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**BOVINE MASTITIS
(AGE, CAUSES AND CONTROL)
IN ASSIUT GOVERNORATE**
(With 4 Tables and 4 Figures)

By

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**التهاب الضرع فى الابقار فى محافظة أسيوط
(السن - الاسباب - الوقاية)**

صديق رشوان

أجريت هذه الدراسة على ٥٠ عينة لبن تم أخذها من أبقار مصابة بالتهاب الضرع الشديد فى مزارع مختلفة بمحافظة أسيوط ، وقد تم دراسة الاعراض الكلينية لهذا المرض ، وتم عزل ٦١ عزلة بكتيرية ، ٢١ عزلة فطرية . وكانت نسبة العزلات كالتالى ٢١ عزلة للميكروب العنقودى الذهبى بنسبة (٣٢,٧٨٪) والميكروب العنقودى الجلدى ٨ (١٣,١٢٪) والميكروب السبحى الاجلجتيا ٨ (١٣,١٢٪) والميكروب السبحى الديس اجلاتيا ٣ (٤,٩١٪) والميكروب القولونى ١٥ (٢٤,٥٩٪) وميكروب الكورنى باكتريم بيوجينز ٧ (١١,٤٨٪) كذلك تم عزل ٦ عزلات للاسبيرجلس فار كلومينارس بنسبة (٢٨,٥٧٪) و ٨ عزلات للكنديديا (٣٨,١٪) و ٣ عزلات للبنسليم كيريزوجينم (١٤,٢٨٪) وثلاثة عزلات للبنسليم سيكلوبيم (١٤,٢٨٪) وعزلة واحدة للكنديديم جيوتيركم (٤,٧٧٪) . وباجراء اختبار الحساسية وجد أن معظم العزلات البكتيرية شديدة الحساسية لكل من اللجنتاميسين والكلورمفينيكول والاريثروميسين وقليلة الحساسية للنتراسيكلين ومقاومة لكل من الكلوستون ونالدسك أسد والاسترتوميسين ، وبتجريب تأثير محلول الثوم المائى باضافته الى الوسط الغذائى بتركيز ٢,٥٪ ، ٥٪ ، ١٠٪ على الفطريات والخمائر المعزولة وجد أن تركيز ٢,٥٪ له تأثير مثبط واضح على كل من الاسبرجلس فار كلومينارس والكنديديم وأن تركيز ٥٪ له تأثير واضح على كل الفطريات والخمائر ماعدا الكنديديم جيوتيركم بينما التركيز ١٠٪ من محلول الثوم المائى كان له تأثير مثبط على جميع الفطريات والخمائر المعزولة.

SUMMARY

This study was carried out on 50 cows clinically affected by acute mastitis in different farms in Assiut Governorate. The clinical symptoms of affected animals have been discussed. The isolated organisms in this study were 61 bacterial isolates and 21 isolates of yeast and fungi, the percentage of bacterial isolates in total infected cases were *Staphylococcus aureus* 20 (32.78%), *Staphylococcus epidermidis* 8(13.12%) *Streptococcus agalactiae* 8(13.12%). *Streptococcus dysagalactae* 3(4.91%) *E.coli* 15(24.59%) and *C. pyogenes* 7(11.48%). The isolated yeast and fungi were *Aspergillus flavous* 6(28.57), *Pencillium chrysogenum* 3(14.28%) *Pencillium cyclopium* 3(14.28), *Candida speices* 8(38.1%) and *Geotricum Candidium* 1(4.77%). Sensitivity test was carried out by different types of antibiotics, the most isolated bacterial organisms were highly sensitive to *Gentamycine*, *Chlormphinicol* and *Erythromycine* while they were less sensitive to *Tetracycline* and resistant to *Colistome*, *Naldizie Acid* and *Streptomycine*. *Aquous Garlic* solution was experminted to determine its effect on isolated yeast and fungi by using different concentration (2.5%, 5% and 10%) of Garlic solution. It was found that 2.5% of Garlic is effective on *Aspergillus* and *Candida sp*, 5% more effective on all species except *Geotericum Candidium*, while 10% concentration causes complete inhibition for all types of fungus and yeast isolates.

