

PREVALENCE OF STAPHYLOCOCCUS SPECIES RECOVERED FROM BOVINE MASTITIS

BY

Ahmed Moustafa Ahmed Moustafa

Animal Health Research Institute , Mansoura Provincial Laboratory

ABSTRACT

Seventy four milk samples represent all affected quarters were taken from forty six dairy cows (holistein breed) suffering from clinical mastitis in a large dairy farm at Dakhalia province – Egypt . The milk samples were examined bacteriologically to study the occurrence of staphylococcus (staph.) microorganisms and determined the susceptibility of coagulase positive staphylococcus for some chemotherapeutics agent. Out of the samples 40 (54.05%) revealed coagulase positive staph. which represent 24(52.17%) mastitic dairy cows , also further identification for coagulase positive staph. isolates revealed that the samples were infected with species of *Staph. aureus* , *Staph. hyicus* and *Staph. intermedius* with percentages 45.94% , 6.46% and 1.35% respectively which represent 21(45.65%) , 3(6.52%) and 1(2.17%) of mastitic cows respectively , on other hand 26(35.14%) of the samples infected with coagulse negative staph . represent 18(39.13%) mastitic dairy cows, also the study showed that the coagulase positive staph. more sensitive to gentamycin , erythromycin , trimethoprim and amoxicillin with percentage 100% , 95% , 92.5% and 65% respectively ,while the organisms less sensitive to sulfamethaxol , oxytetracycline , neomycin and ampicillin with percentage 95% , 72.5% ,62.5% and 52.5% respectively , on the other hand the organisms showed resistance to lincomycin , cefalexin and streptomycin with percentage 100% , 70% and 60% respectively.

INTRODUCTION

Mastitis is one important disease of dairy cattle world wide (**Sargeant et al. 1998**), due to its economic and zoonotic importance ,from economic point of view, the milk production was reduced, discarded milk during treatment, early cow replacement cost, drugs, veterinary service and labour (**Harmon,1995**),also other costs associated with mastitis problems are related to milk quality control, dairy manufacturing, nutritional quality of milk and the losses due to condemnation of the milk and milk products containing antibiotic residues. (**Bramely et al., 1996**). While zoonotic importance attributed to the milk act as a vehicle of infection for some dangerous diseases (**Makar, 1997**). Also *Staph . aureus* cause some infectious diseases in human as skin infection and food poisoning due to ingestion of preformed enterotoxine in milk results in vomiting and diarrhea within 2 to 8 hours (**Hobbs and Roberts,1993**).

Staphylococcus is one of the most important, prevalent and more costly micro-organism which cause clinical and sub-clinical mastitis (**Bramley and Dodd,1984**) and (**Barkema et al.,1998**) . (**Anderson,1976**) reported that different *staph. aureus* strains infected the udder , these organisms had the capacity to penetrate the udder tissue producing deep seated foci and hence intermammary antibiotic therapy was not completely successful in eradication of staphylococcal mastitis.

Staphylococcus are spherical or ovoid , non motile , Gram - positive cells arranged in grape -like clusters on solid media and in pairs ,small groups , or short chains in liquid media .

Therefore this investigation was done to isolate and identify staphylococcal micro-organisms from mastitic cows with special reference to coagulase - positive staph. as well as to determine sensitivity of the isolated coagulase -positive staph. strains to some chemotherapeutic agents

MATERIAL AND METHODS

A total of 74 milk samples were taken from 46 dairy cows (holstein breed) suffering from clinical mastitis in one dairy farm at Dakahlia province . The milk samples were collected aseptically according to Honkanen - Buzalski , 1995 and transported in cool bags to the laboratory and were stored at 4°C and examined bacteriologically .

Bacteriological procedures :-

According to International Commission on Microbiological Specification for Food (ICMSF), 1978, an inoculum of 0.1 ml of each milk sample was spread on to Baird parker medium plates (Oxoid CM 275), incubated at 37°C and examined for bacterial growth after 24 and 48 hours , suspected colonies (Jet black and shiny greater than 1mm in diameter with or without concentric zone of opacity) were picked up, and purified and identified as follows .

1- Microscopical examination :

Gram's stained smears according to Cruikshank et al., 1975

The following tests recorded by Cruikshank et al., 1975, Koneman et al. ,1988 and Quinn et al. , 1994

- a- Haemolysis on blood agar
- b- Catalase test
- c- Coagulase test
- d- Gelatin liquefaction test
- e- Fermentation of glucose , lactose , maltose and arabinose
- f- Growth of manitol salt agar
- g- Pigmentation it was observed on nutrient agar
- h- Acetoin production

Antimicrobial sensitivity test

The susceptibility of the Isolated staph. coagulase positive strains to antimicrobial agents were done on muller hinton agar according to (Cruikshank et al., 1975), using disks (11) of chemotherapeutic agents.(Oxoid).The diameter of inhibition zone of each disk was measured and compared with the standard breakpoints .

