Dept. of Food Control,
Fac. of Vet. Med., Zagazig University.

BOVINE MASTITIS-DIAGNOSIS, BACTERIOLOGICAL STATUS OF MILK AND ANTIMICROBIAL RESISTANCE OF PATHOGENS

(With 7 Tables)

By

S.F.A. ABD EL AAL

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التهاب الضرع في الأبقار طرق التشخيص – الحالة الميكروبيولوجية للبن ومقاومة المسببات الممرضة لمضادات الميكروبات

صلاح فتحي أحمد عبد العال

تم تجميع عدد ٢٠٠ عينة من لبن الأبقار الخام من الأرباع المختلفة للضرع من حوالي ٥٠ بقرة حلوب من سلالة هولشتين في مدينة هوهنهايم – شتوتجارت – ألمانيا. وفحص هذه العينات لمعرفة مدي تواجد التهاب الضرع بالطرق المختلفة ومسبباته البكتيرية ومدي حساسية هذه الميكروبات للمضادات الحيوية المستخدمة في علاج التهاب الضرع في الحقل البيطري. وأوضحت الدراسة أنه باستخدام اختبار كاليفورنيا لتشخيص التهاب الضرع وجد أن حوالي ٧٥ (٣٧،٥ %) من الأرباع المفحوصة مصابة بالتهاب الضرع منهم ٤٥ (٦٠ %) يعطي المستوي رقم ٣ وحوالي ٢٥ (٣٣،٣ %) يعطي المستوي رقم ٢ وحوالي ٥ (٧،٦ %) مستوي رقم ١. وباستخدام العدد الكلي للخلايا النسيجية في اللبن كطريقة من طرق التشخيص وجد أن جميع الأرباع المصابة تحتوي علي خلايا نسيجية بمتوسط ٤٣٧،٣×١٠^{٣} خلية لكل مللي وبهذا تكون أعلي من الحد المسموح به وهو ٢٠٠ ×١٠^{٣} خلية لكل مللي. الفحص الميكروبيولوجي للأرباع الايجابية بالاختبارات السابقة أسفر عن وجود ٦٣ عينة (٨٤،٠ %) تحتوي علي ميكروبات وبعزل الميكروبات وتصنيفها معمليا أوضحت النتائج أن هذه الميكروبات هي المكور العنقودي الذهبي والمكور العنقودي من نوع إبيدرميدس والمكور السبحي المعوي من نوع أوبرس وأجالاكتيا وكوريني بكتريم بوفيز وايشيريشيا كولاي بنسبة (١٤،٢ و ١٩،٢ و ٢٦،٦ و ١٠،٠ و ١٧،٥ و ١٢،٥ %) علي التوالي وبحساب متوسط عدد الخلايا النسيجية في الأرباع المصابة طبقا لوجود كل ميكروب وجد أن متوسط العدد كان (٣٩١،٤ و ٤١٦،٩ و ٤٧٦،٤ و ٧٤٠،٠ و ٣٥٧،٣ و ٥٤٢،٠ ×١٠^{٣}) خلية / مللي علي التوالي. وبإجراء اختبار الحساسية ضد المضادات الحيوية للميكروبات المعزولة في المعمل مع ٢٠ من المضادات الحيوية المستخدمة في المجال البيطري وجد أن المكور العنقودي الذهبي يعطي أعلي مقاومة ١٠٠ % لكل من البنسيلين واللنيكومايسين والكولستين وثلاثي السلفات وكليندومايسين وباستراسين وكلورامفنيكول في

