Isolation and Identification of *Staphylococcus spp.* from Bovine Mastitic milk and their Sensitivity to some Antibiotics at Al-Qadissiya Province.

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Abstract

This study was designed to detect the Staphylococcal bovine mastitis in Al-Qadissiyaprovince and then identify the most effective antibiotic that could be used for inhibit the growth of isolated microorganism invitro. Milk samples have been collected from 120 different cows at Al-Hamza, AL- Shenafia, Nufar, Sumar, AL- Saniah , Al- Sedeer and Afak the results showed different bacterial isolates identified in the present study as :*S.aureus* , *S.intermedius* ,*S. hyicus, S. epidemidis* , *S. chromogenes* , *S .cohnii* , *S. hominis* , *S. xylosus* , *S.sciuri* , *S. simulans* , *S.saprophyticus* in a percentage (15.74%,6.481% , 8.333% , 7.87% , 3.703% , 4.166% , 6.481% , 5.092% , 4.629% , 6.018% , 0.462 %,) respectively .The sensitivity test results showed that *S.aureus,* was more sensitive to Ciprofloxacin in a percentage (91.17%),CNS (Coagulase Negative *Staphylococci*) was more sensitive to Oxytetracycline 83.25%,

Introduction

"Mastitis" is "inflammation of mammary gland tissue". Inflammation of the bovine udder is usually caused by infection, mostly by bacteria, Intramammary infections can be accompanied by visible changes of milk , such as clotting and discoloration, and clinical signs of animal such as swelling and discoloration of the udder, fever, anorexia and even death. When signs are discernible with the naked eye, infection has caused clinical mastitis.Laboratory techniques such as measurement of somatic cell count (SCC) and bacteriological culture are needed to detect inflammation and infection. Mastitis is a major concern to dairy producers and the food industry around the world for reasons of farm profitability, food quality and animal and public health(1).Diagnosis of subclinical forms may be more difficult but is an important part of any herd survey to establish the disease incidence . in addition to bacterial culture of milk ,several indirect tests are employed to ensure the presence of inflammatory exudates and cells in infected milk ,such as California mastitis test (CMT) (2) .This disease can have an infectious or noninfectious etiology, and the infectious pathogen is the most important ones that frequently due to infection by one and/or the other pathogens, such as bacteria, viruses, Mycoplasma, yeasts and algae (3). Fortunately the vast majority of mastitis is of bacterial origin and just a few of species of bacteria account for most cases, such as, *S. aureus*, *E. coli Str.uberis*, *Str. dysgalactiae* and *Str. agalactiae* (4).

Material &Methods

Samples collection:

One hundred and twenty cows were examined, milk samples which positive to mastitis according to result of CMT and other from clinical mastitic cows were collected from cows in Al-Qadissiya province as follow : AL- Hamza , AL-Shenafia ,Nufar, Sumer, AL- Saniah ,Al-Sedeer , andAfak . Milk samples were collected in sterile tubes (2 tubes) for each sample (one for CMT and another for bacteriological test) and aseptic technique used for milk samples collection according to (8).

Bacterial culture and identification:

All milk samples from clinical mastitis and subclinical mastitis which gave a positive reaction with California Mastitis Test (Al-Syria for veterinary preparation .Syria) were submitted to centrifugation at 3000

rpm / 15 minute, and the precipitate was cultured on :Blood Agar ,Nutrient Agar and MacConky Agar ,all the Petri plate that contain this agars were incubated at 37 C° for 24 - 48hrs (6)Diagnosis depend on morphological character & cultural character (9) , then followed by examination with gram stain, after that the colonies were subcultured on selective and differential media according to the type of isolated bacteria then incubated at 37 C° for 24 – 48 hrs. The biochemical test used for diagnosis of *staphylococcus spp*. were include:

- Catalase Test , Oxidase test , Coagulase Test , Urease Test , Heamolysis on blood agar , Gelatin Liquefaction Test (Gelatinase) , Voges – Proskauer Test , Nitrate reduction Test , Sugar Fermentation Test (Mannitol , Lactose , Mannose , Xylose ,Trehalose , Sucrose , Maltose) according to the method of (7 ,8, 9).Production of pigment in Mannitol salt agar and in (Staph 110 media) (LAB – U.K) MAST STAPH™: (Mast Group Ltd , USA)API Staph (biomerioux, France).

