairy herds in Egypt points out the necessity for more effective knowledge on the problem.

There are large gaps in our knowledge of the disease and any progress in understanding the mastitis obscure points which require continuing long-term research. One of these masked point is undoubted the recurrent. Repeated, persist or returned cases of mastitis. **(Malinowski, *et al*., 1992).**

Pathogenic staphylococci is the major etiological agent of mastitis **(Yuji *et al*., 2001)** and its high incidence of *S.aureus* is attributed to

heavy contamination of bedding housing, overcrowding and the increase of moisture **(Roberson *et al*., 1994)**. So rapid diagnosis of pathogenic *S.aureus* strains in milk sample is very essential to control dairy farms infection and the use of new technology in diagnosis will be useful to avoid development of severe damage in udder tissues.

The aim of this work was directed towards the following points:

* Determination of mastitis recurrence with its correlation in the herd.
* Isolation and identification of bacteria associated with recurrent mastitis through using PCR for identification.
* The effect of recurrent mastitis on milk total proteins, fat, minerals (Na, K, Ca, Ph, Cl, Mg) and some heavy metals (Fe, Cu, Zn, Pb).
* Determination of the antibiogram of the isolated bacteria.

Material and Method

1- Milk Samples:

One hundred and ninety two milk samples were collected under aseptic conditions from 192 buffaloes showing clinical mastitis. Also it was recorded the repeat of clinical mastitis 2-3 times.

The samples were collected at three periods / year as follows:

Period I: From March to June, 80 samples showing clinical signs of mastitis.

Period II: From July to October, 45 samples showing clinical signs of mastitis.

Period III: From November to February, 67 samples showing clinical signs of mastitis. (Table 1)

Twenty milk samples were collected from another farm free from recurrent clinical mastitis and examined bacteriologically to be sure they were free from pathogens to use as negative control samples for biochemical analysis.

2-- Bacteriological examination:

Ten ml. of milk were collected aseptically in sterile screw capped bottles then centrifuged at 3000rpm for 20min, the supernatant was discarded and sediment streaked on to nutrient agar, blood agar medium, MacConky agar medium, Mannitol salt agar, psuedomonus base agar with CN supplement and eosin methylene blue agar incubated at 37^0c for 24hrs. Suspected colonies were identified morphologically, Gram staining and biochemically, according to **Quinn *et al*., (1994)**.

The biochemically well identified strains of *S.aureus* were tested for agglutination by using spot kit (Latex agglutination test, Oxoid) for seroidentification of *S.aureus* among the tested strains and were tested for coagulase test using staphylase kit (Oxoid).

Pathogenicity test:

Forty one isolates of *S. aureus*, in addition to seven isolates of staphylococci citrus were examined for their pathogenicity in 98 Swiss mice (mice were divided into 49 groups, each group contain 2 mice) by injection intrapertoneal (I/P) $3x10^8$ cfu/ml (o.5ml for each mouse). The death rate was determined for 7 days post infection **(Quinn 1994)**.

Antibacterial sensitivity test:

Antibiogram susceptibility of *S. aureus* isolates: Forty one isolates of *S.aureus* were examined against different antibacterial agents as amoxycillin, amoxycillin/clavulanic acid, ampicillin, cefoperazone, cefotaxime, ciprofloxacin, enroflaxacin, gentamicin, neomycin, ofloxacin, oxytertracycline and tobramycin disks, using disk diffusion technique was adapted according to **Finegold and Martin (1982).**

Random Amplified Polymorphic DNA (RAPD PCR)

DNA preparation according to **Sambrook *et al*. (1989)**

PCR reaction **(RAPD)** according to **Van Bel Kum *et al*. (1993)**.