حين أن المكور العنقودي من نوع إبيدرميدس أعطي هذا المستوي من المقاومة لكل من ثلاثي السلفات وباستراسين وكليندومايسين لكن في حالة الكوليستين وبولي ميكسين ب قلت هذه النسبة إلي ٩٥،٧ %. وكل المعزولات من نوع المكور السبحي المعوي من نوع أوبرس أعطت ١٠٠ % مقاومة لكل من ثلاثي السلفات وباستراسين وكلورامفنيكول وكليندومايسين وبعد ذلك اختلفت هذه النسبة حيث أصبحت ٩٦،٦ % مع الكولستين وسلفاميثوكسيزول و٨٧،٥ % مع النيومايسين وتراي ميثوبريم. بينما المكور السبحي المعوي من نوع اجالاكتيا كان حساسا جدا لكل من الارثرومايسين والبنسيلين واللينكوسبكتين والأموكسيسيللين والأمبيسيللين والتتراسيكلين والأوكساسللين والسيفوبيرازون وسلفاميثوكسيزول والسيفالكسين بينما معزولات الكوريني بكتريم بوفيز أعطت مقاومة كاملة لأقراص المضادات الحيوية الخاصة بكل من تراي ميثوبريم وثلاثي السلفات وباستراسين وسلفاميثوكسيزول وكلورامفنيكول وكليندومايسين. في حين أن ميكروب الإيشيريشيا كولاي أعطي مقاومة بنسبة ١٠٠ % لكل من الجنتاميسين وثلاثي السلفات و٨٠ % لكل من الأموكسيسيللين والبولي موكسين ب والكولستين والنيومايسين وتراي ميثوبريم وسلفاميثوكسيزول والسيفالكسين. وقد تمت مناقشة الأهمية الاقتصادية والصحية للميكروبات المعزولة وتأثير ذلك علي كمية وجودة الحليب ودراسة المقترحات الخاصة بمقاومة هذه الميكروبات ورفع القدرة الانتاجية لحيوان الحليب وكذلك زيادة جودة اللبن ومنتجاته.

SUMMARY

Quarter milk samples (n=200) from 50 dairy cows (Holstein breed) in Hohenheim region, Stuttgart, Germany, were examined to study the occurrence and causes of mastitis, distribution of mastitis pathogens and in vitro antimicrobial susceptibility of different mastitis pathogens. The study revealed that 75 (37.5 %) quarters had positive California mastitis test (CMT). 45 (60.0 %) of them had CMT score 3, while 25 (33.3 %) showed CMT score 2 and 5 (6.7 %) gave score 1. All positive quarters 75 (37.5 %) had significantly a higher mean value of somatic cell counts (437.3 x10^3 cells/mL). So, all these quarters were considered positive for mastitis (≥200.000 cells/mL). Bacteriological examination of these positive quarters (75) revealed that 63 (84.0 %) quarters yielded bacteria. *Staphylococcus aureus, Staph. epidermidis, Streptococcus uberis, Strept. agalactiae, Corynebacterium bovis* and *E. coli* were the main organisms. These strains were isolated at varying percentages 14.2, 19.2, 26.6, 10.0, 17.5 and 12.5%, respectively. The average somatic cell counts calculated from quarter milk samples in relation to isolated bacterial strains were, 391.4, 416.9, 476.4, 740, 357.3 and 542 x10^3 cells/mL, respectively. According to in vitro antimicrobial susceptibility testing, the *Staphylococcus aureus* demonstrated the highest level of resistance (100.0 %) to Penicillin, lincomycin, Colistin, Triple sulfa, Bacitracin,

Chloramphenicol and Clindomycin. While, *Staph. epidermidis* (*coagulase negative Staphylococci*) gave the same resistance level against Triple sulfa, Bacitracin and Clindomycin. However, in case of Colistin and Polymyxin B it was decreased to 95.7%. All isolated strains of *Streptococcus uberis* (32) gave resistance to Triple sulfa, Bacitracin, Chloramphenicol and Clindomycin by a percentage of 100.0%. This percent was varying with other antibiotics where it became 96.9% with Colistin and Sulfamethoxazol and 87.5% against Neomycin and Trimethoprim. *Streptococcus agalactiae* isolated strains (12) were very susceptible to Erythromycin, Penicillin, Lincomycin, Amoxicillin, Ampicillin, Tetracycline, Oxacillin, Cefoperazon, Sulfamethoxazol and Cefalexin treatment. *Corynebacterium bovis* showed complete resistance (100.0%) with antibiotic discs of Trimethoprim, Triple sulfa, Bacitracin, Sulfamethoxazol, Chloramphenicol and Clindomycin. *E. coli* revealed 100.0 % resistance to Gentamycin and Triple sulfa and 80.0% to Amoxicillin, Polymyxin B, Colistin, Neomycin, Trimethoprim, Sulfamethoxazol and Cefalexin. The economic importance and public health significance of existing microorganisms as well as the suggested measures for improving the keeping quality as well as the sanitary condition of raw milk and its products were discussed.

