COMPARISON OF THREE INDIRECT TESTS FOR THE DIAGNOSIS OF BOVINE SUBCLINICAL MASTITIS CAUSED BY COAGULASE NEGATIVE STAPHYLOCOCCI WITH THEIR SUSCEPTIBILITY TO SEVEN ANTIBIOTICS.

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ABSTRACT

Two hundred and eighty three bovine milk samples were diagnosed as subclinical mastitis by three indirect tests , the results were reported that the California mastitis test was the best one . Forty –four (15.55%) strains were identified as coagulase –negative staphylococci, Bacteriological cultures of coagulase-negative staphylococci were identified as 14 (31.8%) strains of *S. scuiri* , 7 (16%) strains of *S. lentus*,6 (13.6%) strains of *S. gallinarum*, 4 (9%) for each strains of *S. warneri,* and *S. xylosus* , 3 (6.8%) strains of *S. saprophyticus* , 2 (4.5%) for each strains of *S. lugdunensis* , *S. haemolyticus* and strains of *S. cohnii*. The percentage of antibiotic resistant in CNS were 97.7% for Ampicillin , (86.4%)for Novbiocen +Penicillin and (77.2%) for Oxcillin. While the percentage of antibiotic sensative were (100%) for each of Ciprofloxacin , Chloramphenicol and Gentamycin.

INTRODUCTION

Milk is an important diet for human beings it composed of vital components like water, proteins, lactose, minerals and vitamins. The composition of milk varies with species and breed of the animal, feeding regimes, stage of lactation parity and udder health. Milk available to our mass is lower in food value due to high prevalence of mastitis in dairy animals (1). Mastitis is an inflammation of the mammary glands of dairy cows accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue .It is the most common infectious diseases in dairy industry(2). Also, Bovine mastitis is a multi -factorial disease, it imposes serious economic losses for the farmers and dairy industry (3, 4). Moreover the milk from an affected animal may harbor the organisms potentially pathogenic for human (5).

Mastitis or intra-mammary infection can generally be classified as clinical and subclinical (6). Clinical mastitis is characterized by an abnormal secretion containing clots or flakes (7, 8). Moreover the clinical mastitis (acute clinical mastitis) is accompanied by swelling hardness and increase temperature and it also may be accompanied by systemic signs such as loss of appetite ,fever and depression (9).However, subclinical mastitis associated with no apparent changes in the udder or milk composition ,although microorganism can be isolated by appropriate culture techniques .Compositional change and increased somatic cell count in milk usually accompany subclinical mastitis and can be detected by appropriate tests(6,10).

Mastitis may be caused by a wide variety of microrganis including bacteria, fungi, yeast and mycoplasma, however bacteria are the most frequent pathogens of these disease (11,12) .Staphylococci are currently the most frequently isolated microorganisms from bovine and buffaloes mastitis (13,14). Staphylococci are divided in to coagulase-positive (CPS) and coagulase-negative staphylococci (CNS) on the basis of the ability to coagulate rabbit plasma. In diagnosis of bovine mastitis, the clarification has been considered adequate because CNS usually occurs in subclinical mastitis or mild clinical mastitis (11, 12). CNS is mainly involved in inflammatory processes of the bovine udder (15). Moreover during recent years ,CNS have become the most common causes of bovine mastitis isolated in many countries and are regarded as emerging mastitis pathogens (15).

This study aimed to 1) diagnose the bovine subclinical mastitis by using indirect tests California mastitis test (CMT), White side test (WST) and Surf test (ST); 2) Isolation and identification of CNS from positive milk samples; 3) determine antimicrobial susceptibility patterns of the identified isolates.

MATERIALS AND METHODS

Animals

Apparently normal cows were selected to screen for subclinical mastitis by indirect tests CMT, WST and ST. CMT was done according to (16), WST according to (17) and ST according to (18). The reactions was interpreted as follows: score 0 = no reaction; trace = slight slime which disappears with continued swirling; 1+ distinct slime but without gel formation; 2+ = immediate formation of gel which moves as a mass during swirling; 3+ = gel develops a convex surface and adheres to the bottom of the paddle.

Collection of samples

Two hindered eighty three samples were collected aseptically for bacterial studies according to (19). The samples were collected in sterilized tubes; they were marked with

number of animal and position of quarter. Prior to collection of milk samples, the udder washed with water and dried with paper towel then the quarters were disinfected with 70% ethyl alcohol. The first streams of milk discarded then samples were collected. All tubes of samples transported to the laboratory in ice box.