RAPD PCR:

The total reaction volume was 50ml containing 20 ngm of template DNA. A Perkin-Elmar Cetus model 480 thermal cycler was used to carry out the amplification reactions. Reaction conditions were as follows: 94°C for 3 minutes (to make denaturation), then 94°C for 1 minute 25°C for 1 minute 25°C for 1 minute and 72°C for 2 minutes. All are 40 cycle followed by 1 cycle of 72°C for 7 minutes. Then at 4°C thirty

ml ali-quots of amplified DNAs were electrophoresed in 1.5% agarose gels in TAE buffer containing 0.5% ethidium bromide, at 100 volts. Hi-Lo TM DNA marker (LOTM DNA MARKER , CAT. NO.1010,200 1000Bp) were also run in each gel as a standard for size determination of DNA fragments. The DNA was visualized with UV illuminator at 300 nm wave length and photographed.

Sequence of the primer (DPB.05)	2- Sequence of the primer(E.20)
TGC GCC CTTC	AAC GGT GACC

Biochemical analysis:

Milk samples were collected from recurrent mastitic buffaloes which showed repeated clinical signs of mastitis, in addition to twenty normal milk samples were collected from apparently healthy animals (free bacteriological cases). Samples were treated with drops of formalin 10% for each milk sample as a preservative and stored at –4ºC till further analysis.

Determination of total milk protein and albumin were carried out according to **SonnenWirth and Jaret (1980)** and **Drupt (1974)** respectively. Fat percent was determined according to the method of **Aggarwala and Sharma (1961)**. Sodium and potassium were estimated by flame photometer according **Oser (1979).**

Determination of calcium, phosphorous, and magnesium were determined according to **Tietz (1995)** and chloride **Feldkamp (1974)**. Iron, copper, zinc and lead were estimated by using a Unicam 969 Atomic Absorption Sepectrometer with acetylene air flames was used in accordance to manufacture's specification.

Statistical analysis:

Samples were simultaneously analyzed using T-student test according to **Petrie and Watson (1999)**.

Results and Discussion

Mastitis is incriminated as one of the critical problems of the dairy animals leading to severe losses during the lactation period. These losses are primary due to decreased milk yield, reduced milk quality and expensive costs of treatment and control **(Palanivel *et al*., 2008)**.

Milk is a complex biological fluid that contains a wide variety of constituents and possesses unique physical and chemical characteristics.

In addition of being nutritional medium, it also presents a favorable physical environment for the multiplication of microorganisms, therefore frequent assisting the quality of milk is of great importance **(Sadek, 2008)**.

In the dairy herd, Mastitis is nearly always caused by microorganisms, usually bacteria which invade the udder and multiply there. Because most cases of bovine mastitis are the result of such infection, the term mastitis usually implies the presence of an infectious microorganism.

Bovine mastitis has great economic importance in dairy farm animals; in addition mastitis is one of the most important causes of tremendous loss in milk yield in the world. *Staphylococcus aureus* microorganisms is a major etiological agents of mastitis.

One target of the present study was to detect repeated cases under the name "recurrent mastitis" which means the occurring of the disease two or more times in the same quarter of the udder. Yet there was no correlation between the total amount of mastitic cases and number of recurrent cases (Table 1). Twenty six mastitic cases (63.41%) were repeated twice per period and 15 cases with repeated mastitis three times per period (36.58%). This result was found much lower than that reported by **(Ibrahim and Habiballa 1976)** who recorded 76.5% recurrent cases for two times and an incidence of 66.7% recurrent cases for three times.

Generally, the lower incidence of recurrent mastitis recorded in this study could be attributed to the continuous detection and eradication of recurrent cases in the farm.

Staphylococcus aureus was isolated in single

infection of a rate of 75.6% (31 isolates), while it was isolated in double infection with *S.citrus;* streptococcus spp. and *E.coli* at a rate of 7.3%; 4.9 and 2.4% respectively as well as in triple infection with *S.citrus* and *E.coli* at a rate of 9.8%. These results revealed that the main cause of recurrent mastitis was *S.aureus* while E.coli, *S.citrus* and streptococcus spp played a role as secondary infection. **Table (2)**

A higher incidence of *S. aureus* as a cause of mastitis was recorded by different researchers. **(Langenegger and Caelho, 1970)**, found it to reach (52.9%) **(Nada and Goda, 1975)** estimated it as (60.66%), while **(Auxilidora *et al.*, 1984)** reported the highest incidence (67.7%) of *S. aureus* recurrent mastitis.