Sensitivity test:

The sensitivity was done according to the procedure of (10) and the following antibiotic were used (Streptomycin 10mcg, Erythromycin 15mcg, Sulphamethaxazole – Trimethoprim 25mcg (1.25/23.75mcg) , Ampicillin 10mcg, Gentamicin 10mcg, Ciprofloxacin 5mcg, Tetracycline 30mcg ,Oxytetracycline 30 mcg , Amoxicillin / Clavulanic cid 30 mcg (20 mcg / 10mcg)Bioanalyse® ,Germany).The diameter of the inhibition zone of each antibiotic disc (mm) (the clear area that surround the antibiotic disc including diameter of the disc itself which is free of the bacterial growth) by using of calibrated ruler and then compared the result with standard diameter of the inhibition zone of the antibiotic as mention in National Committee for Clinical Laboratory Standard(NCCLS).

Results and discussion

The result showed in *Staphylococcus aureus* was the most isolated bacteria in percentage 15.74% in (table 1) may Due to its contagious nature it has become a major udder pathogen in many parts of the world. It may cause both clinical and sub-clinical mastitis (11), the higher percentage of *S. aureus* isolates in this study was agree with a results of (12).The result of CNS in this study include *S.intermedius ,S. hyicus, S. epidemidis , S. chromogenes , S .cohnii , S. hominis , S. xylosus , S.sciuri , S. simulans , S.saprophyticus* in a percentage of (6.481% , 8.333% , 7.87% , 3.703% , 4.166% , 6.481% , 5.092% , 4.629% , 6.018% , 0.462 %) respectively (table 1).The results of (*S. hyicus , S. epidermidis*, *S. hominis*, *S.sciuri*)in this study was in agreement to the result of (13) who isolates these bacteria from bovine mastitis in a percentage (6.06% , 9.1% , 6,06% , 3.03%), (14) was agree with our result for *S.cohnii* in a percentage,5%. (15)was isolated *S. chromogenes* in a percentage 2.152% which was closed with our result . The result of *S. simulans* was closed to the result of (16) with a percentage 4.245% The result of *S.xylosus* in this study was closed to the result of (17) who isolated this bacteria in percentage 3.66%.%.(18) was closed to our result of *S.saprophyticus* in a percentage (0.68% ,0.3 %) respectively. (19) was closed with our result for *S.intermedius* in a percentage 13.9%.(20) disagree with a result of *S.hyicus ,S.epidemidis ,S.cohnii ,S.hominis*) in a percentage (16.5% , 1.9% , 0.3% ,1.9%) respectively. (21) disagree with our result for *S.saprophyticus , S. simulans* in a percentage (8.176% 1.886%) respectively , (22) also disagree with our result of *S.saprophyticus* in a percentage 10.1%.Among studies isolation of , *S. chromogenes, S. epidermidis,S. hyicus,* and *S. simulans*, seem to be the most common CNS isolated from intra-mammary infections in spite of some variation between herds, countries, and methods used (23). Bovine CNS have traditionally been considered as skin flora opportunists (24).CNS have also been isolated from the cows´ environment (25).*S. chromogenes* was frequently isolated from the teat skin,