Key words: *Bovine mastitis, milk, dairy cows, antimicrobial resistance.*

INTRODUCTION

Bacterial infections are considered the primary cause of mastitis in domestic animals (Abdel Gadir *et al.*, 2006). 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 often never recovered (Gröhn *et al.*, 2004). Also, it is correlated with increased amounts of heat stable protease (plasmin) and lipase (lipoprotein lipase) in milk so will cause protein and fat degradation during refrigerated storage and produce off flavours as well as reduce curd firmness during cheese making (Barbano *et al.*, 2006). Meanwhile, it leads to involuntary culling of lactating cattles (Smith *et al.*, 1985). Clinical mastitis can be detected by examination of the udder, the milk or both. Detection of subclinical mastitis is however, difficult and depends on various test procedures aimed at detecting the cause or products of inflammation in

milk (IDF, 1987). These tests including California mastitis test, somatic cell counts evaluation and bacteriological examination.

California mastitis test (CMT) is a subjective screening test based on scoring the degree of gel formation of a milk and bromocresol reagent mixture. The CMT score has been shown to be positively associated with SCC and with the probability of bacterial infection (Contreras *et al.,* 1996). The CMT has the advantages of being animal-side and of being inexpensive and rapid to perform.

Methods for evaluating somatic cells in milk and their threshold values are developed for dairy cows (Abdurahman, 1998) and used as indicative value of udder infection (Singh and Ludri, 2001). Somatic cell counts (SCC) of normal secretion not more than 100.000 cells/mL and below this figure pathogens can nearly completely be excluded (Heeschen, 2002). If milk SCC of a cow or of a quarter exceeded 200.000 cells/mL, the cow was defined as having mastitis (Barrett *et al.,* 2005; Haltia *et al.,* 2006 and Moroni *et al.,* 2006).

Streptococcus agalactiae, Staphylococcus aureus, coagulase negative Staphylococci (CNS) and *Corynebacterium bovis* which are traditionally considered to be minor mastitis pathogens, have become more common (Huxley *et al.,* 2002) and frequently isolated organisms responsible for bovine mastitis (Naiknaware *et al.,* 1998). *E. coli* mastitis is an increasing problem in many countries and often associated with sever tissue damage and considerable losses in milk yield (Kossaibati *et al.,* 1998).

Contagious bacteria may spread to other quarters or other animals via milking machine. So, treatment with antibiotics should be carried out when clinical mastitis occurs or routinely used to treat all quarters in all cows (Deluyker *et al.,* 2005). The proportion of strains resistant to antimicrobial antibiotics has increased mainly among *Staphylococci* (Myllys *et al.,* 1998). So, susceptibility test should be done before any treatment. This work was carried out to detect udder pathogens and inflammation using CMT and SCC as well as to determine the antimicrobial resistance of isolated bacteria.

MATERIALS and METHODS

Sampling:

Visual observation and palpation of the mammary gland quarters were done, and macroscopic examination of the milk streaks was under taken in strip cups for the presence of abnormal colour, consistency, flakes and other abnormalities. Quarter milk samples were collected

aseptically as described by Honkanen-Buzalski (1995). Before sampling, teat ends were cleaned with apiece cotton moistened in 70% alcohol and allow to dry. The first streams of milk from each quarter were discarded and about 10 mL of foremilk were collected immediately before milking into sterile 10 mL plastic or polyethylene tubes. Some of the milk samples was used for California mastitis test and Somatic cell counts. The remaining milk was cooled and transported in cool bags to the diagnostic laboratory of Environmental and Animal Hygiene Institute, Hohenheim University for further analysis. Samples were stored at 4°C until bacteriological assays were performed.

California Mastitis Test:

CMT was carried out principally according to Schalm and Noerlander's (1957) method. An equal volume of CMT reagent and milk was mixed and the reaction was graded 1, 2, 3, 4, or 5 according to the Scandinavian recommendations (Klastrup and Schmidt Madsen, 1974).

Somatic cell counts (SCC):

The somatic cell values of quarter milk samples were measured by using the Fossomatic Milkoscan System 215 (Foss Electric, Hillerod, Denmark). A quarter was considered to have mastitis when the SCC was $\geq$ 200,000 cells/mL (Schukken *et al.*, 2003). Milk was preserved with one drop of 2-bromo-2-nitro propane–1, 3-diol (preservo liquid, D & F control systems, San Francisco, CA) and incubated for > 16 hrs at 4°C then SCC were determined using 500 µl of this milk.

