

PREVALENCE OF *B*- LACTAMASE PRODUCING *STAPHYLOCOCCUS AUREUS* IN SLAUGHTERED COW'S LUNGS AND LIVER

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ABSTRACT

Fifty liver and lung samples (25 for each) were examend for the presence of *Staphylococcus aureus* . The rate of *S. aureus* isolates were 60 and 56% in livers and lungs respectively.

Identification of *S. aureus* isolates were performed by studying, it's cultural characteristics on mannitol salt agar and testing their pathogenicity factors (Coagulase, haemolysis and

B-lactmase production), 72.4% of isolates were coagulase positive, 58.6% were β -lactamase producer, 62.1% were β -haemolytic and 37.9% were α -haemolytic. There was a difference in the abilty of *S. aureus* isolates to produce *B*-lactamase concerning the time of decolourization when iodometric assay was used.

INTRODUCTION

Staphylococci are wide spread in nature and recoverable from many inanimate sources. One of their hosts is man, and main their ready transference from the human body to a variety of foodstuffs accounts for their role as an important cause of food poisoning. By no means all varieties of *Staphylococcus* are concerned. Only those that are coagulase – positive are capable of producing enterotoxin⁽¹⁾. Food poisoning staphylococci may be isolated from the hands and noses of normal people. Contamination of tables, knives are almost unavoidable. It is not surprising that staphylococci can be isolated from air and dust of contaminated area. Flies in such area may be infected and are probably turned into an important source of food contamination by staphylococci.⁽²⁾

Staphylococcus aureus is one of the major resistant pathogen, it is extremely

adaptable to antibiotic pressure. It was the first bacterium in which penicillin resistance was found in 1947⁽³⁾. Beta – Lactamases constitute the major defense mechanism of these bacteria against beta – lactam antibiotic. When the beta – lactam ring of this antibiotic class is hydrolyzed, antimicrobial activity is destroyed⁽⁴⁾. *S.aureus* carry a wide variety of multi – drug resistant genes on plasmids, which can be exchanged and spread among different species of staphylococci⁽⁵⁾. There is substantial evidence that these resistant bacteria cause antibiotic resistant infection in humans⁽³⁾.

The present study was designated to investigate the prevalence of β -lactamase producing *Staphyococcus aureus* among livers and lungs of slaughtered cows and their fitness for human consumption..

MATERIALS AND METHODS

Samples collection:

A 25 Liver samples and likewise that of lungs samples were collected from slaughtered cows seen in the Basrah abattoir. Liver and lung area used for sampling were 25cm². According to method of (Hall and Maurer)⁽⁶⁾, two test tubes were used for each sample first one containing (10ml) of 0.1% peptone water and the second sterilized tube contain cotton swab. At the time of samples collection a sterile swab was removed from its tube and moistened with peptone water by dipping it into the first tube. The sterile metallic template was pressed against the surface to be sampled. The tip of moistened swab was rubbed over the area to be sampled. The swab was broken off into tube containing peptone water.

Bacteriological analysis:

After the swab samples were shaken thoroughly, aliquets, of 0.1ml was streaked on to surface of mannitol salt agar and spread with a sterile glass spreader, the plates were incubated at 37C° for 2 days. Appearance of yellow colour indicates growth of *S. aureus* on this selective medium.

Tests for the pathogenicity of Staphylococcal isolates:

1-coagulase test

Bound coagulase is detected by the slide test and free coagulase by the tube test. Fresh rabbit plasma is the reagent which was used. This test was performed as described by (Quinn *et al.*)⁽⁷⁾

Coagulase slide test:

A loopful of staphylococcal colony was emulsified in a drop of distilled water on microscopic slide and a loopful of rabbit plasma was added and mixed well with the bacterial suspension. The slide is gently rocked and a positive reaction is indicated by clumping within 1-2 minutes. If the reaction is positive easily visible clumps appear immediately. If no such clumping appears, the reaction is negative and should be checked by the tube test before considering as negative. (Quinn *et al.*)⁽⁷⁾

