رقم البحث (25)

IN VITRO SENSITIVITY OF MASTITIS CAUSING PATHOGENS ISOLATED FROM SOME DAIRY FARMS IN DAKHLIA GOVERNORATE TO CERTAIN ANTIBIOTICS AND ANTISEPTICS COMMONLY USED IN VETERINARY PRACTICES.

BY

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ABSTRACT

This study was carried out in a dairy farm in Dakhlia governorate during the period from June 2009 till end of November 2011 to investigate the occurrence of mastitis and propose a new approach for prevention and control of mastitis in dairy herds. The milking cows were examined clinically and by California mastitis test (CMT) for detection of subclinically mastitic quarters then individual quarter milk samples were taken separately from each cows for isolation and identification of mastitis causing bacteria. The *in vitro* sensitivity of bacterial isolates to some antibiotics and antiseptics were done and a control program for mastitis in dairy farms was proposed. Results revealed the *in vitro* sensitivities of bacterial isolates from mastitic quarters against certain antibiotics and antiseptics indicated that Enroflxacin followed by Cloxacillin were the antibiotics of choice to use for treatment of clinically mastitic cows and dry cow therapy, respectively meanwhile, Iodine was the antiseptic of choice for teat dipping. A control program based early detection and treatment of clinically mastitic cows, dry cow therapy and sound husbandry practices particularly milking time hygiene accompanied by post milking teat dips for all milking cows significantly reduce the extent and severity of mastitis in dairy herds, besides mitigation the rates of new intramammary infection and eliminate existing infection in both treated groups.

INTRODUCTION

Bovine mastitis is one of the most costly diseases in dairy farms which associated with many losses including; discarded milk, increased number of culled cows, cost of antibiotic treatment and reduced milk quality and price (**Kagkli et al. 2007).**

Mastitis causing pathogens classified as "contagious" and "environmental" based on the most common sites of exposure. The most common contagious mastitis pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae, Mycoplasma bovis* and some strains of *Strep. uberis* in which the primary reservoir for the infection is the infected udder meanwhile environmental mastitis pathogens include coliform bacteria such as; *E. coli* and *Klebsiella* spp. and environmental streptococci such as; *Streptococcus uberis* and *Streptococcus dysgalactia* in which the mud and manure are the reservoirs **(Zadoks , 2003)**.

Key elements in the control of mastitis include, sound husbandry practices and sanitation, post-milking teat dipping, treatment of mastitis during non-lactating period and culling of chronically infected animals. Controlling environmental mastitis can be achieved by reducing the number of bacteria to which the teat end is exposed. Post milking teat dipping with a germicidal dip is recommended. To control the environmental mastitis during dry period, proper antibiotic therapy is recommended for all quarters of all animals at drying off (**Khan and Khan, 2006).**

MATERIAL AND METHODS

Study area and period: This study was carried out in two private dairy farm located in Gamasa district; Dakhlia governorate during the period from June 2009 till the end of November 2011.

Study design: The work was planned to determine the antibiotic and antiseptic of choice to be used for dry cow therapy and milking teat dip.

I. Samples collection: Samples were collected from dairy cows including teat apex swabs& individual quarter milk samples from all milking cows in the farm. Before sampling, udders of cows were examined clinically and by using screening test (CMT) for detection of clinical & subclinical mastitic cows, respectively (**Schalm et al., 1971).**

II. Diagnosis of mastitis: Udders and teats of all milking cows were clinically examined then CMT used for detection of subclinical mastitic quarters as technique described by **Schalm et al. (1971)**, individual quarter milk samples were collected aseptically from both apparently normal and clinically mastitic quarters of milking cows as method described by **More (1989)**. Isolation and identification of mastitis causing pathogens was done as methods employed by **Cruickshank et al. (1980)**. Serotyping of *E. coli* isolates was carried out in the central laboratory of the Ministry of Health, Cairo, according to techniques adopted by **Sojka (1965).**

III. *In vitro* sensitivity of bacterial isolates to tested antibiotics:

Minimal inhibition concentration (MIC) values were applied to evaluate the sensitivity of bacterial isolates (*Staph.aureus, CNS, Strept. Agalactiae, Strept.*

Uberis and E. coli ; O157, O55&O103) to 13 different antibiotics commonly used in mastitis prevention and control were tested in this study including; penicillin-G (10 µg), chloramphenicol (30 µg), gentamycin (10 µg), enrofloxacillin (10µg), streptomycin (10µg), colistin (25µg), cloxacillin (30µg), neomycin (30µg), erythromycin (15µg), kanamycin (30µg), amikacin (30µg), amoxycillin (30µg) and ampicillin (30µg).

