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-Qadissiya province 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. chromogens , 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 MacConkey 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, Hemolysis 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 –

The result of CNS in this study include S.intermedius, S.hyticus, 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 CNS 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.epidemidis, 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-Qadissiyaprovine, 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

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>15.740 %</td>
</tr>
<tr>
<td>S.intermedius</td>
<td>6.481 %</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>8.333 %</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>7.870 %</td>
</tr>
<tr>
<td>S. chromogenes</td>
<td>3.703 %</td>
</tr>
<tr>
<td>S. cohnii</td>
<td>4.166 %</td>
</tr>
<tr>
<td>S. hominis</td>
<td>6.481 %</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>5.092 %</td>
</tr>
<tr>
<td>S. sciuri</td>
<td>4.629 %</td>
</tr>
<tr>
<td>S. simulans</td>
<td>6.018 %</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>0.462 %</td>
</tr>
</tbody>
</table>
Table (3): Sensitivity of *Staphylococcus Spp.* Isolates to Antibiotic

<table>
<thead>
<tr>
<th>AB</th>
<th><em>S.aureus</em> %</th>
<th><em>CNS</em> %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>23.35</td>
<td>56.41</td>
</tr>
<tr>
<td>S</td>
<td>58.82</td>
<td>62.81</td>
</tr>
<tr>
<td>CIP</td>
<td>91.17</td>
<td>74.41</td>
</tr>
<tr>
<td>CN</td>
<td>70.58</td>
<td>73.64</td>
</tr>
<tr>
<td>AMC</td>
<td>79.41</td>
<td>70.58</td>
</tr>
<tr>
<td>T</td>
<td>67.64</td>
<td>69.67</td>
</tr>
<tr>
<td>OT</td>
<td>82.35</td>
<td>83.25</td>
</tr>
<tr>
<td>E</td>
<td>88.23</td>
<td>71.93</td>
</tr>
<tr>
<td>SXT</td>
<td>73.52</td>
<td>63.16</td>
</tr>
</tbody>
</table>

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

Figure (1) : API Staph

**References**


