International Journal of Chemical and Biomolecular Science
Vol. 2, No. 1, 2016, pp. 32-37
http://www.aiscience.org/journal/ijcbs
ISSN: 2381-7372 (Print); ISSN: 2381-7380 (Online)

Detection of β-lactamases Enzymes in *Staphylococcus aureus* Isolated from Nasal Cavity of Healthy Students

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Abstract

20 *S. aureas* isolates isolated from 80 students, specimens were collected from the anterior narse using small sterile swabs placed in a screw-cap container containing transport (brain heart infusion broth) medium, All 20 *S. aureas* isolates capable of fermenting manitol were sub cultured on blood agar, only 10 isolates were ß-hemolytic representing 50% Whereas 7 isolates were found to be non hemolytic or γ-hemolytic representing 35%, and 3 isolates were α-hemolytic representing 15%, All *S. aureus* (coagulase positive) isolates (20) had a β-lactamase activity (100%) according to Detection of β-lactamase Enzyme by Rapid Iodometric Method.

Keywords

Staphylococcus aureus, β-lactam, Gram Positive

Received: September 15, 2015 / Accepted: December 16, 2015 / Published online: January 17, 2016

1. Introduction

Staphylococcus aureus is a Gram positive bacteria (GPB) causing diseases in humans and animals. It is a normal flora of humans especially, in the skin and nasal vestibule. Besides, this bacterial colonization is very common in certain areas in the body namely – axilla, umbilicus, perineal region and mammary folds (Coller *et al.,* 1996*). Staphylococcus aureus* mainly cause opportunistic infections acquired from different sources like patients, hospital staff mainly through their hands and also from their normal flora. The common types of disease caused by *Staphylococcus aureus* are various types of skin infections including Staphylococcal scalded skin syndrome (SSSS), Osteomyelitis, Meningitis, Pneumonia, Septicemia, Gastroenteritis etc (Maxwell *et al.,*1969). *S. aureus* is also the leading gram positive bacteria causing the hospital acquired infections, especially in the Intensive care patients. The other gram positive bacteria causing such infections are Enterococcus, Pneumococcus and Coagulase negative Staphylococcus. Due to many invasive procedures among the critically ill patients, the bacteria can cause serious bacterimia leading the septicaermia.

The carrier rate of *Staphylococcus aureus* in the nasal canal among the healthy people range from 20-30%. From the healthy carriers among the hospital health care personnel, there are more chances of spreading from their hands, nose or throat by touching, sneezing, talking, coughing etc. (Stubbs E *et al.,* 1994).

2. Material

2.1. Penicillin G Solution for Iodometric Method

Penicillin G solution was prepared freshly by dissolving 0.6

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gm of penicillin G powder in 60 ml of phosphate buffer, then the volume completed to 100 ml and kept in a dark container.

2.2. Soluble Starch Solution (WHO, 1978)

The starch solution (1%) was freshly prepared by dissolving 1 gm of starch powder in 90 ml distilled water, and then completed to 100 ml. The container was placed in a water bath at 100°C with shaking until completely dissolved.

2.3. Iodine Solution (WHO, 1978)

Iodine solution was prepared by dissolving 2.03 gm of iodine and 5.32 gm of potassium iodide in 90 ml distilled water and the volume completed to 100 ml and kept in a dark container.

2.4. Preparation of Human Plasma

Take 5 ml of human blood by sterile syringe, mix it with 0.5 ml of sodium citrate in a clean test tube, and then centrifuge it for 5 min. to obtain plasma.

3. Methods

3.1. Sample Collection

A total of 20 samples (nose swabs) were collected from 80 students, specimens were collected from the anterior narse using small sterile swabs placed in a screw-cap container containing transport (brain heart infusion broth)medium .the swabs were immediately transported to the microbiology laboratory for bacteriological examination.

3.2. Cultivation and Isolation

1. The samples were cultured in brain heart infusion broth and incubated over night (o/n). at 37°C.

2. Each sample was sub cultured on human blood agar and mannitol salt agar (MSA) and incubated (o/n) at 37°C.

3. Purified isolated single colonies were streaked on brain heart infusion agar incubated (o/n) at 37°C. Subcultures from isolated single colonies formed were made on brain heart infusion agar.

4. A small isolated single colony was smeared on a clean slide, fixed, gram stained and examined by light microscope.

5. All plates were kept at 4°C, as stock cultures for further investigation.

3.3. Characterization and Identification of Bacterial Isolates

3.3.1. Morphological Characterization

Shape, consistency, colour, surface, elevation, size, edge diameter and odder of the suspected colonies, as well as Hemolysis on blood agar and growth on selective media were examined.

3.3.2. Microscopic Characterization

Size, response to Gram stain (Gram positive or Gram negative) and shape (cocci, bacilli or coccobacilli) of isolated bacteria were studied.

3.3.3. Biochemical Tests

Many biochemical tests were used for characterization and identification of bacterial isolates.

Catalase Test: (Collee *et al*, 1996)

A loopful of the bacterial growth was taken from the top of the colonies avoiding contact with blood agar medium .The bacterial cells were placed on a clean microscopic slide using a sterile wooden stick; bacterial cells were mixed with a drop of 3% H2O2. The production of gas bubbles within few seconds indicates a positive reaction.

Coagulase Test: (Sperber *et al*., 1975)

This test was performed by adding 0.1ml of overnight broth culture to 0.3 ml of human blood plasma in a small test tube incubated at 37°C .The tubes were observed every 4 hours for clot formation and final reading was taken after 24 hours and any tube that did not form clot was regarded as a negative result.

