ISOLATION AND BIOTYPING OF *STAPHYLOCOCCUS AUREUS* FROM WHITE CHEESE IN BASRAH LOCAL MARKETS

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ABSTRACT

Fifty samples of white cheese were collected from 3 different local market of Basra city AL-basra, (15), AL-ashar(20) and AL-jumhurya(15 ) samples respectively, After being examined by culturing on MSA media, the results reveal that 53.33%, 50%, and 13.33% of Staph aureus were isolate from white cheese respectively. Depending on the biotyping, the percentage of biotype A Staphylococcus aureus was 90% and biotype C was 10%. Antibiotic sensitivity test showed that 55%, 55%, 45%, 35% of isolates were more sensitive to Erythromycin, Chloramphenicol, Tetracycline and Ciprofloxacin respectively.

Key words: White cheese, microbiological quality, Microbiological contamination

INTRODUCTION

Milk, is one of our most nutritionally complete foods adding high quality protein, fat, milk sugar, essential minerals and vitamins to our diet[1]. However, milk contains bacteria that when improperly handled may create conditions for multiplication, most of bacteria in fresh milk of healthy animals are either harmless or beneficial but rapid changes in health of an animal or milk handler or contaminated from polluted water, air, manure, air, cuts and wound can make raw milk potentially dangerous[2]. Raw milk cheese is more likely to harbor harmful bacteria because raw milk cheese is made with milk that has not been pasteurized as a result, if the milk becomes contaminated with any bacteria during milking or cheese making processes that bacteria will present in the final product and consumers would then be exposed to bacteria which could cause anything from mild stomach distress to death[3].
Staphylococcus aureus is a leading cause of gastroenteritis resulting from the consumption of contaminated food[4]. Contamination of dairy products with staphylococcus aureus bacteria may influence considerably their harmlessness, decrease their shelf-quality and endanger the health of consumers. Of Staphylococcus aureus causes disease both in people and animals[5,6]. And thus attracts considerable attention particularly from the point of view of food hygiene[7]. For this reason, the aim of this study were done to isolation Staphylococcus aureus from cheese samples collected randomly from local area in Basrah city.

**MATERIALS AND METHODS**

**Sample collection**

A total of 50 samples of white cheese were collected from different shops of Basrah city in three local markets (Al-Basrah, Al-Ashar and Al-Jumhuriya). Microbiological analysis of these samples were made. Investigation were take place in the laboratory of microbiology college of veterinary medicine, Basrah University. Samples were transported in an ice chest and stored at 5°C before analysis.

**Microbiological analysis:**

Appropriate dilutions of procedure of homogenization and reference samples were made by transferring 10g in 90ml of aqueous solution 2% sodium citrate. After that serial dilutions were performed using 9ml of 0.1% pepton water as adilunt. The cheese samples were analysed for the presence of S. aureus by transferring 0.1ml of each dilution onto the dry surface of prepared plates of Mannitol salt agar and spreading with a sterile glass spreader, the plates was inverted and incubated at 37°C for 2 days. After incubation suspected colonies of Staphylococci were sub cultured again on Nutrient agar.

**Identification of Staphylococcus aureus :**

The isolates were identified on the basis of cultural characteristics. Pigment production, Haemolysin, plasma coagulation and Susceptibility to crystal violat were performed as follow: The isolates of S. aureus were cultured on the blood agar and incubated at 37°C for 24hrs presence of zone of haemolysis around the colonies was
considered as a positive result. These isolates also cultured on the milk agar and incubated at 37°C for 24h., after that yellow pigment appearance production indicated positive results. The S. aureus was tested for coagulase production done by adding 0.1ml of an overnight Brain heart infusion broth culture of the Staphylococcus to the 0.3ml of human plasma.

The tube is rotated gently to mix the contents and then incubated at 37C for 4 hrs. A positive test with clotting of the plasma, can occur in 2 to 4 hours and or after overnight incubation, the isolates revealed positive results, by culturing of isolates on the crystal violate medium and incubating at at 37C for 24hrs., different biotypes were classified depending on the color of the growth on this medium, biotype A appeared positive to crystal violate, biotype C appeared yellow on this medium[8].