Bacteriological analysis:

Bacteriological culturing of milk samples was performed according to standards of the National Mastitis Council (NMC, 1999). Ten microliters of each milk sample was spread on blood agar plates (5% defibrinated sheep blood). Plates were incubated aerobically at 37°C and examined after 24 hrs. If no growth, incubated for another 24 hrs to insure that it is negative. Colonies were provisionally identified on the basis of Gram stain, morphology and haemolysis patterns. Representative colonies were then subcultured on blood agar plates and incubated aerobically at 37°C for 24 hrs to obtain pure cultures. Catalase and coagulase production was tested for Gram positive cocci. Specific identifications of Staphylococci, Streptococci and Corynebacterium were made using Commercial micro methods (API Staph, API 20 Strept and API Coryne., Bio Merieux, France). Gram negative isolates were identified by using colony morphology, Gram staining characteristics, oxidase and biochemical reactions on MacConkey's agar and API 20 E (Bio Merieux).

Susceptibility testing:

The in vitro susceptibility of the isolates to antimicobials was determined according to standards of the National Committee for Clinical Laboratory Standards (NCCLS, 2002). Loopfull from pure culture of each isolated microorganism was mixed well with 9 mL of sodium chloride solution then spreading over the surface of nutrient agar or blood agar plates (4 plates) then suction the excess fluid. Twenty antibiotic discs (antibiotics approved for use in treatment of bovine mastitis) were spread on the surface of inoculated plates (5 for each plate). Plates were incubated at 37°C for 24 hrs. The diameter of inhibition zone of each disc was measured (in mm) and compared with the standard breakpoints.

RESULTS

Table 1: Results of detection of mastitic animals using California mastitis test (CMT).

Examined animals (n=50)		Examined quarters (n=200)		Score 3 quarters		Score 2 quarters		Score 1 quarters	
Positive		Positive							
No.	%	No.	%	No.	%	No.	%	No.	%
25	50.0	75	37.5	45	60.0	25	33.3	5	6.7

n: Number of examined samples.

Table 2: Statistical analytical results of somatic cell counts/mL in examined milk samples.

	Positive (200.000) or more cells/mL		Min. $x10^3$	Max. $x10^3$	Mean $x10^3$	±S.E.M. $x10^3$
	No.	%				
Examined Animals (n=50)	25	50.0	207.5	567.5	353.9	14.9
Examined quarters (n=200)	75	37.5	200	860	437.3	11.5

Table 3: Bacteriological findings of examined milk samples.

Examined animals (n=25)		Examined quarters (n=75)				No of isolated mos.	Total No of isolated stains
Positive (grow)		Positive (grow)		Negative (not grow)			
No.	%	No.	%	No.	%	4	120
25	100.0	63	84.0	12	16.0		

Table 4: Prevalence of isolated bacterial strains in examined milk samples.

Isolated Bacterial strains	No.	%
Staphylococcus aureus	17	14.2
Staphylococcus epidermidis	23	19.2
Streptococcus uberis	32	26.6
Streptococcus agalactiae	12	10.0
Corynebacterium bovis	21	17.5
E. coli	15	12.5
Total	120	100.0

Table 5: Somatic cell count distribution in relation to isolated bacterial strains in examined milk samples.

Isolated Bacterial strains	Min. $x10^3$	Max. $x10^3$	Mean $x10^3$	±S.E.M $x10^3$
Staphylococcus aureus	280	710	391.4	30.9
Staphylococcus epidermidis	210	810	416.9	32.5
Streptococcus uberis	240	860	476.4	34.7
Streptococcus agalactiae	670	810	740	19.8
Corynebacterium bovis	210	540	357.3	23.8
E. coli	430	620	542	14.1

Table 6: Breakpoints of antibiotics used in vitro susceptibility testing (NCCLS)