Coagulase tube test:

A 0.3ml of rabbit plasma was placed in a small test tube with 0.1ml of an overnight brain heart infusion broth culture of the staphylococcus isolate. The tube was rotated gently to mix the contents and then incubated overnight at 37°C. A positive reaction with clotting of the plasma, was occurred in 2 to 4hr. (Quinn *et al.*)⁽⁷⁾

2- Haemolysis test

Blood agar base was prepared from dehydrated powder, sterilized in an autoclave at 121°C for 15 minutes then cooled to 50°C. A 10% vol/vol of sterile blood was added to the cooled agar base and mixed well before the plates were poured.

Staphylococcal haemolytic activity was detected after streaking of staphylococcal colony on the surface of blood agar and incubation of the inoculated medium at 37°C for 24hr. A zone of blood haemolyses should be noted if the tested staphylococcal isolates produce haemolysin (Quinn *et al.*)⁽⁷⁾.

3- Detection of β - lactamase production:

For detection of β - lactamase the following Solution have been used:

- A) Iodine solution: Iodine (2.03g) and (5.32g) potassium iodide were dissolved in 100ml of distilled water.
- B) Pencillin – G. Solution: penicillin – G powder (0.089g) was dissolved in (10ml) of distilled water.
- C) Starch solution: soluble starch (1g) was suspended in 100ml distilled water and incubated in a water bath at 100°C for 10 min.

The procedure:

Rapid iodometric method was used for detection of β - lactamase as described by (Lian *et al.*)⁽⁸⁾. As follows:

- 1) *Staphylococcus aureus*^o was streaked on blood agar medium and incubated at 37C for 24hr.
- 2) A 0.1ml of pencilline solution was added to the wells of microtiter plates.
- 3) To the same wells except the last two horizontal rawas (7) colonies of 24hr blood agar culture of *S. aureus* were added and mixed with pencillin solution.
- 4) The mixture was incubated at 37C for 30 minutes until formation of turbed suspension.
- 5) The turbed suspension was left at room temperature for (1hr) then 0.2ml of (1%) starch solution and 0.1ml of iodine solution were added to the suspension, due to the reaction of these materials the colour of the mixture should be blue.
- 6) The transformation of the mixture blue color into white colour with in one minute should be Consider positive.

RESULTS

The prevalence of *S. aureus* in liver and lung samples:-

The distribution of *S. aureus* isolates in liver and lung samples was shown in Table(1). The rate of *S. aureus* isolates in liver was 60% and in lung samples was 56%.

Table (1):- The distribution of *S. aureus* in liver and lung samples.

Samples	No.of samples	positive	
		No	%
Liver	25	15	60
Lung	25	14	56
Total	50	29	58

Identification of *S. aureus* isolates:-

S. aureus isolates were identified by it's cultural characteristics on mannitol salt agar as the change of pink colour of the medium to yellow colour ensure the growth of *S. aureus*. Also the identification of the isolates depend on the test of pathogenesis as displayed in Table (2).

Table (2):- The result of *S. aureus* identification tests:-

Samples		*+Ve Samples	* M. S.A	Coagulase	β -lactamase	haemolyses	
						β	α
Liver	No.	15	15	11	13	10	5
	%	60	100	73.3	86.7	66.7	33.3
Lung	No.	14	14	10	11	8	6
	%	56	100	71.4	78.6	57.1	42.9
Total	No.	29	29	21	17	18	11
	%	58	100	72.4	58.6	62.1	37.9

*+Ve : positive results

* M. S. A: Mannitol salt agar

The ability of *S. aureus* to produce β - lactemase:

Table 3. explained the differences in the ability of *S. aureus* isolates to produce β -lactamase enzyme in different periodes of time when iodometric assay was used.