The bacteriological characterization of *S.aureus* was clarified in Table (3), all isolates were catalase positive, sensitive to novobicin and the rest of characterizations are varied according to the used techniques as **Schleifer, (1986); Thomson *et al*., (1989) and Quinn, *et al*. (2002).**

Regarding hemolytic activity of *S. aureus* isolates on human and sheep blood agar as shown in Tables (3), it was clear that 39 isolates (95.12%) had hemolytic activity on sheep blood agar. Meanwhile 9 isolates only (21.95%) had hemolytic activity on human blood agar. **Boerlin *et al*., (2003)** concluded that testing for the presence and type of hemolysis on blood agar plates represents a first simple and rapid method for identification.

b-Hemolysis also represents important criteria for rapid presumptive identification of *S. aureus* in primary cultures. With regard to this criteria, the results of **Lam *et al*., (1995) and Aarestrup *et al*. (1999)** showed that approximately one fifth to one fourth of the *S.aureus* isolates from bovine mastitis do not present any detectable beta-hemolytic activity in primary cultures. Intramammary infections in dairy cows by *S. aurues* have been studied extensively by **Laevens *et al*. (1996),** most of *S. aureus* produced typically b-haemolysin and the staphylococcal clumping factor.

Staphylococcus aureus strains are usually identified as b-haemolysin producing, catalase-positive and gram-positive cocci. Additional tests are needed to identify b-haemolysin negative *S.aureus* strains. A test that examines the ability of the strains to clot plasma by a tube test for free coagulase or a slide test for bound coagulase (clumping factor) has been suggested **(Harmon *et al*., 1991)**. Other tests, such as the manitol production gelatinase and production of crystal violet can be used together with the clumping factor test for the identification of *S.aureus* **(Laevens *et al*., 1996).**

For studying the pathogenicity of 48 strains of Staphylococci in mice, I/P injection of $3x10^8$CFU of each strains into (2mice) was carried out and the death rate was determined during 0-7 days as well as pathogenic strains were reisolated from dead mice, all positive latex agglutination strains (38) proved to be pathogenic for mice with a mortality reached 76 mice, while 7 out of 48 strains were negative to latex test (*Staphylococci* citrus) were non pathogenic to mice (14 mice survived), Table (4). These results agree with **Selim *et al*. (1983).**

Antimicrobial agents of various classes are widely used for therapeutic intervention of diseases in animals caused by *staphylococci*, but also for prophylactic measures, e.g. for drying off buffaloes at the end of the lactation period.

Unfortunately, this development has not been documented continuously in the veterinary field. Publications differ distinctly in respect to the number of isolates and the staphylococcal species investigated, as well as the animal source, the clinical status of the animal, and the available information on antibiotic pretreatment.

Moreover, different antimicrobial agents have been tested for their invitro efficacy, and a variety of methods (disk diffusion, agar dilution, micro-and macro broth dilution) as well as different standards for the interpretation of results have been used as a consequence evolution frequency of staphylococci resistant to antimicrobial agents has been recorded over the last decades **(Devriese *et al*., 1997 and Normand *et al*., 2000)**.

In the present investigation high resistance was

recorded to ampilcillin (95. 12%). Followed by enrofloxacin (90.24%) Amoxicillin(73.17%)). Meanwhile (97.56 %) of the examined *S. aureus* isolates were sensitive to cefoperazone, (87.80%) to cefotaxime and (75.61%) to ciprofloxacin Table (5).

Stephan *et al*., (2001) recorded that the antibiotic resistance testing revealed that all 34. *S. aureus* strains were susceptible to cefoperazone, oxacillin, cephalothin, gentamicin, neomycin, tetracycline, chloramphenicol, lincomycin and erythromycin. A total of 31 isolates (91.2%) were susceptible to all antibiotics tested and two of the remaining three isolates were resistant to penicillin G/ampicillin, one to polymyxin B.