Antibiotics	Appreviation	Concentration/µg	High sensitivity +++	Low sensitivity ++
Erythromycin	E-15	15	≥ 21(mm)	17-20 (mm)
Penicilline	P-10	10	≥ 29	28
Lincomycin	L-15	15	≥ 23	15-22
Amoxicillin	AMX-25	25	≥ 27	21-26
Ampicillin	AM-10	10	≥ 22	15-21
Gentamycin	GM-10	10	≥ 21	15-20
Tetracycline	TE-30	30	≥ 22	17-21
Oxacillin	OX-5	5	≥ 16	15
Polymyxin B	PB-300	300	≥ 12	9-11
Colistin	CL-25	25	≥ 11	9- 10
Neomycin	N-30	30	≥ 17	13-16
Cefoperazon	CFP-30	30	≥ 18	15-17
Trimethoprim	TMP-5	5	≥ 16	11 – 15
Triple sulfa	SSS-25	0.25	≥ 17	13-16
Sulfamethoxazol	SXT	23.75	≥ 16	11- 15
Cefalexin	CN-30	30	≥ 18	15-17
Pirlimycin	PIR-2	2	≥ 13	12
Bacitracin	B	10	≥ 13	9-12
Chloramphenicol	C	30	≥ 21	20
Clindomycin	CC	10	≥ 21	15-20

Table 7: Resistant bacterial strains isolated from examined milk samples to twenty selected antimicrobial agents.

Strains / Antibiotics	*Staph. aureus*		*Staph. epidermidis*		*Strept. Uberis*		*Strept. agalactiae*		*Coryne. bovis*		*E. coli*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Erythromycin	0	0.0	11	47.8	19	59.4	0	0.0	13	61.9	6	40.0
Penicillin	17	100.0	19	82.6	15	46.9	0	0.0	17	80.9	6	40.0
Lincomycin	17	100.0	15	65.2	20	62.6	0	0.0	12	57.1	6	40.0
Amoxicillin	0	0.0	12	52.2	12	37.5	0	0.0	13	61.9	12	80.0
Ampicillin	0	0.0	12	52.2	12	37.5	0	0.0	11	52.4	3	20.0
Gentamycin	0	0.0	11	47.8	25	78.1	12	100.0	11	52.4	15	100.0
Tetracycline	0	0.0	0	0.0	17	53.1	0	0.0	11	52.4	6	40.0
Oxacillin	0	0.0	0	0.0	13	40.6	0	0.0	16	76.2	6	40.0
Polymyxin B	0	0.0	22	95.7	15	46.9	6	50.0	11	52.4	12	80.0
Colistin	17	100.0	22	95.7	31	96.9	12	100.0	11	52.4	12	80.0
Neomycin	0	0.0	12	52.2	28	87.5	12	100.0	14	66.7	3	80.0
Cefoperazon	0	0.0	11	47.8	14	43.75	0	0.0	11	52.4	3	20.0
Trimethoprim	0	0.0	11	47.8	28	87.5	6	50.0	21	100.0	12	80.0
Triple sulfa	17	100.0	23	100.0	32	100.0	12	100.0	21	100.0	15	100.0
Sulfamethoxazol	0	0.0	11	47.8	31	96.9	0	0.0	21	100.0	12	80.0
Cefalexin	13	76.5	17	73.9	18	56.3	0	0.0	12	57.1	12	80.0
Pirlimycin	0	0.0	0	0.0	19	59.4	12	100.0	11	52.4	6	40.0
Bacitracin	17	100.0	23	100.0	32	100.0	12	100.0	21	100.0	6	40.0
Chloramphenicol	17	100.0	18	78.3	32	100.0	12	100.0	21	100.0	6	40.0
Clindomycin	17	100.0	23	100.0	32	100.0	12	100.0	21	100.0	6	40.0

DISCUSSION

The results tabulated in Table 1 revealed that 25 animals of total examined 50 dairy cows had mastitis. 75 quarters of these positive cows yielded positive CMT. 45 (60.0 %) of them gave score 3, 25 (33.3 %) had score 2 and 5 (6.7 %) represented score 1. These results were inagreement with that reported by Abdurahman (2006) where 80% of infected quarters gave score 2 or more. Meanwhile, higher results were detected by Kivaria *et al.* (2006).

It is evident from the previous results that CMT used as indicator of bovine mastitis and bacteriological status of milk. The CMT has the advantages of being animal-side and of being inexpensive and rapid to perform (Contreras *et al.*, 1996). This test may give positive with non infected quarters (Abdurahman, 2006). So, it should carried out with other tests as somatic cell counts and bacteriological examination to detect the cause or products of mastitis.