Key words: *Bovine masitis in Assiut*

INTRODUCTION

Mastitis is of a great economic importance in farm animals. The disease remains one of the most important causes of tremendous loss in milk yield in many countries. The economic significance of the disease varies among herds and to some extent depends on the system of management and the degree of intensification (Blood *et al.*, 1983), it reduces production of milk with an average 225\$ per caw each year (Philip, 1991). In addition mastitis may have some public health importance since

occasionally milk harbouring human pathogens may cause infection to consumers of raw or inadequately heated milk.

In concerning clinical symptoms the affected animal suffering from a rise of body temperature, swelling of udder, hotness, hardness, some times the animal refused the food. the secreted milk becomes watery, clotted, gelatinous and some times tinged with blood or bloody (Zaitoun *et al.*, 1991 and Naser and Abdel-Moghney, 1994).

The main causative agents of mastitis were *Staphylococcus aureus* followed by *E.coli*, *Streptococcus species* and *Corynebacterium pyogens* (Waage and Aursio, 1992) and Ameh *et al.* (1993). Mycotic mastitis in dairy cow, either alone or in association with other pathogens has gained importance in recent years. Various fungi including *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus terreus*, *Cladosporium species*, *Pencillium species* as well as *Candida species* are frequently associated with bovine mastitis (Misra and Panda, 1986).

In case of treatment and antimicrobial activity Char *et al.* (1993) revealed that (85.38-90.8%) of bacterial isolates were sensitive to *Gentamycine* and *Chlorumphenicol* while pencillin was the least effective.

* From the previously mentioned the aim of the present work was the isolation and identification of bacterial or mycotic causitive agents of bovine mastitis.

* Antimicrobial activity and treatment of affected mimals.

MATERIAL and METHODS

1. Animals:

This study was carried out on 50 caws clinically affected with acute mastitis, these animals were classified according to age to 3 groups:

- A) First group (21) Animals aging between 2½-4 years).
- B) Second group (14) Animals aging between 4-6 years).
- C) Third group (15) Animals aging above 6 years).

2. Collection of Samples:

Milk samples were collected aspetically according to the known routine procedure. The udder was throughly washed with running water and dried with clean towel, then the teats sprayed with 70% ethonol. After that the first few squirts of milk was

discarded then 5 ml of milk samples from each quarter was collected in a sterile McCartney bottle. All samples were cooled at (4°C) and transported to the laboratory.

The milk samples were activated by incubation for 12 hours at 37°C. Then milk samples centrifugated at 300RPM for 30M. before bacteriological cultivation.

3. Isolation and Identification:

A) Bacterial causes:

10 ul from milk samples was streaked on the surface of a blood agar plate and MacConkeys agar plate and incubated at 37°C for 48hrs. Purified isolates were identified according to their different feature which included, morphological, biochemical characters, Cown and Steel (1973), Chruichshank et al., (1975) Coles (1986) and Collee, et al. (1989).

B) Fungal causes:

Part of the centrifugated milk samples was cultured on Sabourad's dextrese agar (Moss and Mcquown, 1979) containing Chlormphenicol and Cyclohexamide (0.5g/L for each) to inhibit the bacterial growth and to minimize the development of some saprophytic fungi. Cultured plates were incubated at 25°C and examined daily until growth become evident.

Identification of fungal colonics was made depending on macroscopic appearance of fungal colonies, colour, and microscopic characters (Shape and dimensions of Conidia and chlamydospores, mode of hyphal branching) (Raper and Feenel, 1965 and Frey et al., 1979).

The antibiogram of the isolated organisms were applied using several types of antibiotics by disc diffusion method described by Stokys, (1986). These antibiotics were *Gentamycine* (10 ug), *Erythromycine* (10 ug), *Chlormphenicol* (30 ug), *Rifampicine* (30 ug), *Teteracycline* (30 ug), *Ampicillin* (10 ug), *Flumaquine* (30 ug), *Colistone* (10 ug), *Naldizic acid* (30 ug) and *Streptomycine* (10 ug).

