

## **ISOLATION OF *Staphylococcus aureus* FROM BUFFALO MILK IN BASRA GOVERNORATE AND DETECTION OF THEIR ANTIBIOTIC SUSCEPTIBILITY**

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### **ABSTRACT**

This study was conducted on 215 milk samples collected from apparently healthy lactating buffalo during the period from August 2012 to March 2013. Isolation and identification of bacterial isolates were carried on the basis of their morphology, staining, cultural and biochemical properties. Antibiotics susceptibility of the isolated organisms were also done . Out of 215 milk samples examined 22 isolates were identified as *Staphylococcus aureus* . Results of antibiotics sensitivity in this investigation showed that *Staphylococcus aureus* were sensitive to kanamycin , azithromycin , gentamycin , vancomycin , nitrofurantoin , streptomycin and resistant to oxacillin and ampicillin.

### **INTRODUCTION**

Milk is a major component in human diet all over the world, but it also serves as a good medium for growth of many microorganisms, especially pathogenic bacteria. Thus, the quality of milk is considered essential to the health and welfare of a community. Also, all cases of dairy illness continued to be of bacterial origin. Pathogens that have involved in communicable diseases associated with the consumption of milk include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter*, *Yersinia* (1)

The detection of coliform bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used (2; 3).

Milk of buffaloes constituting an important source of market milk has some unusual qualities. It meets certain specific food requirements of human population (4). The fat content can exceptionally be as high as 15 percent and the overall average may be 7 %. Bacteria need

considerable amounts of nutrient such as water, carbohydrate, fat and other substances for their growth and milk contains all of these nutrients (4). Microorganisms are always undesirable in milk and its products. They are capable of causing deterioration in flavor, physical appearance of milk and transmission of infectious diseases to the consumers. The various organisms get into milk through unhygienic, carelessness and unsanitary practices of the farmers, processors and distributors. Discoloration, sliminess, rancidity, putrefaction, rancidity and many other defects are caused by various microorganisms growing in the milk and milk products.

To date, there are 32 species and eight sub-species in the genus *Staphylococcus*. *Staphylococcus aureus* is the most characterized and studied strains (5). Pathogenic staphylococci are commonly identified by their ability to produce coagulase. This distinguishes the coagulase positive strains, *S. aureus* (a pathogen), from the other staphylococcal species such as *S. epidermidis*, that are coagulase-negative (CoNS). *Staphylococcus aureus* is a bacterium found passively colonizing skin and nasal passages of healthy humans and animals (6), though this opportunistic pathogen colonizes without causing disease (7).

*Staphylococcus aureus* is a major pathogen of increasing importance due to the rise in antibiotics resistance (8). It is distinct from the CoNS (e.g. *S. epidermidis*), and more virulent despite their phylogenic similarities (5; 9)

*Staphylococcus spp.* causes severe disease such as mastitis, arthritis and urinary tract infection by introducing numerous virulence factors such as extracellular toxins and enzymes into animal (5). *S. aureus* infection continues to present a difficult problem and is the main etiological cause of mastitis in cattle, sheep and goats (10). The various strains of *S. aureus* can produce a number of potential virulence factors such as hemolysins in addition to lipase (11)(12)

The present study aims for isolation and identification of *S. aureus* from buffalo milk, detect their susceptibility to some antibiotics by disk diffusion method and determine their ability for production of hemolysin and lipase enzymes.

## **MATERIALS AND METHODS**

### **Samples collection**

A total of 215 samples of buffalo milk were collected randomly during the period from August 2012 up to March 2013 from different geographical regions of Basrah city. Samples were collected from buffalo by hand milking, as normally practiced by the farmers, in sterile screw bottles kept in cool boxes until transported immediately to laboratory for isolation and identification of bacteria(13).

### **Laboratory Identification**

The specimens were directly inoculated by streaking on to mannitol salt agar (MSA) and incubated at 37 °C for 24 h. All colonies from primary cultures were purified by subculture onto MSA medium and incubated at 37 °C for 24- 48 h. (13).Gram stain slides were investigated .

### **Biochemical tests :**

**Free coagulase Test:** Zero point one (0.1) ml of 18 h. culture broth was added to 0.1 ml of human plasma without dilution and incubated at 37 °C for 4 h. The clotting hourly noticed. The appearance of the clotting indicates a positive result comparable to control (14).

**Catalase Test:** A small amount of pure growth of *S. aureus* was transferred with a wooden stick from nutrient into clean slide, then a drop of catalase reagent was added. The evolution of gas bubbles indicates a positive result (14).

**Oxidase Test:** A filter paper was moistened with several drops of oxidase reagent 1% then a small portion of the colony was removed with a sterile wooden stick rubbed on moistened filter paper. A positive reaction is indicated by the appearance of dark or deep purple color within 10-20 sec. (14).

**Nitrophenl-B-D-galactopyranoside (ONPG):** Small portion from the colony was mixed with 1 ml of D.W in sterile tube and homogenized then a disc of ONPG was added. The incubation took place at 37 °C and the results were observed after 1- 4-24 h. The white color indicates that was *S. aureus* (14).