A representative field strains isolated from mastitic cows were purified and identified biochemically and serologically then tested *in vitro* for their sensitivities to different types of antibiotics and commonly used in veterinary field using standard procedures as described by **Quinn et al. (1994)**.

IV. In vitro sensitivities of bacterial isolates to tested antiseptics.

Three commercial types of antiseptic commonly used as teat dips in veterinary practice and listed with the Food and Drug Administration (FDA) and are of proven effective under field conditions including: Chlorhexidine gluconate (**0.5%Teisen products Ltd, Worcestershire,UK**); Bovadine iodine (**7.5% available iodine, Downland Marketing Ltd, Carlisle,UK**) and Sodium Hypochlorite **(4% available chlorine, Emprasan Chemicals Ltd, Merseyide, UK)**. Different dilutions of antiseptics were used based on previous studies (**Jasper, 1976**).

RESULTS AND DISSCUSION

Table (1): Antibiotic sensitivities of different bacterial isolates from mastitic cows'quarters in vitro, using Minimum Inhibitory Concentration (MIC)

Antibiotic tested / Bacterial isolates	*Staph. aureus*	CNS.	*Strept. agalactiae*	*Strept. Uberis*	*E. coli*		
					O157	O103	O55
Enrofloxacin (10 µg)	++	++	++	++	++	+	+
Cloxacillin (30 µg)	++	++	+	++	++	++	+
Gentamycin (10 µg)	++	++	+	++	++	+	+
Chloramphenicol (30 µg)	++	++	+	++	+	+	-
Kanamycin (30 µg)	++	+	++	++	++	+	+
penicillin-G (10 µg)	-	-	++	+	-	-	-
Ampicillin (30 µg)	-	-	++	+	-	-	-
Streptomycin (10 µg)	+	+	++	++	+	+	-
Colistin (25 µg)	-	-	-	-	-	-	-
Erythromycin (15 µg)	-	-	++	++	+	+	-
Amoxicillin (30 µg)	-	-	-	-	-	-	-
Neomycin (30 µg)	++	++	+	+	+	+	-
Amikacin (30 µg)	+	+	+	+	+	+	-

(++): Sensitive **(+):** Intermediate **(-):** Resistant

Concerning antibiotic sensitivities of different bacterial isolates from mastitic quarters, Table (1) showed that enrofloxacin followed by cloxacillin, gentamycin and kanamycin were the most effective antibiotic against the majority of bacterial isolates, after while the less effective antibiotics were streptomycin followed by neomycin, chloramphenicol, amikacin, ampicillin, erythromycin and penicillin- G. On other hand all bacterial isolates showed resistance to colistin and amoxicillin.

These results revealed that *Staph. aureus* was highly susceptible to enrofloxacin, cloxacillin, gentamycin, chloramphenicol, kanamycin and neomycin, while intermediately sensitive to streptomycin and resistant to other tested antibiotics, which are in agreement with **Adwan (2006).** On other hand, Coagulase Negative Staphylococci (CNS) were sensitive to enrofloxacin, cloxacillin, gentamycin, chloramphenicol and neomycin, while intermediately

sensitive to kanamycin and streptomycin and resistant to other antibiotics, this finding are nearly similar to that determined by **Sawant et al. (2009).** Regarding; *Strept. agalactiae* was more susceptible to enrofloxacin, kanamycin, penicillin- G and ampicillin, while intermediately sensitive to gentamycin, chloramphenicol, cloxacillin, neomycin and amikacin but resistant to colistin and amoxicillin, these results were similarly to previous results of **Malinowski et al. (1997).** *Strept. uberis* was susceptible to enrofloxacin, cloxacillin, gentamycin, chloramphenicol, kanamycin, streptomycin and erythromycin, while intermediately sensitive to penicillin- G, ampicillin, neomycin and amikacin but resistant to colistin and amoxicillin.

Regarding testing of *E. coli* isolates, *E. coli* (O157) was highly susceptible to enrofloxacin, cloxacillin, gentamycin and kanamycin, while intermediately sensitive to chloramphenicol, streptomycin, erythromycin, neomycin and amikacin but resistant to penicillin- G, ampicillin, colistin and amoxicillin. On other hand, *E. coli* (O103) was highly susceptible to cloxacillin and intermediately sensitive to enrofloxacin, gentamycin, chloramphenicol, kanamycin, streptomycin, erythromycin, neomycin and amikacin while resistant to other tested antibiotics. *E. coli* (O55) was resistant to all tested antibiotics, but intermediate to enrofloxacin, cloxacillin, gentamycin, and kanamycin. These results are similarly to results of **Kalmus et al., (2011).**

Table (2): In vitro antiseptic sensitivities of different bacterial isolates from mastitic quarters in farms I &II using minimum inhibitory concentration (MIC).