and teat canal, but also from extra-mammary sites like nares, hair coat and vagina in heifers (26).According to(27) *S. cohnii, S. saprophyticus, S.sciuri,* and *S. xylosus*, were the most common in the cows' environment (e.g.alfalfa hay, straw and bedding).The variations in the percentage of infection ratio of different CNS may be due to geographical areas and climatic differences.The results of present study showed that there were differences in the percentage of bacterial isolates between villages and township of Al-Qadissiyaprovince , these differences could be explain may be due to variation in geographical areas and climatic condition, according to the differences in temperature , humidity ,environment and nature of society (28) (table 2 ,3).The result of this study for sensitivity test showed that the *S. aureus* was more sensitive to Ciprofloxacin, Erythromycin, Oxytetracycline, Amoxicillin/ Clavulanic acid followed by Sulphamethaxazole/ Trimethoprim then Gentamicin (91.17%, 88.23% , 82.35% , 79.41%, 73.52% ,70.58%) respectively and less sensitive to Streptomycin 58.82% and the lower sensitivity to Ampicillin 32.35% these result was closed to the results of (29) who found that *S. aureus* was more resistance to Ampicillin and 80 % sensitive to Gentamicin.The resistance of bacteria to Ampicillin may caused by random using of this antibiotic (30). The results of sensitivity of Gentamicin was closed to the results of (31; 32) while disagree with them in the sensitivity of Ampicillin.(33) was closed with our results of Ciprofloxacin, Gentamicin, Tetracycline, Sulphamethaxazole / Trimethoprim sensitivity with a percentage of (100% , 76.88% , 71.19% , 72.26%) respectively while disagree with sensitivity of Ampicillin in a percentage 0%.It has been found that amoxicillin with clavulanic acid are the very efficient in inhibiting the growth of *Staph. aureus* (34) which was very closed to our results he also reported that 75% of the *Staph. aureus* strains were resistant to tetracycline and 6.2% of the isolated strains were susceptible which was disagree with our results, while (29) mentioned that 58.33% of isolates susceptible to tetracycline which was closed to our result. (35) found that *Staph. aureus* was resist to Streptomycin in a percentage 42.9% which was very closed to our results .(20) was found that *Staph. aureus* sensitive to Erythromycin in percentage 74.4% which closed to our results . (36) found the sensitivity of *Staph. aureus* to Sulphamethaxazole / Trimethoprim was 74.7% which was very closed to our result . (14) closed with our result in Ciprofloxacin with a percentage 100% while disagree with our result for Oxytetracycline in a percentage 60 % while the result of Oxytetracycline closed with (30) in 2nd station in a percentage 83.33%.(34) was closed with our result of sensitivity of CNS for Amoxicillin/ Clavulanic acid with a percentage 75% . (20) found that the percentage of sensitivity of CNS for Ampicillin, Streptomycin, Tetracycline was (58.2% ,62.7% , 76.1%) respectively which was closed to our results while disagree with our results of Erythromycin 51.4%. (14) closed to our result of (Ciprofloxacin, Oxytetracycline,Erythromycin,Sulphamethaxazole/Trimethoprim) in a percentage (80% , 90% , 70% , 73%) respectively also(37) closed to our result of (Oxytetracycline, Erythromycin, Sulphamethaxazole / Trimethoprim, Amoxicillin/ Clavulanic acid , Tetracycline) in a percentage (77.61% , 71.64% , 65.67%, 80.6% , 71.64%) respectively while disagree with Gentamicin in a percentage 100%in table (4) .Resistance to antibiotic mediated most commonly by the production of enzymes that modified the drug e.g β- Lactamases Hydrolyse Penicillin other mechanism include decrease the passage in to or increase the efflux of drug from the bacterial cell , modification of the target site so that the antimicrobial bound less effective and by passing of inhibited metabolic pathways as resistance to trimethoprim in many bacteria (38) .

Table(1): percentage of bacterial isolates

Bacterial isolates	%
S.aureus	15.740 %
S.intermedius	6.481 %
S . hyicus	8.333 %
S . epidermidis	7.870 %
S . chromogenes	3.703 %
S . cohnii	4.166 %
S . hominis	6.481 %
S . xylosus	5.092 %
S . sciuri	4.629 %
S . simulans	6.018 %
S . saprophyticus	0.462 %

Table (2):Distribution of the *Staphylococcus spp*. according to regions

Region	*S. aureus*	*S. intermedius*	*S . hyicus*	*S . epidermidis*	*S . chromogenes*	*S . cohnii*	*S . hominis*	*S . xylosus*	*S . sciuri*	*S . simulans*	*S. Sapro.*
AL- Hamza	26.470 %	5.882 %	2.941%	8.823 %	2.941%	5.882%	2.941%	0	5.882 %	5.882%	0
AL- Shenafia	12%	4%	4 %	4%	0	4%	12%	12%	4%	8%	4%
Nufar	14.814%	11.111%	11.111 %	3.703%	11.111%	11.111 %	0	0	7.407 %	7.407%	0
Sumer	9.090%	0	9.090%	9.090%	0	0	9.090%	0	0	9.090%	0
AL- Saniah	10.416%	4.166%	22.916 %	12.5%	2.083%	0	8.333%	4.166%	4.166 %	4.166%	0
Al- Sedeer	25.0%	7.142%	0	3.571%	3.571%	0	7.142%	7.142%	3.571 %	14.285%	0
Afak	11.627%	9.302%	2.325%	9.302%	4.651%	6.976%	6.976%	9.302%	4.651 %	0	0