RESULTS

Table (1) breakpoints of chemotherapeutics used in vitro susceptibility testing

Chemotherapeutic agent	Disc potency	Zone diameter(mm)	
		High sensitive +++	Low sensitive ++
Gentamycin	10 ug	≥ 21	15-20
Erythromycin	15 ug	≥ 21	17-20
Trimethoprim	5 ug	≥ 16	11-15
Amoxicillin	25 ug	≥ 27	21-26
Sulfamethaxol	23.75 ug	≥ 16	11-15
Oxytetracycline	30 ug	≥ 22	17-21
Neomycin	30 ug	≥ 17	13-16
Ampicillin	10 ug	≥ 22	15-21
Streptomycin	10 ug	≥ 24	21-23
Cefalexin	30 ug	≥ 18	15-17
Lincomycin	15 ug	≥ 23	15-22

Table (2) prevalence of staphylococcus microorganisms in examined mastitic milk samples
(n = 74)

Type of staphylococcus organism	Examined mastitic cows			Examined mastitic quarters		
	Number	Positive	%	Number	Positive	%
Coagulase positive staph.	46	24	52.17	74	40	54.05
Coagulase negative staph.	46	18	39.13	74	26	35.14

Table (3) prevalence of coagulase positive staph. in examined mastitic milk samples (n= 74)

Type of Coagulase positive staph. organism	Examined mastitic cows			Examined mastitic quarters		
	Number	Positive	%	Number	Positive	%
<i>Staph. aureus</i>	46	21	45.65	74	34	45.94
<i>Staph. hyicus</i>	46	3	6.52	74	5	6.76
<i>Staph. intermedius</i>	46	1	2.17	74	1	1.35

N.B. One mastitic cow was infected with mixed infected of *Staph. aureus* and *Staph. intermedius* .

Table (4) sensitivity of coagulase positive staphylococcus isolates from milk samples (n=40) to chemotherapeutics .

Chemotherapeutic agent	High sensitive		Low sensitive		resistant	
	Number	%	Number	%	Number	%
Gentamycin	40	100	-	-	-	-
Erythromycin	38	95	2	5	-	-
Trimethoprim	37	92.5	3	7.5	-	-
Amoxacillin	26	65	14	35	-	-
Sulfamethaxol	-	-	38	95	2	5
Oxytetracycline	-	-	29	72.5	11	27.5
Neomycin	2	5	25	62.5	13	32.5
Ampicillin	-	-	21	52.5	19	47.5
Streptomycin	-	-	16	40	24	60
Cefalexin	-	-	12	30	28	70
Lincomycin	-	-	-	-	40	100

DISCUSSION

Table 2 showed that the coagulase positive staph. Strains were the main cause of mastitis in the farm as it could be isolated from 24 out of 46 mastitic cows with percentage 52.17% , farther more 40 out of 74 mastitic quarters of this animal were infected with coagulase positive staph. With percentage 54.05% . higher results reported by Roberson et al. (1994) but lower results obtained by Mikhail (2006), On the other hand the coagulase negative staph ,were isolated from 26 out of 74 mastitic quarters with percentage 35.14% (18 out of 46 mastitic cows with percentage 39.13%) , higher results reported by Enany et al.(2008), while lower results obtained by schukken et al.(1989). Further identification for the coagulase positive Staph. declared that *Staph. aureus* could be identified from 34 out of 74 of mastitic milk samples with percentage 45.94% (21 out of 46 examined mastitic cows) as showed in (Table 3), the obtained data nearly agreement with that those recorded by Ali et al.(1990), meanwhile higher finding were reported by Auxiliadora et al.(1984) and lower results recorded by Calvinho et al.(1991). Also the results tabulated in Table 3 revealed that the species of *Staph. hyicus* and *Staph. intermedius* were isolated from 5(6.76%) and 1(1.35%) of the samples respectively. In Egypt Mikhail (2006) could isolate *Staph. hyicus* and *Staph. intermedius* which in agreement with that reported herein . The high prevalence of staphylococcus bacteria in mastitic cows may be attributed to the wide distribution of the organism inside the mammary gland and skin of the teats and udder (Leslie and schukken ,1993) .

Also the staph. is one of cause the recurrent mastitis (Labib,1994) , beside the organism belong to the contagious mastitis pathogens (Roberson et al. 1998).

Lastely the staph. cause a form of difficult mastitis, previously infected quarter were the major source of that infection and the infection was transferred through poor hygiene conditions (John ,1991) .