These results of sensitivity test clarify the occurrence of recurrent mastitis where highly resistant strains of *S.aureus* still persist and accurate treatment didn`t apply due to lack of sensitivity test applied prior to treatment and the misuse of antibiotic agents without determining the exact microbe causing the infection and its sensitivity to antibacterial agents.

Nucleic acid amplification by PCR has applications in many field of biology and medicine, including the detection of viruses, bacteria, and other infectious agents (**Thiele, 1990**).

In the present study, the use of PCR amplification of different *staphylococcal* isolates genomic DNAs resulted in reproducible DNA fragment patterms as shown in Photos. (1 and 2).

The use of PCR amplification in primer one showed the pattern of *S.aureus* bands. The first isolate (320,562,928), the second isolate (300,416,709,928) and the third (320,562,928). But in second primer the *S.aureus* bands were the first isolate (240,416), the second isolate (240,342,630) and the third(240,400,514,811).

The same results were reported by **Belkum *et al*., (1992)** and **Lipman *et al*. (1995)** who found that the DNA fingerprinting technique using RAPD-PCR proved to be useful in differentiating isolates of *S. aureus* in a rapid and accurate manner as shown in photo (1) and (2)

Milk is an important part of the human diet, the nutritional significance of milk is indicated by the fact that daily consumption of a quart (1.14 liters) of milk furnishes approximately all the fat, calcium, phosphorus, riboflavin, one half of the protein, one third of vitamin A, ascorbic acid, thiamine and one fourth of calories needed daily by average individual **(Bilal and Ahmad 2004,** and **Uallah *et al*., 2005)**.

Table (6) showed significant increase in total protein and albumin in milk of recurrently mastitic buffaloes. These results are in agreement with the results previously recorded in milk from cow with clinical mastitis by **Rashed *et al*. (2002)**, **Safinaz *et al*. (2006)** in buffaloes' milk. The increase in total protein and albumin content in mastitic milk could be attributed to serum albumin, immunoglobuline, transferrin and other serum proteins pass into milk as a result of increased vascular permeability **Khan & Khan (2006)**. Casein, the major milk protein of high nutrional quality, decline due to the presence of proteolytic activity by more than 2-fold during mastitis. Plasmin and enzymes derived from somatic cells can cause extensive damage to casein in udder before milk removal. In addition lower quality whey proteins increase, which adversely affects the quality of dairy products such as cheese.

The increase of serum albumin and immunoglobins resulting in reduction in heat stability of mastitic milk and pasteurization gives lower grade scores after storage **Khan and Khan (2006)**.

As regards to fat concentration, the results revealed significant drop in fat concentration in mastitic milk table (6). This result is in agreement with those of **Abdel Galil and Nassib (1980)** who found a slight decrease in fat content in mastitic milk. **Mandel and Raheja (1985)** reported that fat content decreased from 5.18 to 5.06% with severity of mastitis. **Shahin and Haggag (1987)** found that the average fat content in milk of healthy and mastitic buffaloes were 7.28$\pm$0.14 and 4.02$\pm$0.8% respectively. Similarly, **Mahran *et al*., (1992)** indicated 18% less in fat in buffaloes' mastitic milk as compared to normal. **Hortel *el al*., (1998)** reported that there was a slight reduction in milk fat content in dairy cows due to mastitis.

On the other hand, **Uallah *et al*., (2005)** found significant difference in fat content in buffalos' milk according to the severity of mastitis. They also mentioned that maximum milk fat content were found in control and minimum in P_3 grade mastitis quarter (P_3 = Rapid/ heavy clumping).

