

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 thate 53.33% , 50% , and 13.33% of Staph aureus were isolate from white chees respectively . Depending on the biotyping , the percentage of biotype A Staphylococcus aureus was 90% and biotype C was 10% . Antibiotic sensitivety 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 poluted 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 a 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. *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 5C° before analysis.

Microbiological analysis:

Appropriate dilutions of procedure of homogenization and refrence 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 ager and spreading with a sterile glass spreader, the plates was inverted and incubated at 37C for 2days.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, Heamolysin, plasma coagulation and Susceptibility to crystal violat were performed as fallow: The isolates of *S.aureus* were cultured on the blood agar and incubated at 37C 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 37C° 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 36C°± 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 revealed that the isolates showed β- haemolysis in blood agar media , producing pigment on milk agar [Fig 2] and about 90% of 20 positive isolates appear as biotype A in crystal violate media while only 10% percentage appears 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 chees were found to be abute 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 apercentage 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 Gentamycine, 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 than 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

No.	Source of sample	Examined sample	Growth on MSA	
			No.	%
1	Al-Basrah	15	8	53.33
2	Al-Ashar	20	10	50
3	Al-Jumhuriya	15	2	13.33

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

β -Hemolysin Total 20	pigment	Coagulase	biotype A	biotype C
100%	100%	100%	90%	10%

Table 3 :Antibiotic susceptibility of S.aureus to different type of antibiotics discs.

NO.	Antibiotic	Susceptible Percentage	Intermediat percentage	Resistance percentage
1	Erythromycin	55%	25%	20%
2	Tobramycin	20%	30%	50%
3	Ciprofloxacin	35%	40%	25%
4	Tetracyclin	45%	25%	30%
5	Penicillin	30%	30%	40%
6	Vancomycin	–	10%	90%
7	Chloramphenicol	55%	20%	25%
8	Nitrofurantion	10%	–	90%
9	Clindamycin	20%	45%	35%
10	Gentamycin	25%	–	75%

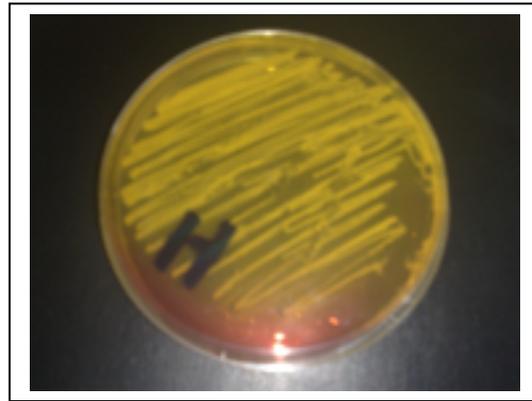


Fig 1- Growth on Mannitol salt agar



(2)

(1)





Fig 2- Biotyping test {growth on ,(1) ,(2) crystal violate ,(3) blood agar ,(4)milk agar, (5) coagulase test}



Fig 3- Antibiotic test

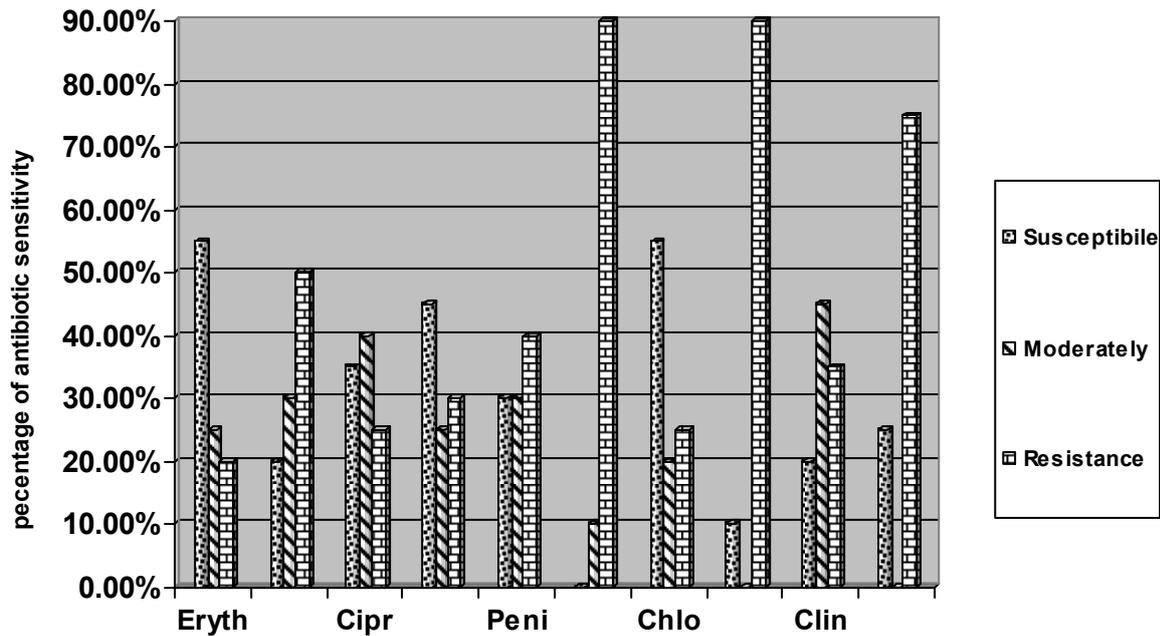


Fig 4 - Antibiotic sensitivity of *staph aureus* from white chees samples

Conclusion : According to the present results *Staphylococcus aureus* isolates were obtained from white chees in different percentage and the A biotype was isolates in higher percentage in comparison to biotype C.

العزل والتصنيف الحيوي لجرثومة *Staphylococcus aureus* من الجبن الابيض لاسواق البصرة المحلية

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الخلاصة

جمعت خمسون عينة جبن ابيض من ثلاثة اسواق محلية في محافظة البصرة (١٥ عينة من سوق البصرة ، ٢٠ عينة من سوق العشار ، ١٥ عينة من سوق الجمهورية) زرعت عينات الجبن على وسط اكار ملح المانيتول حيث عزلت جرثومة *Staphylococcus aureus* بنسب مختلفة (٥٣,٣٣% ، ٥٠% ، ١٣,٣٣%) على التوالي اعتمادا على التصنيف لجرثومة *Staph aureus* كانت نسبة النمط A 90% ونسبة النمط C 10% ، بعد اجراء فحص الحساسية للمضادات الحيوية اظهرت النتائج بأن العزلات كانت اكثر حساسية للمضادات الحيوية الارثرومايسين ، الكلورامفينكول ، تتراسايكلين وسبروفلوكسين (٥٥% ، ٥٥% ، ٤٥% ، ٣٥%) على التوالي .

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