Table (3): Sensitivity of *Staphylococcus Spp.* Isolates to Antibiotic

AB	*S.aureus*	*CNS*
	%	%
AM	23.35	56.41
S	58.82	62.81
CIP	91.17	74.41
CN	70.58	73.64
AMC	79.41	70.58
T	67.64	69.67
OT	82.35	83.25
E	88.23	71.93
SXT	73.52	63.16

AM = Ampicillin , S = Streptomycin , CIP = Ciprofloxacin CN = Gentamicin , , AMC = Amoxicillin/ Clavulanic acidT =Tetracycline, OT = Oxytetracycline, E =Erythromycin
, SXT = Sulphamethaxazole/Trimeprime

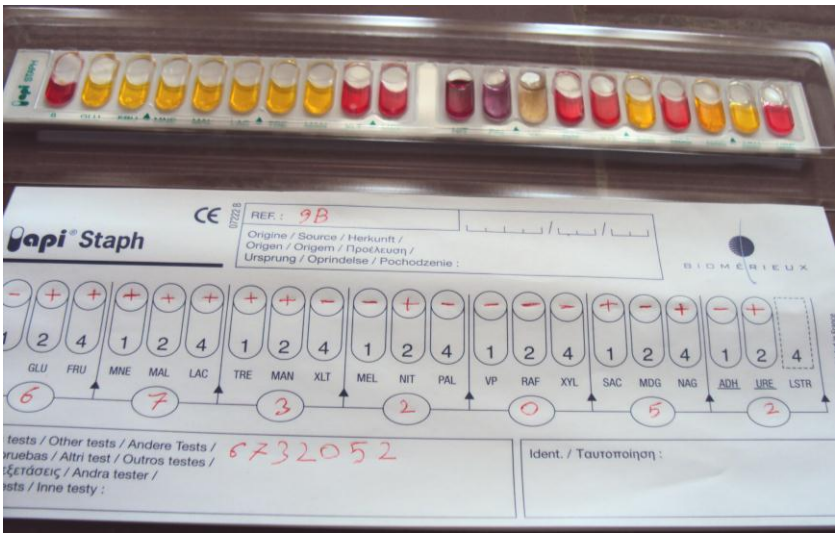

Figure (1) : API Staph

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العزل والتشخيص ألمختبري لجراثيم المكورات العنقودية المسببة لالتهاب الضرع البقري وحساسيته لبعض المضادات الحياتية في محافظة القادسية

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الخلاصة

صممت هذه الدراسة للكشف عن التهاب الضرع ألبقري المتسبب بواسطة المكورات العنقودية في محافظة القادسية والكشف عن المضاد الحيوي الأكثر فعالية في تثبيط نمو هذه الجراثيم مختبريا. جمعت عينات الحليب من 120 بقره في مناطق مختلفة من المحافظة (الحمزة ,الشنافية ,نفر, سومر ,السنية , السدير , عفك) والنتائج بينت عزل وتشخيص: المكورات العنقودية الذهبية, المكورات العنقودية انترميديس , المكورات العنقودية هايكس , المكورات العنقودية ابيديرميدس, المكورات العنقودية كروموجينس , المكورات العنقودية كوهني , المكورات العنقودية هومينس , المكورات العنقودية زايلوسس , المكورات العنقودية سيميولنس , المكورات العنقودية سابروفايتكس وبنسبة (15.74% ,6.481% , 8.333% , 7.87% , 3.703% , 4.166% , 6.481% , 5.092% , 4.629% , 6.018% , 0.462%) على التوالي. ان نتائج فحص الحساسية للمضادات الحياتية الذي اجري مختبريا أظهرت إن المكورات العنقودية الذهبية كانت أكثر حساسية للسايبروفلوكساسين بنسبة (91.17%) أما المكورات العنقودية السالبة للاختبار التجلط كانت اكثر حساسية للاوكسي تتراسايكلين بنسبة 83.25%.