**Antibiotic susceptibility test**

Susceptibility to antibiotics was tested according to disk diffusion method using Muller Hinton Agar. Erythromycin, Clindamycin, ciprofloxacin, Tobramycin, penicillin, Vancomycin, Cloramphenicol, Nitrofurantion, Tetracyclin and Gentamycin antibiotic disks in this testing. The plates were incubated at 36°C± 1 for 24h, and the measurements of the inhibition zone compared to special standard table was done[9].

**RESULTS AND DISCUSSION**

Table 1 showed that 53.33%, 50% and 13.33% of cheese sample were collected from different local market Al-Basrah, Al-Ashar and Al-Jumhuriya respectively gave positive results able to grow on mannitol salt agar, [10] found that about 88% from 120 raw milk samples contained staphylococci, [11] also found that 58 of 93 samples from nomadai herds [Fig 1].

In table 2 biotyping study was reveled that the isolates showed β- haemolysis in blood agar media, produsing pigment on milk agar [Fig 2] and about 90% of 20 positive isolates apper as biotype A in crystal violate media while only 10% percentage appers as biotype C on this media [Fig3]. In another study [13] done in France showed that two pasteurized milk samples were contain biotype A staphylococcus from 250 pasteurized milk samples.
while [13] mentioned that the majority of the Staph aureus isolated from raw milk and cheese were found to be about 62% biotype C, [14] showed that 80% percentage of 20 Staph aureus isolates were biotype C and 20% percentage were biotype A. This study showed also the ability of isolates to coagulate to human plasma [Fig 4][Table 2]. The coagulase positive Staphylococci constitute the well known pathogenic Staph aureus [15].

A variety of disease may be potentially transmitted through milk and its product, the source of pathogenic agents occurring in milk may be either of cows or human, and it may be transmitted by both [16]. The milk drawn from healthy animals may be free of bacteria but it becomes contaminated by hands of milkier or from udder of animals hand boring microorganism, Dairy teats with dung mud are the direct source of various types of bacteria but the main source is the contaminated water that was added to milk to increase its quantity, the raw milk passes through unhygienic conditions during its transportation, Moreover, it takes long time to reach the consumer and during that time it becomes highly contaminated because of high temperature, which causes the proliferation of bacteria [15].

**Antibiotic susceptibility:** This study revealed different percentage of susceptibility to different antibiotics. The present result showed that (55%,55%,45%,35%,30%) of isolates were more sensitive to Erythromycin, Chloramphenicol, Tetracyclin, Ciprofloxacin, Pencillin and a percentage of (25%,20%,25%,40%) were moderately susceptible to these antibiotics [15] showed that about 96.10% Staph aureus isolates from raw milk were Susceptible to Erythromycin, only 1.3% from 77 isolates was moderately Susceptible bacteria. [16] mentioned that Staph aureus isolates from raw milk and milk product appeared sensitive to Erythromycine 90%, Chloramphenicol 95%. On the other hand the results in this study showed that about 90% of the isolates were more resistance to Vancomycine, Nitrofurantion and 75% resistance to Gentamycin, while 40% were resistance to Pencilline. The resistance of bacteria to antibiotics may be due to the excessive rational uses of this antibiotics that has developed resistance, Moreover, when low doses of antibiotics were used against bacteria, they inhibit the growth of susceptible bacteria and leave smaller number of already resistance bacteria, which thrive and grow. These bacteria spread their
resistance traits to other previously non-resistant cells thane eventually affecting other cells [17, 18,19, 20, 21].

The resistant strains may have been transferred to cow then to milk, which can be the reason of infection in human beings if we take raw milk, these can be treated by improving hygienic condition and careful handling of cow during milking.