Listed results in Table 2 decleared that all cows positive to CMT had somatic cell counts ≥200.000 cells/mL so, all these cows defined as

having mastitis (Haltia *et al.*, 2006 and Moroni *et al.*, 2006). All positive quarters 75 (37.5 %) had significantly a higher mean value of somatic cell counts (437.3 $x10^3$ cells/mL). Nearly similar findings were reported by Janosi and Baltay (2004) and Trevisi *et al.* (2006). While, higher results were recorded by Anne and Olav (2006) and Severino *et al.* (2007). Green *et al.* (2006) reported lower values.

It is achieved that somatic cell count was a better predictor of bacteriological status of milk than CMT score (McDougall *et al.*, 2001) because bacterial infection mainly correlated with sever tissue damage (Kossaibati *et al.*, 1998). Meanwhile, somatic cell counts may be increased in other cases as faulty milking by milking machine or trauma of the udder. So, we should apply bacteriological examination.

Increasing somatic cell counts produces high economic losses because it is mainly correlated with increased amounts of heat stable protease and lipase in milk so, will cause protein and fat degradation during refrigerated storage of milk and produce off flavours as well as reduce curd firmness during cheese making (Barbano *et al.*, 2006).

Results presented in Table 3 showed that the bacteriological examination was carried out to milk of quarters which gave positive CMT and SCC only (n=75) because Heeschen (2002) reported that when SCC not more than 100.000 cells/mL undesired pathogens can be nearly completely be excluded. Sixty-three (84.0 %) quarters yielded bacteria and others not grow. This ratio was nearly higher than that reported by Moroni *et al.* (2006) and Bradley *et al.* (2007).

There are several causes of this elevated percentage, the first explination is the movement of pathogens from animal to another and from quarter to another through milking machines as a result of inefficient cleaning and sanitization of milking machines compartments mainly teat cups. Also, it is may be due to absence of teat dipping after milking and dry cow therapy. Calf suckling practice not present and it plays a role in reducing bacteria in milk *(Faecal Coliforms)* by the elimination of foremilk which is known to be the most contaminated by bacteria (Srairi *et al.*, 2006). Environmental bacteria contaminating the milking cluster might be considered as potential risk factor for movement of pathogens (Feldmann *et al.*, 2006). While, Zdanowiez *et al.* (2004) mentioned that *Coliforms* and *Streptococci* causing mastitis on teat ends of lactating cows come mainly from bedding.

Inspection of Table 4 revealed that the main organisms isolated from examined milk samples were *Staphylococcus aureus, Staph. epidermidis, Streptococcus uberis, Strept agalactiae, Corynebacterium*

bovis and *E. coli*. The obtained data were inagreement with that reported by Janosi and Baltay (2004); Haltia *et al.* (2006) and Bradley *et al.* (2007). Meanwhile, these bacterial strains were isolated with lower percentages than that mentioned by the authors where, the obtained percentages were 14.2, 19.2, 26.6, 10.0, 17.5 and 12.5%, respectively. The problem of these microorganisms not only economic or disturb animal health but also, produce a public health hazard to human being.

Staphylococcus aureus is the most important human pathogen among the *Staphylococci* under certain circumstances. *Staph. aureus* may cause a variety of infectious diseases ranging from relatively skin infections to life threating systemic illness due to production of thermostable enterotoxins (A to E), Leucocidin and toxic shock syndrome toxin (TSST) that are responsible for the clinical feature of *Staphylococcal* food poisoning. Ingestion of preformed enterotoxins in food results in vomiting and diarrhea within 2 to 8 hrs sometimes followed by collapse (Hobbs and Roberts, 1993). Although coagulase positive is the most dangerous *Staphylococci*, but nowadays coagulase negative has been recognized as important agent of human disease which include an nosocmial and community-acquired urinary tract infections, bacteraemia in compromised hosts, osteomylitis and post surgical infections.