As observed in the present study, decrease in fat content in milk from recurrent mastitic buffaloes may be attributed to breakdown of milk fats resulting from mastitis. Milk from recurrent mastitic animals had hyper activity of lipase enzyme. The predominant type of fat in milk is in the form of triglycerides **(Barbano, 1989).** Lipase attaches the triglycerides and release free fatty acids. At a very low concentration free fatty acids produce off flavors in milk and dairy products that are characterized as rancid off flavors **(Schmidt *et al*., 1988 and Uallah *et al*., 2005).**

Concerning the content of minerals, results indicated a significant increase in sodium and chloride accompanied with significant decrease in potassium, calcium and phosphorus as compared to apparently healthy animals (table 7). This result agree with the finding of **Janota-Bassatik *et al*., (1978)** who found that mastitis increased milk sodium contents from 402 mg to as much as 2770 mg/l and decreased potassium content from 1649 to 522mg/100ml. Similarly**, Banga *et al* (1989)** in sheep**, Charjan – Ku *et al*., (2000)** in cow, **Rawdat and Omima (2000)** in buffaloes reported an increase in sodium contents and decrease in potassium, calcium and magnesium contents in mastitic milk of buffaloes. **Safinaz *et al*., (2006) and Ahmad *et al*., (2007)** found significant increase in sodium, on the other hand potassium, calcium and phosphorus were significantly decreased. **Guha *et al*., (2010)** reported a significant elevation in sodium, chloride and decrease in potassium in milk of buffaloes with sub clinical mastitis.

The probable reason for these changes in mineral contents in mastitic milk may be due to the break down of the secretary epithelium, causing blood constituents to appear directly into milk by passing between the cells as a result of break down of the junctional complexes that normally hold the secretary cells together. Moreover infections of the mammary glands with microorganisms that produce mastitis have a profound effect on the synthesis of milk and its composition. In severe mastitis, with breakdown of secretary epithelium, blood component can appear in milk, resulting in high amounts of sodium and reduction of other minerals **Ahmad *et al*., (2007)**. **Safinaz *et al*., (2006)** was mentioned that inflammation of udder caused disturbance in milk minerals.

Concerning heavy metals content in milk, table (8) showed significant decrease in iron, copper and zinc content in mastitic milk compared to healthy group. These results were in agreement with **Rawdat and Omima (2000), Safinaz *et al*., (2006)** and **Ahmad *et al*., (2007)** who recorded significant decrease in iron, zinc and copper in buffaloes mastitic milk. This decrease may be attributed to disturbance in absorption of these elements from gastro intestinal tract or due to loss of appetite in mastitic animal **(Safinaz *et al*., 2006)**.

It is concluded from the present study:

* The recurrent mastitis attributed to the miss use of antibacterial agents as well as treatment without determining the actual microbe causing mastitis.
* PCR is considered a new technology that confirms the identification of *S.aureus* as well as using of specific species primer.
* On the basis of the present study it can also be concluded that there was a remarkable variation on total proteins, fat, minerals and essential heavy metals between mastitic and normal milk.
* Mastitis lowered the food value of milk in term of reduction in fat, potassium, calcium, phosphorus, iron, copper and zinc content and increase in sodium and chloride levels.
* The isolated *S.aureus* strains were highly sensitive to cefoperazone, cefataxine and ciprofloxacin.

Table (1). The number of cases of recurrent mastitis in relation to period.

Period of study	Time of study	Total No. of examined samples	Recurrent mastitis cases		No. of Repetitions			
			No.	%	2		3	
					No.	%	No.	%
I	From march to June	80	13	16.25	9	69.23	4	30.77
II	From July to October	45	9	20	5	55.55	4	44.44
III	From November to February	67	19	28.35	12	63.16	7	36.84
Total		192	41	21.35	26	63.41	15	36.58

Table (2). Occurrence of different bacteria species isolated from recurrent Mastitis.

Type of infection	Isolated organism	No. of isolates	%
Single infection	*S.aureus*	31	75.6
Double infection	*S.aureus+S.citrus*	3	7.3
	S.aureus+streptococcus spp.	2	4.9
	S.aureus +E.coli	1	2.4
Triple infection	*S.aureus+S.citrus+E.coli*	4	9.8
Total		41	100

Table (3). The characteristic features of the examined *S.aureus* isolates.