In referring to yeast and mycotic isolates. The isolates were inoculated in S.D.A. plates containing (2.5%), (5%) and (10%) Garlic aqueous solution and incubated at 28°C till the colonial groth become evident. The degree of inhibition was compared to control one.

RESULTS

The obtained results were tabulated in Tables 1, 2, 3 & 4 and Figures 1, 2, 3 & 4.

DISCUSSION

Mastitis or inflammation of the udder has a direct effect on the quality and quantity of milk, the main clinical signs of mastitis were painful swelling, hotness, hardness of udder, rise of body temperature and reduced milk yields. The mastitic milk of infected quarter was pale and yellowish in colour in *E. coli* infection and blood or tinged with blood in *Staphylococcus aureus*, in case *Streptococcus sp.*, the milk was gelatinous with clots, while in *Corynebacterium* the milk contained pus and straw yellow and mucoid in mycotic infection. These clinical symptoms in agreement to that mentioned by Zaitoun *et al.* (1991) and Nasar and Abdel-Moghney (1994).

In the Table (1). The obtained results cleared that the main bacterial isolates were *Staphylococcus aureus*, *E. coli*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Corynebacterium pyogens* their percentage in total infection were 20 (32.78%), 15 (24.59%), 8(13.12%), 8(13.12%) 3(4.91%) and 7(11.48%) respectively. These results were nearly similar to that previously mentioned by Ahmed *et al.*, (1988), Ameh *et al.*, (1993) Kothe *et al.* (1993), Waage and Aursio (1992) and Chotiah (1993), while, Ibsam *et al.* (1993) stated that the predominant organisms were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *E. coli* with Lesser percentage (23.56%), (9.77%) and (6.32%) respectively.

From table (1). In the 1st group (2½-4 years) and 2nd group (4-6 years) *Staphylococcus aureus*, *E. Coli*, *Staphylococcus epidermidis*, and *Streptococcus agalactiae* represent the main causative agents and were isolated with high percentage (35.48-31.25%), (25.91-25%), (16.13-12.5%) and (12.90-18.75%) respectively in comparing with lesser percentage in 3rd group above 6 years (18.58%), (21.42%), (7.14%) and (7.14%). This results insure that IMI (Intermammary infection) increased in age

between 2½-4 years in 1st and 2nd group (4-6 years) and decline above 6 years (3rd group).

These results were nearly similar to that recorded by Rober *et al.* (1994), Coasta *et al.* (1994), Fox *et al.* (1995) and Lescort, *et al.* (1995) whose mentioned that *Staphylococcus aureus*, *E. Coli*, *Staphylococcus sp.*, and *Streptococcus agalactia* were the most prevalent causative agent at early age.

Smith (1983); Waagee and Aursio (1992) and Nickroson *et al.* (1995) isolated *Staphylococcus epidermidis* and *E. coli* from clinically affected dairy heifers at breeding age (2-5 years) and their percentage were (15%) and (24%) respectively. These results were nearly similar to that recorded in this study in 1st and 2nd group.

From this study, the presence of mixed infection mainly *Staphylococcus aureus*, *staphylococcus epidermidis* with *E. coli*, demonstrate the complexity of the disease and this result agree to that obtained by Jain (1979) who stated that *Staphylococcus aureus* may predispose the dairy herd to infection by coliform organisms or other pathogens.

The high incidence of *Staphylococcus aureus* and *E. coli* may be attributed to heavy contamination of bedding, housing, food materials, water, air and equipments. Also *Staphylococcus aureus* was isolated from teat skin, external orifices of teats and teat canal before and after parturition. Also *Staphylococcus aureus* has wide spreading among seasonal year except summer. the over crowded, increase housing time, and increase the moisture, humidity increase IMI with *E.coli* pathogens, this was supported by Roberson *et al.* (1994).

The high incidence of *Corynebacterium pyogens* (28.58%) in 3rd group incompared to lower percent in 1st and 2nd group (6.45-6.25%) attributed to that most of these samples were taken in summer and later age of animals, this was supported by Untermann (1965), Yass *et al.* (1983) and Waage and Aursio (1992) whose mentioned that the prevalence of IMI with *Corynebacterium pyogens* increased with lactation number (9 lact-number) and age of animal up to (9-10 years).