### Table 1: Number and percentage of Staph aureus isolate from cheese samples that showed growth on MSA

<table>
<thead>
<tr>
<th>No.</th>
<th>Source of sample</th>
<th>Examined sample</th>
<th>Growth on MSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Al-Basrah</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53.33%</td>
</tr>
<tr>
<td>2</td>
<td>Al-Ashar</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>3</td>
<td>Al-Jumhuriya</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.33%</td>
</tr>
</tbody>
</table>

### Table 2: percentage of Heamolysin, Coagulase, pigment positive reactions and Percentage of biotype A and biotype C of the positive isolates

<table>
<thead>
<tr>
<th>β-Heamolysin</th>
<th>pigment</th>
<th>Coagulase</th>
<th>biotype A</th>
<th>biotype C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 20</td>
<td>100%</td>
<td>100%</td>
<td>90%</td>
<td>10%</td>
</tr>
</tbody>
</table>
Table 3: Antibiotic susceptibility of S. aureus to different types of antibiotics discs.

<table>
<thead>
<tr>
<th>NO.</th>
<th>Antibiotic</th>
<th>Susceptible Percentage</th>
<th>Intermediate percentage</th>
<th>Resistance percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Erythromycin</td>
<td>55%</td>
<td>25%</td>
<td>20%</td>
</tr>
<tr>
<td>2</td>
<td>Tobramycin</td>
<td>20%</td>
<td>30%</td>
<td>50%</td>
</tr>
<tr>
<td>3</td>
<td>Ciprofloxacin</td>
<td>35%</td>
<td>40%</td>
<td>25%</td>
</tr>
<tr>
<td>4</td>
<td>Tetracyclin</td>
<td>45%</td>
<td>25%</td>
<td>30%</td>
</tr>
<tr>
<td>5</td>
<td>Penicillin</td>
<td>30%</td>
<td>30%</td>
<td>40%</td>
</tr>
<tr>
<td>6</td>
<td>Vancomycin</td>
<td>_</td>
<td>10%</td>
<td>90%</td>
</tr>
<tr>
<td>7</td>
<td>Chloramphenicol</td>
<td>55%</td>
<td>20%</td>
<td>25%</td>
</tr>
<tr>
<td>8</td>
<td>Nitrofurantion</td>
<td>10%</td>
<td>_</td>
<td>90%</td>
</tr>
<tr>
<td>9</td>
<td>Clindamycin</td>
<td>20%</td>
<td>45%</td>
<td>35%</td>
</tr>
<tr>
<td>10</td>
<td>Gentamycin</td>
<td>25%</td>
<td>_</td>
<td>75%</td>
</tr>
</tbody>
</table>
Fig 1- Growth on Mannitol salt agar
Fig 2- Biotyping test {growth on (1), (2) crystal violat, (3) blood agar, (4) milk agar, (5) coagulase test}

Fig 3- Antibiotic test
Conclusion: According to the present results Staphylococcus aureus isolates were obtained from white cheeses in different percentages and the A biotype was isolated in higher percentages in comparison to biotype C.

Staphylococcus aureus

العزل والتصنيف الحيوي لجرثومة
من الجين الأبيض لأسواق البصرة المحلية

نورس نوري جابر
فرع الأحياء المجهري، كلية الطب البيطري، جامعة البصرة، البصرة، العراق

الخاتمة

جمعت خمسون عينة جبن أبيض من ثلاثة أسواق محلية في محافظة البصرة (15 عينة من سوق البصرة، 20 عينة من سوق العشائر، 15 عينة من سوق الجمهورية) زرعت عينات الجبن على وسط استكر مله المانيتوول حيث عزلت جرثومة Staphylococcus aureus المانيتوول، ونسبة النطاق A المانيتوول كانت نسبة النطاق 90%، ونسبة النطاق C المانيتوول كانت نسبة النطاق 10%. بعد إجراء فحص الحساسية للمضادات الحيوية، أظهرت النتائج أن العزلات كانت أكثر حساسية للمضادات الحيوية الأيزوثياميسين، الكلورامينكول، تتراساكتين، وسبيروفوكسين (55%، 55%، 45%) على التوالي.
REFERENCE