Table (3):- The results of iodometric assay

Sample	*+Ve staph isolates	+Ve β -Lactamase production	Time (minutes)					
			1	3- 4	4- 7	7-10	10 -20	20 - 25
Liver	* 15	13	3	6	1	1		2
	60	86.7	20	40	6.7	6.7	-	13.3
Lung	* 14	11	3	5	1			2
	56	78.6	21.4	35.7	7.1	-	-	14.3

*Number and percentage of *S. aureus* isolates

* +Ve: positive results

DISCUSSION

Isolation of *S. aureus* from liver (60%) and lung (56%) of slaughtered cows represent health hazard to human consumers. The first and important health hazard is the food poisoning. As staphylococcal food poisoning is an intoxication the onset is rapid and the incubation period can be as short as 2 hours. *S. aureus* strain isolated from cows is implicated in an outbreak of food poisoning, these bacteria produce enterotoxin which is resistant to most proteolytic enzymes and are heat – tolerant *S. aureus* it self is destroyed by cooking but enterotoxin is destroyed gradually at 100C° and can survive light cooking⁽⁷⁾.

The majority of food poisoning strains are of human and animal origin, they colonies nasal cavity, skin and mucus membrane and can be transient in the intestinal tract. Mostly carcasses are contaminated with staphylococci at the time of slaughtering or during handling after slaughtering. (Minor and Marth)⁽⁹⁾.

The results of coagulase test of the present study show that 73.3% of liver *S. aureus* isolates and 71.4% of lung's isolates were coagulase positive. These results ensure the health hazard for human consumers as recored by Quinn *et al.*,⁽⁷⁾who stated that coagulase – positive *S. aureus* produce enterotoxin involved in food poisoning outbreaks.

The results of coagulase test in this study was numerically lower than that of Abdul – Wadood,⁽¹⁰⁾. Alaa, ¹¹⁾ who found that 100% of staphylococcal isolates were coagulase positive.

On the other hand determination of β -lactamase production by staphylococci isolates poses a threat to public health as *S. aureus* carry multi –drug resistant genes on plasmids that can be spread among other staphylococci and lead to antibiotic resistant bacterial infection⁽⁵⁾.

In this study determination of β - lactamase production achived by micro – iodometric assay. This method is sensitive for measuring the rate of hydrolysis of penicillin to penicilloic acid by β -lactamase and depended upon the reduction of iodine by penicilloic acid but not by penicillin and it was carried out by measuring the rate of decolourization of the blue starch iodine complex when the enzyme and substrate react in the presence of the starch – iodine⁽⁸⁾. The results of β -lactamase detection reveald that

86.7% liver isolates and 78.6% of lung isolates produce this enzyme but there was variation in their decolourization times.

Fast positive results indicated high production of the enzyme and delayed positive results mean slow enzyme production (Sykes and Mathew)⁽¹²⁾.

انتشار المكورات العنقودية الذهبية المنتجة لأنزيم البيتا لاكتيميز في رئة وكبد الأبقار المذبوحة

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الخلاصة

للكشف عن وجود المكورات العنقودية الذهبية تم فحص (25 عينة من رئات و (25 عينة أكباد تم تشخيص عزلات المكورات العنقودية الذهبية بواسطة دراسة خصائصها الزرع على وسط آكار ملح المانيتول وكذلك بأجراء اختبارات الكشف عن الأمراض (اختبار التخثر وتحلل الدم وأنتاج انزيم البيتا لاكتيميز). اوضح البحث أن نسبة عزل المكونات العنقودية الذهبية من الاكباد كانت 60% وكانت نسبة العزل من الرئات 56%. وظهر أن 72.4% من العزلات كانت ذات نتائج موجبة في اختبار التخثر و 58.6% كانت منتجة لانزيم البيتا لاكتيميز وان 62.1% من العزلات كانت محلله لدم من نوع β وأن 37.9% كانت ذات تحلل من نوع α وقد وجدت اختلاف في قدرة عزلات المكورات العنقودية الذهبية على انتاج انزيم البيتا لاكتيميز فيما يتعلق بوقت ازالة اللون عند استخدام طريقة iodometric.

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