The data present in Tables (1,4) allow to conclude that the coagulase positive staph. high sensitive to gentamycin with percentage 100% followed by erythromycin , trimethoprim , and amoxicillin with percentages 95% , 92.5%, and 65% respectively , also

the results showed that the organisms less sensitive to sulfamethaxol (95%) followed by oxytetracycline , neomycin , and ampicillin with percentages 72.5% , 62.5% and 52.5% respectively , on the other hand results mentioned that the coagulase positive staph. were resistant to lincomycin (100%) followed by cefalexin and streptomycin with percentages 70% and 60% respectively , these finding results are in general inagreement with that reported by Labeb , 1994 and ABD EL AAL , 2008 , the variation may be attribute to proportion of strains resistant to antimicrobial antibiotics has increased among staph. Organisms (Myllys et al.,1998) therefore susceptibility test to chemotherapeutic agent must be done in mastitic infections before any treatment .

REFERENCES

- Abd EL AAL , S.F.A. (2008)** : Bovine Mastitis –Diagnosis, Bacteriological status of Milk and Antimicrobial Resistance of Pathogens. Assuit Vet Med.J.54(116):90-104.
- Ali,S.L.; superkar, P.G. and shukla,P.C.(1990)**: pathogens involved In subclinical mastitis . Indian Vet Med.J.,140(1):86-87
- Anderson, J.C. (1976)**: Mechanisms of staphylococcal in relation to bovine mastitis . Brit. Vet J. 132-229.
- Auxiliadora, A.N.M.; Carvalho, D.F.F. and Costa, R.R.D.(1984)**: Bovine mastitis in the dairying region of Goiania. A nais dos Escalas de Agronomiae de veterinaria .Goias,Brazil,14,69-78.
- Barkema, H.W. ; Schukken , Y. H. ; Lam ,T.J.G.M. ; Beiboer, M.L.; Wilmink, H. ;Benedictus,G. and Brand ,A. (1998)** : Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell count. J.Dairy Sci.81:411-419.
- Bramley, A.J. and Dodd,F.H. (1984)**: Reviews of the progress of dairy science : mastitis control –progress and prospects. J. Dairy Res. 51:481.
- Bramely ,A.J.; Cullor,J.S. ; Erskine,R.J. ; Fox, L.K. ; Hamon, R.J.; Hogan, T.S.; Nickerson, S.C.; Oliver, S.P.; Smith, K.L. and Sordillo, L.M.(1996)**: current concepts of bovine mastitis . 4th Ed. national mastitis. council
- Calvinho,L.F.,Delgado, A.P.,Vitulich, C.A., Occhi, H.L.,Canaveso, V.R., Zubriggen, M.A. and Tarabla, H.D.(1991)**: Drug resistance of bovine mastitis pathogens isolated in herds of the sante Fedairying region . veterinaria Argentina, 8:667-680.
- Cruickshank, R.; Duguid, J.R.; Marmion, B.D. and Swain, R.H.A. (1975)**: Medical microbiology. The practice of medical microbiology VIII 11th and 12th Ed., Churchill Living Stone Edinburgh, London and New York.
- Enany, M.E.; Eid ,H.M.; EL-Kholy, A.A.M.A., and Abd ELazeem, N.H. (2008)**: Application of PCR as A diagnostic tool of clinical Mastitis in dairy cattle in comparison to traditional methods .Suez Canal Vet. Med. J. XIII(1): 61-68 .
- Harmon, V. R. (1995)**: Mastitis and milk quality.Milk quality edited by F. Harding.

- Hobbs, B.C. and Roberts, D. (1993):** Food poisoning and food hygiene. 6 Ed. Singular publishing group. Inc. , San Diego, California.
- Honkanen-Buzalski, T. (1995):** Sampling technique, transportation and history. In: Sandholm M, Honkanen-Buzalski T., Kaartinen L., Pyorala S., editor. The bovine udder and mastitis. Gummerus Kirjapaino Oy, Jyväskylä, Finland; 1995. p.112.
- International Commission on Microbiological Specification for Food "ICMSF" (1978):** Microorganisms in foods: Their significance and method of enumeration, 2nd Ed, Univ. of Toronto. Press, Toronto, London.
- John H. K., (1991):** Diagnosis and Treatment of difficult Mastitis cases, *Staphylococcus aureus*, *Agri-practica*. 12,(1):5-7.
- Koneman, E. W. ; Allen, S. D. ; Dowell, V.R. and Summers, H.W. (1988):** Color atlas and text book of diagnostic microbiology. J.B. Lippincott company Philadelphia.
- Labib , Sahar, R.M. (1994):** Bacteriological Studies on Recurrent Mastitis in Cows. M.D. thesis Department of microbiology, Faculty of Veterinary Medicine, Zagazig University .
- Leslie, K.E. and Schukken, Y.H. (1993):** Herd programs for elimination and preventing *staphylococcus aureus* mastitis. in: national mastitis council annual meeting Kansas city . 36-47
- Makar, Azza, N.F. (1997):** Some studies on bovine mastitis, M.D. Thesis, Infectious diseases Department, Fac. Vet Medicine , Zagazig University
- Mikhail , Magda, M.S. (2006):** Immunological and Bacteriological Studies on *Staphylococcus aureus* recovered from Bovine Mastitis. M.D. thesis , Microbiology Department, Faculty of Veterinary Medicine , Cairo University.
- Myllys, V.; Asplund, K.; Brofeldt, E.; Hirvela-Koski, V.; Honkanen- Buzalski, T.; Junttila, J.; Kulkas, L.; Myllykangas, O.; Niskanen, M.; Saloniemi, H.; Sandholm, M. and Saranpaa, T. (1998):** Bovine mastitis in Finland in 1998 and 1995-changes in prevalence and antimicrobial resistance. *Acta vet Scand* 39:119-126.
- Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G.R. (1994):** Clinical Veterinary Microbiology 4th Ed. Wolf published, an important of Mosby-Year book. Europ. limited ISBN 0 7234-17113.