Our results cleared that lower incidence of *Streptococcus agalactiae* in 1st and 2nd group (12-90, 18.75) agree to that

obtained by Schukken *et al.* (1993) who mentioned that *Streptococcus agalactiae* was fallen in old years.

The results of isolated yeast and fungal pathogens from mastitic milk of caws as identified in table (2) were *Aspergillus flavous* (28.57%), *Pencillium Chrysogenum* (14.28%), *Pencillium cyclopium* (14.28%), *Candida sp.* (38.1%) and one isolates of *Geotricum Candidium*. *Aspergillus flavous* and *Candida sp.*, represent the main causitive agents of mycotic and yeast mastitis, where *Candida sp.* isolated in pure culture from 8 cases and *Aspergillus* isolated in pure culture from 4 clinical cases. These obtained results in table (2) were in agreement to that mentioned by Misra and Banda (1986); Gammoudi *et al.* (1992); Zaitoun *et al.*, (1991); Kothte *et al.* (1993) and Guhad *et al.* (1995).

The high incidence of *Candida sp.* (44.45%), *pencillium Chrysogenum* (22.22%) and *Aspergillus flavous* (33.33%) in 3rd group than that in 1st and 2nd group insured that IM by yeast and fungi decreased in younge age (2½-6) and increased in later age above 6 years in 3rd group as in Table (2). This agree to that obtained by Sreeramulu *et al.* (1992) who mentioned that the incidence of mycotic and yeast mastitis increased with age (8-10 years).

The variation in isolated causitive agents (bacterial, fungus and yeast) of acute mastitis were attributed to age of animal, Immune deffensive mechanism of the udder, laction stage, heavy contaminated bedding, season 0, dirty udder, high line milking machine, wide sprcading of flies among the animal form, and over crowded house Waage and Aursio (1992), Sreeramulu *et al.* (1992), Bortlett and Miller (1993) and Liebisch, *et al.* (1994).

In refering to treatment sensitivity was carried out on several types of antibiotics using dise diffusion method as in (Table 3). The results indicate that most bacterial isolates *Staphylococcus aureus*, *Staphylococcus epiderndis*, *Streptococcus agalactiae* and *Corynebacterium pyogens* which represent the major causes of mestitis were highly sensitive to *Gentamycine*, *Erythromycine* and *Chlormphenicol* except *Corynebacterium pyegenes* which was resistant to *Chlormphenicol*. Also these organisms were less sensitive To *Rifampcine* and *Ampicilline* and *Flumaquine* and *Teteracycline* (30 mg) and resistant to *Colistone*, *Naldizic acid* and *Steptomycine* (Table 3). these results were supported by Saxena,

et al. (1993), Char et al. (1993), Kothe et al., (1993) and Gentilini et al. (1994). The field treatment of infected cow with *Gentamycine* for 6 days give good results (more than 90% of clinical cases were cured). This agree with reported by Saxena et al. (1993) who mentioned that all cows were treated with *Gentamycine* were cured (100%). In table (4) both isolated yeast and fungi were *Aspergillus flavous*, *Candida sp.*, and *Penicillium* (*P. Chrysogenum* and *P. Cyclopium*) which represent the main cause of bovine mycotic mastitis were highly sensitive to *Garlic* solution 5% and 10% while *Candida sp.* and *Aspergillus flavous* the most prevalant cause were highly sensitive to 2.5% of *Garlic* solution. These results agree to that obtained by El-Badry and Sokkar (1988), Ziatoun, et al. (1991) and El-Shanawany (1993).

In conclusion, mastitis in dairy form still present one of the commonest and serious diseases, so more efforts must be done to eleminat the accurence of that problem which represent good mongement practices such as milkers hygine, sanitization of milking machine, healthy enviroment as well as controlling the other pridisposing disease should be put in consideration among the major prophylactic measures. Identification of the causitive agent and sensitivity testing are very important in the tratment of mastitis and elemenation of the disease. Also teat dipping with suitable dairy antiseptic and scrubbing of teat orifice with alcohol soaked pad diminshed the prevalance of the disease.