Microbiological examination

Loop full of milk samples were inoculated on mannitol salt agar (MSA, Himedia) by streaking procedure and incubated for 24h-48h at 37°C and on blood agar(BA , Himedia) by the same technique for 24h at 37°C for pure culture according to (20). Suspected colonies on MSA and BA were further studied through macroscopic examinations by stained preparation Gram's stain. The isolates which give microscopic appearance of staphylococci cells were examined by catalase test according to (21), and oxidase test by using oxidase discs from (Himedia ,India) according to (22).

Tube coagulase test

The suspected colonies were inoculated in nutrient broth for 18hr at 37°C, 0.8 ml of broth was mixed with 0.2 ml of rabbit plasma in sterile tubes and were incubated at 37°C.Positive result indicated by clot formation within 4-24 hr according to (23).

Biochemical identification test

The suspected isolates were identified by using HiStaph^{TM} identification kit (Himedia,India). The results were compared with standard chart from the producer.

Determination of antimicrobial susceptibility

The identified isolates were tested for their susceptibility to 7 antibiotics. The disc diffusion procedure was used according to (24). The tested discs included Oxacillin (1µg), Penicillin (10U),Ampicillin (25µg), Novobiocin (30µg), Ciprofloxacin (5µg), Gentamycin (30µg) and Chloramphenicol (30µg), all these discs were from (Bioanalyse/Turkey).

Statistical analysis

- The statistical calculation was carried out with statistical package Minitab V.14.

RESULT

All isolated CNS were detected from milk samples which were positive to indirect tests. Table (1) shows efficacy of three indirect tests used in primary detection of subclinical mastitis. The tests were differ in strength of response to inflammation of udder, the difference was statistically significant (Chi-square =12.864, DF=6, P-Value <0.05).

Table (1): Comparison between the efficacies of three different indirect tests.

Tests name	Trace	+1	+2	+3	Total*
CMT	0	18	17	9	44
WST	8	19	14	3	44
ST	8	20	12	4	44

(Chi-square =12.864, DF=6, P-Value <0.05)

*All identified isolates were 44 CNS.

Forty four coagulase-negative staphylococci (15.5%) were isolated from 283 milk samples which were positive to indirect tests. The samples were taken from 103 lactating cows. CNS isolates include 9 species as shown in table (2).

Table2: Number of CNS isolates which identified by HiStaph™ identification kit and the species they belong to it.

Species	Number of isolated Bacteria	Percentage
S. sciuri	14	31.8%
S. lentus	7	16%
S. gallinarum	6	13.6%
S. warneri	4	9%
S. xylosus	4	9%
S. saprophyticus	3	6.8%
S. lugdunensis	2	4.5%
S. haemolyticus	2	4.5%
S. cohnii	2	4.5%
Total	44	100%

Nine species of CNS were detected from sub-clinical mastitis cases, including: *S.sciuri* (31.8%) was the most frequently identified isolates (figure: 1), followed by *S.*

lentus (16%), *S. gallinarum* (13.6%), *S.warneri* ,*S.xylosus* (9%) for each, *S.Saprophyticus* (6.8%),*S. lugdunensis* , *S.haemolyticus* and *S.cohnii (4.5%)* for each .

Voges proskaers	Alkaline phosphotase	ONPG	Urea	Arginine utilization	Mannitol	Sucrose	Lactose	Arabnose	raffinose	Trehlose	Maltose
-	-	-	-	-	+	+	-	+	-	+	+

Staphylococcus sciuri

Figure (1)*: Staphylococcus sciuri* identified by HiStaph^{TM} identification kit.

The CNS isolates were screened by Kirby-Bauer method to testing antibiotic resistance pattern, table (3) and figure (2) show the results of antibiotic susceptibility. Substantial antibiotic resistance was shown against Ampicillin (97.7%), Novobiocen (86.4%), Penicillin (86.4%), and Oxacillin (77.2%). All isolates were found to be highly sensitive to Chlormphenical , Ciprofloxacin and Gentamycin(100%) for each .

Table (3): Antibiotic susceptibility of CNS isolates screened with disk diffusion method.