Streptococci are thermoduric microorganisms, can grow at a wide range of temperature and tolerate sodium chloride, hence they can grow and produce undesirable changes affecting the keeping quality of the products (Seidel and Muschter, 1967). Moreover, *Streptococci* has been incriminated in cases of food poisoning specially when it was predominating in the food (ICMSF, 1978) and associated with a large number of outbreaks of gastroenteritis and implicated in urinary tract and wound infections, intra-abdominal abscesses and endocarditis (Eley, 1996). It is thought that their toxins giving symptoms similar to but less acute than those of *Staphylococcal* enterotoxins (Hobbs and Roberts, 1993).

E. coli is one of *Coliforms* which have probably received more attention than the most other groups of bacteria for their significance as indicator organisms for faecal contamination and their ability to grow well over a wide range of temperature below 10°C to 46°C (Frazier and Westhoof, 1978). High levels of *Coliforms* (10^6 or more) believed to be necessary for foodborne illness to occur (Doyle and Cliver, 1990). The infective dose of Enterotoxogenic *E. coli* strains required to induce

diarrhea was found to lie between 10^8–10^{10} cells (Frank and Marth, 1978).

Corynebacterium bovis may contribute to many problems to human beings as hydrocephalus, acute nephritis, nephrotic syndrome, decreased complement levels of circulating immune complexes and diminished creatinine clearance (Bolton *et al.*, 1975).

Results given in Table 5 revealed that the average somatic cell counts calculated from examined quarter milk samples in relation to the isolated bacterial strains were 391.4, 416.9, 476.4, 740, 357.3 and 542 x10^3 cells/mL for *Staphylococcus aureus, Staph. epidermidis, Streptococcus uberis, Strept agalactiae, Corynebacterium bovis* and *E. coli*, respectively. These results were nearly similar to the findings obtained by Haltia *et al.* (2006). Higher values were reported by Klossaowska *et al.* (2005).

It is evident that all isolated strains were accompanied by increased number of somatic cell counts as a result of sever tissue damage, subsequently considerable losses in milk yield and production of milk with off flavour (Kossaibati *et al.*, 1998 and Gröhn *et al.*, 2004). Also, it is insured the fact that the main cause of increased somatic cell counts is the bacterial infection of the udder mainly pathogenic microorganisms (Janosi and Baltay, 2004 and Abdurahman, 2006).

Data reported in Tables 6 and 7 mentioned the breakpoints of twenty antimicrobial agents used in treatment of mastitis and the resistant bacterial strains isolated from examined milk samples of mastitic animals. The results achieved allow to conclude that *Staph. aureus* exhibited high resistance to Penicillin, lincomycin, Colistin, Triple sulfa, Bacitracin, Chloramphenicol and Clindomycin. These results were inagreement with that reported by Pitkälä *et al.* (2004) and Mohammed (2006). *Staph. epidermidis* (*coagulase negative Staphylococci*) were very susceptible to Tetracycline, Oxacillin and Pirlimycin. Nearly similar results were obtained by Gentilini *et al.* (2000). All isolated strains of *Streptococcus uberis* were very resistant to Triple sulfa, Bacitracin, Chloramphenicol and Clindomycin. While, *Streptococcus agalactiae* strains were inhibited by 10 antimicrobial agents (Erythromycin, Penicillin, Lincomycin, Amoxicillin, Ampicillin, Tetracycline, Oxacillin, Cefoperazon, Sulfamethoxazol and Cefalexin). *Corynebacterium bovis* produced a relatively high level of resistance against Trimethoprim, Triple sulfa, Bacitracin, Sulfamethoxazol, Chloramphenicol and Clindomycin. The same findings were reported by Jeffry and Silvia (2000). *E. coli* revealed complete resistance to

Gentamycin and Triple sulfa. From the previous mentioned data it is concluded that all isolated bacterial strains exhibit different levels of resistance to B-lactam (Penicillin, Ampicillin), Glucoside (Streptomycin, Neomycin), Macrolide (Erythromycin), Tetracycline, Chloramphenicol and Sulphonamides and this could be related to the fact that these antimicrobial drugs are extensively used for mastitis therapy. While, highly susceptible to another agents and this may be due to these drugs are not yet available as veterinary preparation and not used routinely in mastitis therapy (Mohammed, 2006).

CONCOLUSION

In our results suggest that to improve udder health and milk quality the general measures of hygiene including, teat disinfection, antibiotic dry cow therapy, correction of milking machines faults, antibiotic treatment during lactation in clinical cases and removal of treatment resistant animals(culling) should be applied to improve product quality and protect consumers.

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