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Fig. [1]: Bacterial causes and their incidence in bovine mastitis.

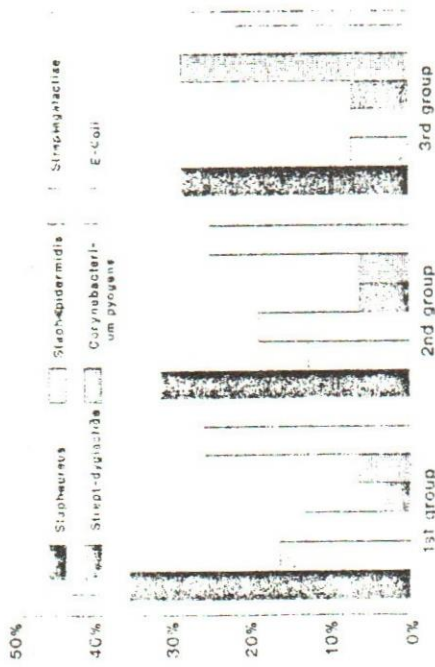


Fig. [2]: Bacterial causes and their incidence in bovine mastitis.

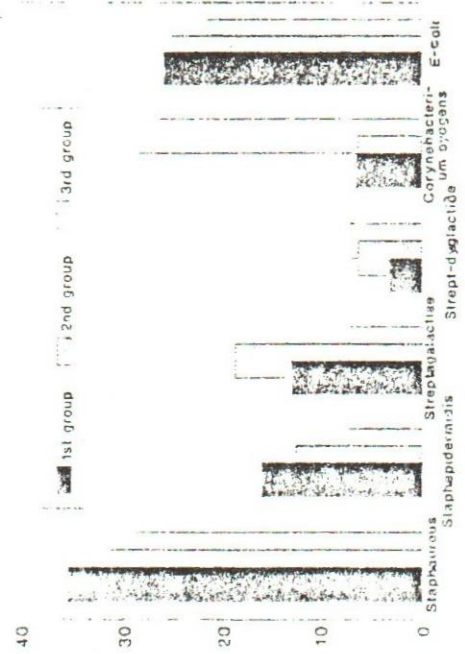


Fig. [3]: Yeast and Mycotic causes and their incidence in bovine mastitis

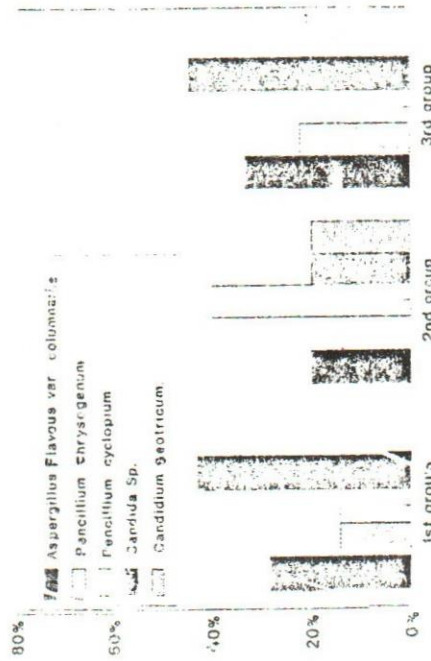


Fig. [4]: Yeast and Mycotic causes and their incidence in bovine mastitis

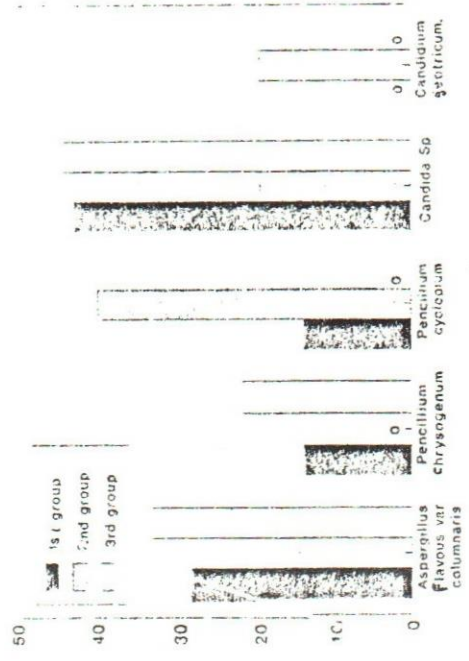


Table (1): Number and percentage of isolated bacteria from mastitic cow milk in relation to age.