**DNase Production Test:** Overnight incubated of bacterial isolates were streaked on DNase agar and incubated at 35 °C for 24h. The bacterial growth was covered by HCl 1N solution. The appearance of clear zone around of the colonies indicates positive result (14).

#### **Antibiotics susceptibility test**

Ten antibiotics were used in this study to conduct antibiotic susceptibility test. The antibiotics were: Ampicillin/cloxacillin, APX, 30 µg . Azithromycin , AZM , 15 µg . Chloramphenicol,C ,30 µg . Clindamycin , DA , 10 µg. Gentamycin , CN , 10 µg . Kanamycin , K, 30 µg. Nitrofurantoin , F, 100 µg. Oxacillin , OX, 1 µg. Streptomycin, S , 10 µg . and Vancomycin , VA, 30 µg.

. The antibiotic susceptibility testing was done by the agar discs diffusion method as described by, (15). Five isolated colonies of *S. aureus* isolates were selected from the agar plate culture. The top of each colony was touched with a loop and the growth was transferred into a tube containing 5 ml BHI broth and incubated at 35 °C for 15 min. The turbidity of the broth was adjusted to the 0.5 McFarland standards.

Sterile cotton swab was dipped into adjusted suspension, then rotated several times and pressed firmly on the side of the tube above the fluid level. This removed excess inoculum from the swab.

The dried surface of the a Mueller–Hinton agar (MHA) plate was inoculated by streaking the swab over the entire sterile agar surface .This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculum. The predetermined antimicrobial disks were dispensed on to the surface of the inoculated agar plate. Each disk was pressed down individually to ensure complete contact with agar surface.

The plates were placed in an incubator for 18h. at 35 °C. The resulting zone of inhibition was uniformly circular with confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including diameter of the disk. The size of inhibition zones were interpreted by referring to zone diameter interpretive standard from (Bioanalyse sensitivity discs Ankara/Turkey).

### **Enzyme Production tests**

#### **Hemolysin production.**

Overnight incubated of *S. aureus* isolates were streaked on blood agar plates BAP that supplemented with sheep blood 7%. Plates were incubated at 37°C for 24 h. different types of hemolysin production were seen on the pates.

#### **Lipase production.**

*S. aureus* strains were inoculated on Tween 80 medium. Plates were incubated at 37°C for 72 h. The emergence of opaque zone around the colonies evidence of the positive result

## **RESULTS**

During the period of the study, out of 215 tested buffalo milk samples, 22(10.23%) were found to be infected with *S. aureus* (table 1).

**Table (1) Number and percentage of *S. aureus* isolated from buffalo milk samples**

Sample	No.of samples	No. <i>S. aureus</i>	(%)
Buffalo milk	215	22	(10.23)

### **Enzyme Production tests**

Production of virulence factors such as hemolysin and lipase showed that 2/10 (20%) of *S. aureus* isolates were produce hemolysin, but there were no *S. aureus* isolates produced lipase enzymes as shown in table 2.

**Table (2): Production of hemolysin and lipase enzymes by *S. aureus* strains**

Isolate No.	Hemolytic Production /Day			Isolate No.	Lipase Production / Day		
	1	2	3		1	2	3
1	-	-	-	1	-	-	-
2	-	-	-	2	-	-	-
3	-	-	-	3	-	-	-
4	-	-	-	4	-	-	-
5	-	-	-	5	-	-	-
6	-	-	-	6	-	-	-
7	-	-	-	7	-	-	-
8	-	-	-	8	-	-	-
9	+	+	+	9	-	-	-
10	-	+	+	10	-	-	-
11	-	-	-	11	-	-	-
12	-	-	-	12	-	-	-
13	-	-	-	13	-	-	-
14	+	+	+	14	-	-	-
15	-	-	-	15	-	-	-
16	-	-	-	16	-	-	-
17	-	-	-	17	-	-	-
18	-	+	-	18	-	-	-
19	-	-	-	19	-	-	-
20	-	-	-	20	-	-	-
21	-	-	-	21	-	-	-
22	+	+	+	22	-	-	-

**Antibiotics susceptibility testing of *S. aureus* isolates**

Table (3) provides antibiotic susceptibility results of 10 different antibiotics by disc diffusion test against 8 *S. aureus* isolates. In the present study, there were various resistance of *S. aureus* isolates to the different antibiotics. All *S. aureus* strains 8/8 (100%) were resistant to oxacillin and ampicillin/cloxacillin, and all *S. aureus* isolate 8/8(100%) were sensitive to gentamycin and kanamycin. 4/8(50%) of *S. aureus* strains were resistant to clindamycin, but 3/8(37.5%) of *S. aureus* isolate were resistant to chloramphenicol, nitrofurantoin and streptomycin, while 2/8(25%) of *S. aureus* strains were resistant to azithromycin and vancomycin (Figures 1, 2, and 3)