- Roberson, J.R.; Fox, L.K.; Hancock, D.D. and Gay, J.M. (1994):** Coagulase positive Staphylococcus Intramammary Infections In Primiparous Dairy Cows. J.of Dairy Science 77(4):958-969 .
- Roberson, J.R.; Fox, L.K.; Hancock, D.D.; Gay, J.M and Besser, T.E. (1998):** Sources of intramammary infections from *staphylococcus aureus* in dairy heifers at first parturition, J. Dairy Sci 81:687-693 .
- Sargeant, J.M.; Scott, H.M.; Leslie, K.E.; Ireland, M. J. and Bashiri, A. (1998):** Clinical mastitis in dairy cattle in ontario: frequency of occurrence and bacteriological isolates. Can. Vet J. ,39(1):33-38
- Schukken, Y.H.; grommers, F.J.; Van De geer, D. and Brand, A.(1989):** Incidence of clinical mastitis on frames with low somatic cell counts in bulk milk .vet Rec., 125:60-63.

الملخص العربي

مدى تواجد المكورات العنقودية في الابقار المصابة بالتهاب الضرع.

احمد مصطفى احمد مصطفى

معهد بحوث صحة الحيوان – المنصورة

أجريت هذه الدراسة على ٧٤ عينة لبن خام تمثل ٤٦ بقرة حلب مصابة بالتهاب الضرع (٧٤ ربع مصا (ب) سلالة هولشتاين تم تجميعها من مزرعة البان في محافظة الدقهلية- مصر حيث تم فحصها بكتريولوجيا للوقوف على مدى تواجد ميكروبات المكورات العنقودية وكذلك اجراء اختبار الحساسية لتلك الميكروبات . اوضحت النتائج ان ٤٠ عينة لبن بنسبة ٥٤,٠٥% تمثل ٢٤ بقرة مصابة بنسبة ٥٢,١٧% اجابية لمكورات العنقودية المجلطة للبلازما وتصنيف ميكروبات المكورات العنقودية المجلطة للبلازما وجد أن عينات اللبن إيجابية لميكروبات المكورات العنقودية الذهبية والمكورات العنقودية الهيكس و المكورات العنقودية الانترميديس بنسب ٤٥ و ٩٤% و ٦٤ و ٦٥% و ٣٥ و ١% علي التوالي وتمثل ٢١ (٦٥ و ٤٥%) و ٣ (٥٢ و ٦٥%) و ١ (١٧ و ٢%) بقرة مصابة علي التوالي من ناحية

أخرى وجد أن ٢٦ عينة لبن بنسبة ٣٥,١٤% تمثل ١٨ بقرة بنسبة ٣٩,١٣% اجابية لميكروبات المكورات العنقودية الغير مجلطة للبلازما واطهرت الدراسة ايضا ان ميكروبات المكورات العنقودية المجلطة للبلازما اكثر حساسية للمضادات الحيوية الاتية :

الجينتاميسين والارثرومايسين والتراى ميزوبريم والاموكساسلين بنسب ١٠٠%، ٩٥% ، ٩٢,٥%، ٦٥% على التوالي بينما تلك الميكروبات اقل حساسية للسلفاميثايسول والاكسي تتراسيكلين و النيومايسين والاميسلين بنسب ٩٥%، ٧٢,٥%، ٦٢,٥%، ٥٢,٥% على التوالي . من ناحية اخرى اظهرت ميكروبات المكورات العنقودية المجلطة للبلازما مقاومة المضادات الحيوية اللنكومايسين والسيفالكسين والاستربتومايسين بنسب ١٠٠%، ٧٠%، ٦٠% على التوالي .