Isolates	First group age (2½-4 years) 21 cases		2nd group age (4-6 years) 14 cases		3rd group age (above 6 years) 15 cases		Total Number of Isolates	
	No	%	No	%	No	%	Total No	%
<i>Staphylococcus aureus</i>	11	35.48	5	31.25	4	28.58	20	32.78
<i>Staphylococcus epidermidis</i>	5	16.13	2	12.5	1	7.14	8	13.12
<i>Streptococcus agalactiae</i>	4	12.90	3	18.75	1	7.14	8	13.12
<i>Streptococcus dysgalactiae</i>	1	3.23	1	6.25	1	7.14	3	4.91
<i>Corynebacterium pyogenes</i>	2	6.45	1	6.25	4	28.58	7	11.48
<i>E.coli</i>	8	25.81	4	25	3	21.42	15	24.59
Total Isolates	31	50.82	16	26.23	14	22.95%	61	-

Table (2): No and percentage of isolated yeasts and fungi from mastitic milk in relation to age.

Isolates	First group aging (2½-4 years) 21 cases		2nd group aging (4-6 years) 14 cases		3rd group aging (above 6 years) 15 cases		Total Number	
	No	%	No	%	No	%	No	%
<i>Aspergillus flavous</i> var <i>columnaris</i>	2	28.58	1	20	3	33.33	6	28.57
<i>Penicillium chrysogenum</i>	1	14.28	-	-	2	22.22	3	14.28
<i>Penicillium cyclopium</i>	1	14.28	2	40	-	-	3	14.28
<i>Candida</i> sp	3	42.86	1	20	4	44.45	8	38.1
<i>Candidium geotricum</i>	-	-	1	20	-	-	1	4.77
Total Isolates	7	33.33	5	23.81	9	42.86	21	-

Table (3): Antibiogram for isolated bacteria from mastitic cow milk.

Isolates	Antibiotic Sensitivity									
	Gentamycine (10 ug)	Erythromycine (10 ug)	Chloramphenicol (130 ug)	Rifampicine (30 ug)	Tetracycline (30 mg)	Ampicillin (10 ug)	Fluvasigone (30 mg)	Colistone (10 ug)	Nalidixia acid (30 ug)	Streptomycine (10 ug)
<i>Staphylococcus aureus</i>	+++	+++	+++	+++	+	+++	++	-	-	-
<i>Staphylococcus epidermidis</i>	+++	+++	+++	++	-	---	---	-	-	-
<i>Streptococcus agalactiae</i>	+++	+++	++	++	+	++	+	+	-	-
<i>Streptococcus dysgalactiae</i>	+++	+++	++	+	+	-	-	-	-	-
<i>E. coli</i>	+++	+++	+++	++	-	++	+	-	-	-
<i>Cornebacterium pyogenes</i>	+++	+++	-	++	+	+	+	-	-	-

Table (4) Sensitivity of isolated yeast and fungi from mastitic milk of cows to 2.5%, 5% and 10% of aqueous garlic solution.

Isolates	Garlic (2.5%)			Garlic (5%)			Garlic (10%)		
	<i>Aspergillus flavous var columnaris</i>	-	-	-	-	-	-	-	-
<i>Penicillium chrysogenum</i>	+	-	-	-	-	-	-	-	-
<i>Penicillium cycloptium</i>	+	-	-	-	-	-	-	-	-
<i>Candida sp.</i>	-	-	-	-	-	-	-	-	-
<i>Candidium geotricum</i>	++	-	-	+	-	-	-	-	-

- Complete inhibition of yeast and mycotic colony.
 +, ++ The growth of mycotic colony equal and more than control.