**Table 3. Antibiotics susceptibility test of 8 strains of *S. aureus* against different antibiotics**

Sample No.	APX	AZM	C	DA	CN	F	K	OX	S	V
1	R	R	R	R	S	S	S	R	R	R
2	R	S	S	R	S	S	S	R	R	S
3	R	S	R	S	S	S	S	R	S	S
4	R	S	R	S	S	R	S	R	S	S
5	R	S	S	S	S	R	S	R	S	S
6	R	S	S	R	S	S	S	R	R	S
7	R	S	S	S	S	S	S	R	S	R
8	R	R	S	R	S	R	S	R	S	S
*%	100	25	37.5	50	0.0	37.5	0.0	100	37.5	25

\*Total of antibiotic resistant



**Figure 1. Antibiotic susceptibility of *S.aureus* to Nitrofurantoin.**



**Figure 2. Antibiotic susceptibility to oxacillin of *S.aureus* isolats.**



**Figure 3. Antibiotic susceptibility of *S.aureus* to Ampicillin**

## DISCUSSION

*Staphylococcus aureus* is one of the most commonly identified pathogens in human medicine and is the major cause of infections for nosocomial and animals and community-acquired infections (1, 6, 16, 17). It colonizes healthy mucous membranes and the skin of humans and many animals (6), and causes one of the most common types of chronic mastitis. Though some cows may flare up (especially after calving) with clinical mastitis (2).

The present study showed that *S. aureus* reached 22/215 among samples collection, This confirms the fact that these bacteria endemic in most studeied areas. This result is in line with Hanon (18), who reported that *S. aureus* was isolated from bovine milk in a percentage of 48.57%. At the study of Jakee et al., (19), 409 samples were collected and the highly isolation rate was 12% observed in chicken. The causes of this distribution of *S. aureus* in different hosts beyond to a number of virulence factors which enable it to colonize, invade and infect different hosts.

Treatment is usually economically justified by antibiotic sensitivity testing. In the present study, 8 *S. aureus* isolates were tested against various antibiotics( Table 3). The results demonstrated that 100% of *S. aureus* isolates were resistant to oxacillin. This result is agreed with the study of Bendahou *et al.*, (20) who found that the percentage of oxacillin was 85% from raw milk and milk products. While it is not agreed with the study of (18), who mentioned that the percentage of susceptibility to oxacillin was 52.5% and to the findings reported by (21) who detected that the MRSA is in the percentage of 50% from the buffalo milk.

In the present study, susceptibility of *S. aureus* isolates to vancomycin was 75%. Our study is a pit similar to the study of (22)(23) who demonstrated that all *S. aureus* isolates were (100%) sensitive to vancomycin.

This study reveals that , the antibiotic susceptibility testing of *S. aureus* isolates were resistant in a percentage of 37.5% to the chloramphenicol, nitrofurantion and streptomycin for each one. These results are in line with the study of (24), who mentioned that the sensitivity to chloramphenicol was 100%. And to those of (18), in bovine isolates who reported that *S. aureus* isolates are (62.5%) susceptible to chloramphenicol, and less susceptible results were shown for nitrofurantion (47.5%).

In the present study, susceptibility of *S. aureus* isolates to gentamycin was (100%). A similar finding was obtained by the study of (25) and (20) who reported that the highest sensitivity (100%) was found, but (10), who recorded sensitivity to gentamycin was 76.8% of *S. aureus* isolates from mastitis.

On the basis of the present study, we conclude that *S. aureus* is the most important pathogen that isolated from buffalo milk. The antibiotic susceptibility of *S. aureus* isolates showed high resistance to oxacillin and ampicillin/cloxacillin. All *S. aureus* isolate 8/8(100%) were sensitive to gentamycin and kanamycin. But there are various resistant to other antibiotics.

### عزل جراثيم المكورات العنقودية الذهبية من حليب الجاموس في محافظة البصرة وتقييم

#### حساسيتها للمضادات الحيوية

بسام ياسين خضير ، اسراء تلفان عناد ، باسل عبدالزهره عباس  
فرع الاحياء المجهرية ، كلية الطب البيطري ، جامعة البصرة ، البصرة ، العراق.

#### الخلاصة

تم إجراء هذه الدراسة على ٢١٥ عينة حليب جمعت من حيوانات الجاموس السليمة ظاهرياً خلال الفترة من آب ٢٠١٢ لغاية آذار ٢٠١٣، تم كشف المجاميع البكتيرية وعزلها اعتماداً على الخواص المظهرية، والتصبيغ، والزرع والخواص الكيموحيوية. كذلك تمت دراسة الحساسية الدوائية للبكتيريا المعزولة. تم عزل ٢٢ عزلة بكتيرية تعود للنوع *Staphylococcus aureus* من المجموع الكلي للعينات. أظهرت نتائج فحص حساسية البكتيريا للمضادات الحيوية ان هذه البكتيريا المعزولة حساسة لكل من المضادات الحيوية التالية ، kanamycin ، azithromycin ، oxacillin ، nitrofurantoin ، Streptomycin ، gentamycin ، vancomycin ، ampicillin .

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