Antibiotic	Resistance		Intermediate		Sensitive		Total
	Number	(%)	Number	(%)	Number	(%)	
Ampicillin	43	97.7	0	0	1	2.3	44
Novobiocen	38	86.4	0	0	6	13.6	44
Penicillin	38	86.4	2	4.5%	4	9.1	44
Oxacillin	34	77.2	0	0	10	22.8	44
Chlormphenicol	0	0	0	0	44	100	44
Ciprofloxacin	0	0	0	0	44	100	44
Gentamycin	0	0	0	0	44	100	44

(Antibiotic discs = Ampicillin=25µg ,Pencillin = 10U , Oxacillin= 1µg ,Novobiocen =30µg , Chlormphenicol=30µg ,Ciprofloxacin= 5µg and Gentamycine=30µg)

Figure (2): Antibiotic susceptibility of CNS strains isolated from subclinical mastitis.

DISCUSSION

Results of this study indicate that the CMT was the most efficient in diagnosis of subclinical mastitis, table (1). Forty four samples which tested by CMT were gave result ≥ 1+, however, the samples tested by other two tests were gave 34 ≥ 1+. This result is in accordance with (25), who concluded that the efficiency of CMT is better than that of other indirect tests.

CNS were more common in subclinical mastitis than in clinical cases of mastitis (26).*The* isolation rates of CNS from bovine subclinical mastitis ranged from 7.4% to 53.5% (27 and 28). In this study , the isolation rate of CNS from subclinical mastitis was (44/283) 15.55%, this result is nearly similar to that reported by(29), who reported that the isolation rate of CNS from bovine subclinical mastitis was (11.7%) , and in agreement with(30), who examined 123 cow milk samples in Argentina and found 13.6% were positive to CNS .

The isolated species from subclinical mastitis including; *S. sciuri, S. lentus, S. gallinarum, S. warneri, S. xylosus, S. saprophyticus, S. lugdunensis, S. haemolyticus* and *S. cohnii,* table (2). These results are in agreement with (31,32,33,34, 35 and 36).

One of the important reasons for failure of treatment is assumed to be indiscriminate use of antibiotic without using *in-vitro* sensitivity of causative organism.This practice at one hand increases of economic loses and on the other hand , results in development of resistance to commonly used antimicrobial (37).In this study CNS species were highly resistant to Ampicillin, Penicillin, Novobiocen and Oxacillin, whereas these isolates

were highly sensitive to Chloramphenicol, Ciprofloxacin and Gentamycin, table (3). These results are in accordance with (31,38.,39 and 40).

مقارنة لثلاثة اختبارات غير مباشرة في تشخيص التهاب الضرع ألبقري تحت ألسريري الناتج عن المكورات العنقودية السالبة لإنزيم التجلط وحساسيتها تجاه سبعة أنواع من المضادات الحياتية.

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الخلاصة

تم تشخيص مائتين وثلاثة وثمانون عينة من الحليب ألبقري لأبقار مصابة بالتهاب الضرع تحت ألسريري بواسطة ثلاثة اختبارات غير مباشرة،أوضحت نتائجها بان اختبار كاليفورنيا الأكثر فعالية في تشخيص التهاب الضرع . تم تشخيص العزلات طبقا للفحوص الزرعية والمجهرية والكيموحيويةو HiStaph^{TM} identification kit, أربعة وأربعين (15.55٪) سلالة من المكورات العنقودية السالبة لانزيم التجلط. وقد تم تحديد العزلات البكتيرية من المكورات العنقودية السالبة لانزيم التجلط إلى 14 (31.8٪)عزلة من *S. scuiri*، 7 (16٪) من عزلة *lentus* *S.* 6 (13.6٪) عزلة من *S. gallinarum* ، 4 (9٪) من عزلة *S. warneri* 4, (9٪) سلالة من *S. xylosus*، 3 (6.8٪) من عزلة *S. saprophyticus* ،2 (4.5٪) عزلة من S *S. lugdunensis* ، 2 (4.5٪) عزلة من *S. haemolyticus* و 2 (4.5٪) من عزلة *S. cohnii*. أظهرت نتائج اختبار الحساسية للمضادات الحيوية ان سلالات المكورات العنقودية السالبة لانزيم التجلط كانت مقاومة للأمبيسلين (97.7٪)، النوفوبيوسين + البنسلين (86.4٪) وأوكساسيلين (77.2٪). في حين كانت العزلات حساسة (100٪) للسيبروفلوكساسين، الكلورامفينيكول والجنتاميسين.

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