Serial	Characteristic features (41 isolates)	Positive		Negative	
		No.	%	No.	%
1	Colony pigment				
	White	2	4.88	39	95.12
	Creamy	11	26.83	30	73.17
	Golden yellow	28	68.29	13	30.71
2	Hemolytic activity				
	Sheep blood	39	95.12	2	4.88
	Human blood	9	21.95	32	78.05
3	Gelatinase activity	39	95.12	2	4.88
4	Catalase test	41	100	0	0
5	Novobiocin Sensitive	41*	100	0	0
6	Crystal violet medium				
	violet	9	(21.95%)	32	78.05
	yellow	26	(63.41%)	15	36.59
	white	6	(14.63%)	35	85.37
7	Mannitol production	41	100	0	0

Table (4). Prevalence of staphylococci from mastitic milk in correlation to agglutination and pathogenicity in mice.

Types of isolates	No. of isolates	Latex test		Mortality in mice	
		No. of +ve isolates	No. of -ve isolates	No. of Survived mice	No. of dead mice
S.aureus	41	38	3	6	76
S. citrus	7	0	7	14	0

Table (5). Results of antibiogram of 41 *S. aureus* isolates

Antimicrobial agents	**Sensitive**		**Resistant**	
	No.	**%**	**No.**	**%**
Amoxicillin	11	26.831	30	73.17
Amoxicillin + clavulonic acid	12	29.27	29	70.73
Ampicillin	2	4.88	39	95.12
Cefoperazone	40	97.56	1	2.44
Cefataxime	36	87.80	5	12.70
Ciprofloxacin	31	75.61	10	24.39
EnroFloxacim	4	9.76	37	90.24%
Gentamicin	13	31.71	28	68.29
Neomycin	16	39.02	25	60.98
Ofloxacinne	27	65.85	14	34.15
Tobramycin	28	68.29	13	31.71

Table (6). Total Proteins and fat concentrations in milk from apparently healthy and recurrent mastitic buffaloes.

Parameters	**Apparently healthy**	**Recurrent mastitis**
Total proteins (g/d1)	4.06±0.10	4.7^{**}±0.21
Albumin (g/dl)	0.66±0.07	0.83^{*}±0.04
Fat %	6.32±0.29	5.3^{**}0.09

Table (7). The content of minerals in milk of apparently healthy and recurrent mastitic buffaloes

Parameters	**Apparently healthy**	**Recurrent mastitis**
Na (mEq/L)	81.09 ± 1.70	92.47^{**} ± 1.7
K (mEq/L)	145.43±3.50	126.2^{**}±2.28
Ca(mg/dl)	40.43±0.86	28.59^{**}±0.83
Ph(mg/dl)	44.41±0.99	36.05^{**}±0.815
Cl (mg/dl)	117.88± 2.83	126.43^{**}±2.93
Mg (mg/dl)	7.95±0.09	7.73±0.08

Values represent means ± standard errors * Significantly different ($p<0.05$)
** Significantly different ($p<0.01$)

Table (8). Essential heavy metals in milk of both apparently healthy and recurrent mastitis buffaloes.

Parameters	Apparently healthy	Recurrent mastitis
Fe (ppm)	1.9 ± 0.24	1.14* ± 0.17
Cu (ppm)	0.86 ± 0.08	0. 68* ± 0.02
Zn (ppm)	2.72 ± 0.26	1.5** ± 0.093
Pb (ppm)	N.D	N.D

Values represent means ± standard errors.
** Significantly different (P<0.01).
* Significantly different (p<0.05)
N.D not detected