Germicide		**Bacterial isolates**						
Product	**dilution**	**Staph. aureus**	**CNS.**	**Strept. Agalactiae**	**Strept. Uberis**	**E.coli**		
						O103	**O157**	**O55**
Iodine	0.062	-	+	+	+	+	-	+
	0.125	-	+	+	+	+	+	+
	0.25	-	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+
Sod. Hypochlorite	0.5	-	-	+	+	-	-	+
	1.0	-	-	+	+	+	+	+
	2.0	-	+	+	+	+	+	+
	4.0	+	+	+	+	+	+	+
Chlorhexidine-gluconate	0.031	+	+	+	+	-	-	-
	0.062	+	+	+	+	-	-	-
	0.125	+	+	+	+	+	+	-

(+): Sensitive **(-):** Resistant

Results in table (2) concerning the *in-vitro* sensitivity of seven bacterial strains isolated from quarter milk samples of dairy cows against three antiseptics; Bovadine iodine, Chlorhexidine gluconate and Sodium hypochlorite. All bacterial isolates were sensitive to iodine with different dilutions (0.5, 0.25, 0.125 and 0.062%) except *Staph. auraus* was sensitive only to 0.5% iodine, while *E. coli* (O157) was resistant only to 0.062% iodine. On other hand all bacterial isolates were resistant to 0.5% Sodium hypochlorite except *Strept. agalactiae*, *Strept. uberis* and *E. coli* (O55), meanwhile all bacterial isolates were sensitive to Sodium hypochlorite with dilutions; 1.0, 0.2 and 0.4%, except *Staph. auraus* was resistant to Sodium hypochlorite 0.1 and 0.2% while CNS was resistant to 0.1% Sodium hypochlorite only. Meanwhile all bacterial isolates were sensitive to different dilutions of chlorhexidine gluconate (0.031, 0.062 and 0.125%), except *E. coli* isolates which were resistant to chlorhexidine gluconate, while *E. coli* (O157 & O103) were sensitive only to 0.125% chlorhexidine gluconate.

These results proved that all bacterial isolates were sensitive to 0.5% iodine. This indicates that iodine is antiseptic of choice for pre-milking and post-milking teat dip. These findings were in agreement with **Foret et al., (2005)** who stated that iodophors represented more than 90.0% of post milking teat antiseptics which used for control of mastitis in dairy farms.

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الملخص العربي

اختبار حساسية المسببات المرضيه لالتهاب الضرع المعزوله من بعض مزارع الالبان فى محافظة الدقهلية لبعض المضادات الحيويه والمطهرات الاكثر استخداما فى المجال البيطرى

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أجريت هذه الدراسة خلال الفترة من يونيو٢٠٠٩ وحتى نهاية نوفمبر٢٠١١ في مزرعة أبقار حلابة تقع فى مدينة جمصة بمحافظة الدقهلية بهدف وضع برنامج للسيطرة على المرض لتقديمه كنموذج يمكن تطبيقه فى مزارع الألبان .

وعلى هذا فقد تمت الدراسة

بتحديد معدل انتشار المرض بالقطعان الحلابة وبعض المؤشرات الدالة على حدوث المرض حيث تم فحص جميع الأبقار الحلابة إكلينيكيا وباختبار الكاليفورنيا للكشف عن التهاب الضرع الإكلينيكي وتحت الإكلينيكى على التوالي ثم أخذت عينات من الأبقار (مسحات من الحلمات وعينات ألبان) حيث أجريت الفحوص البكتريولوجى لعزل وتصنيف البكتريا المسببة للمرض بالطرق البيوكيميائية والسيرولوجية .

وقد خلصت النتائج فى هذا الجزء الى مايلى:

اختبار حساسية بعض العزلات البكتيرية من حالات التهاب الضرع لبعض المضادات الحيوية والمطهرات وجد أن الانروفلوكساسين والكلوكساسيلين هى أفضل المضادات الحيوية لعلاج حالات التهاب الضرع وكذلك اليود هو المطهر الأفضل لتغطيس الحلمات قبل وبعد عملية الحلب.