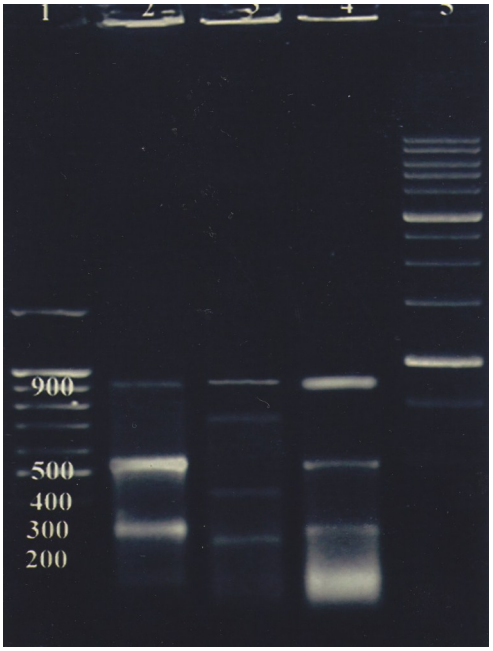

Photo. (1). DNA banding patterns following amplification with primer (1)
Lanes (1and 5) ladder, lanes (2,3 and 4) *S.aureus* isolates.

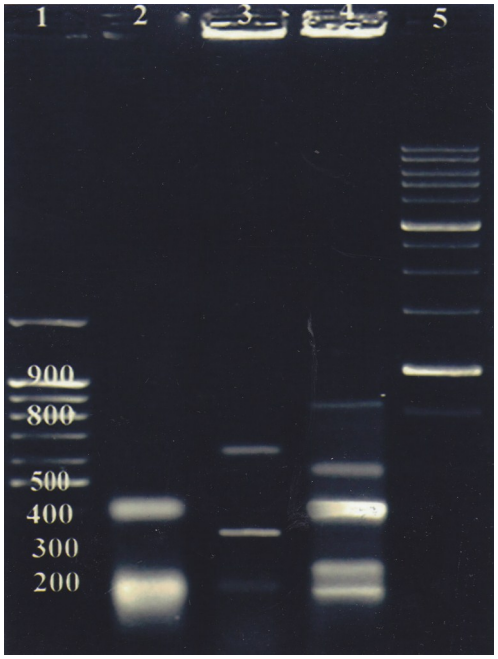

Photo. (2). DNA banding patterns following amplification with primer (2)
Lanes (1 and 5) ladder, lanes (2,3, and 4) *S.aureus* isolates.

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دراسات بكتريولوجية وبيوكيميائية على التهاب الضرع المتكرر فى الجاموس

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الملخص العربى

وجد ان الميكروب العنقودى الاكثر شيوعا فى حالات التهاب الضرع المتكرر فى الجاموس الحلاب . اظهرت النتائج خلال فترة البحث ان 41 معزولة تم تصنيفها حسب الشكل الظاهرى والبيوكيميائ الى الميكروب العنقودى الذهبى بالاضافة الى 14 عترة من الميكروب العنقودى الاصفر والميكروب الاشيريشيا القولونى .

من بين المعزولات العنقودية وجد ان 38من بينهم ايجابى لاختبار التلزن . وقد تم دراسة العلاقة بين اختبار التلزن واحداث النفوق فى الجرزان وتم مناقشة النتائج وكانت تشير الى ان كل العترات الايجابية لاختبار التلزن كانت ممرضة وادت الى نفوق الجرزان بنسبة 100% . تم اجراء اختبارات الحساسية للمضادات الحيوية المختلفة وكانت النتائج تشير الى ان الميكروب العنقودى الذهبى عالى الحساسية لكل من سيفوبيرازون, سيفوتاكسيم, سيبروفلوكساسين , توبراميسيين والافلوكساسيين. وباستخدام اختبار البلمرة المتسلسل العشوائى وجد ان الميكروب العنقودى الذهبى لها 4 انواع من الاوزان الجزئية .

والتحليل الكيميائى لعينات اللبن المجمعة من التهاب الضرع وجد اختزال فى مكونات الدهون , البوتاسيوم ,الكالسيوم ,الفوسفور , الحديد , الزنك , النحاس وزيادة فى البروتين الكلى والالبيومين والصوديوم والكلوريد