Deoxyribonuclease Test (DNase): (Platt *et al*., 1948).

DNase agar plates were streaked with the suspected bacterial isolates, incubated at 37°C for 24 hours. Plates were then flooded with 1% HCl and left for about 10 min. The appearance of a clear zone around the colonies indicated a positive result.

Hemolysin Production:

Suspected colonies were streaked on blood agar and incubated (o/n) at 37°C. The appearance of clear zone around the growing colonies was regarded as positive result.

3.3.4. Antibiotic Susceptibility Test: (Bauer *et al.*, 1966; Atlas *et al.*, 1995)

All bacterial isolates were tested against 10 antibiotic discs as in the following:

1. Four or five similar colonies were selected and transferred to a tube containing 3-5ml of Brain Heart Infusion broth and incubated at 37°C for 3-6 hours.

2. The broth culture was diluted to obtain a turbidity equivalent to that of the McFarland no.1 tube standard, which is about ($1x10^8$) cell/ml.

3. A sterile cotton swab was dripped into the adjusted inoculums and rotated several times with firm pressure on the inside wall of the tube to remove excess inoculums.

4. The swabs was streaked over the entire surface of Muller-

Hinton agar evenly in three directions and then plates were left for 3-15 min. on the bench (at room temperature).

5. The antibiotic discs(5 discs) were placed on the agar surface using sterile forceps, distributed evenly and gently pressed down to ensure complete contact with the agar surface .

6. Plates were incubated at 37°C /24 hours. The diameters of the inhibition zones were measured in mm. using a transparent ruler. Interpretation of the inhibition zones was determined according to the Interpretation chart provided by National Committee of Clinical Laboratory Standard (NCCLS, 2006).

3.3.5. ß-lactamase Detection by Rapid Iodometric Method (Collee *et al.,* 1996)

Rapid iodometric method was performed to determine the ability of bacterial isolates to produce ß –lactamase enzymes as in the following:

1. Overnight culture of all bacterial isolates was prepared on a selective media (Manitol salt agar for *Staphylococci* and MacConky agar for different *Enterbacteriaceae)*, a small portion of a single isolated colony of each bacterial isolate was transferred to an eppendroff tube containing 0.1ml of penicillin G solution, tubes were incubated at 37°C for 60 min.

2. Fifty microliters of starch solution was added to each tube, and followed by adding 25 micro liters of iodine solution, and the tubes were shacked for homogenization.

3. The rapid changing of the color from blue to white indicates a positive result.

4. Results and Discussion

4.1. Morphological and Microscopical Characterization

Primary isolation of *S.aureus* was performed by streaking all collected samples on Manitol Salt Agar. Results from table (1) showed that the 20 *S. aureus* isolates representing 100% were capable of mannitol salt agar fermentation. Suspected bacterial colonies appeared rough, bright yellow. All these isolates were gram positive cocci occurring in clusters under the microscope (Jewetz *et al., 2007)*. Since all isolates of *S. aureus* from human sources ferment mannitol, mannitol fermentation may be considered a professional identification of *S. aureus* from human origin, because *S. aureas* could ferment mannitol sugar and grow in high salt concentration. (Cruickshank, *et al.,* 1975.; Jawetz *et al.,* 2007).

All 20 *S. aureas* isolates capable of fermenting manitol were sub cultured on blood agar, only 10 isolates were ß-hemolytic representing 50% Whereas 7 isolates were found to be non hemolytic or γ-hemolytic representing 35%, and 3 isolates were α-hemolytic representing 15% table (1). Since this reaction is variable, therefore hemolysis cannot be considered as a reliable index for *S. aureas* identification (Woo *et al., 2001).*The interaction between α and β toxins increases the adherence to epithelial cells and proliferation of *S. aureas* (Shana *et al., 2009).*

Results from table (1) showed that all 20 *S. aureus* isolates were coagulase, catalase, and DNase producers. A positive catalase test provided a rapid indicator of *Staphylococci,* to distinguish between *S. aureus* and the remaining staphylococcal species required further testing. This was commonly performed using coagulase test and DNase test. These results were in agreement with those obtained locally and worldwide from other studies in which all *S. aureus* isolates from clinical origin or any other origin were positive to coagulase, catalase, and DNase tests (Al-Barwary, 2006; Othman, 2008; Rahman *et al.,* 2005; Schaberg *et al.,* 1982; Zunita *et al.,* 2008; Khan *et al.,* 2007).

Table (1). Results of biochemical tests used for identification and characterization of *S. aureus* isolated from internal narse of healthy persons.

Tests	No. of *S. aureus* isolates	%
Manitol fermentation	20	100
ß-hemolysis	10	50
δ-hemolysis or non	7	35
α-hemolysis	3	15
Catalase	20	100
Coagulase	20	100
Deoxyribonuclease	20	100
β-lactamase	20	100

4.2. Antibiotic Sensitivity

The standard disk diffusion method was used to determine the sensitivity of all twenty isolates towards ten antibiotics.

Results from table (2) showed that all isolates 100% were resistant to ampicillin, amoxicillin, on the other hand most of these isolates were highly sensitive towards vancomycin, rifampicin, fusidic acid.

S. aureus is one of the bacteria that have a dramatic increase in resistance to antibiotics in the last decade (Bush, 1988; Cookson and Phillips, 1988).Penicillin resistant *S. aureus* strains emerged in the early 1940, shortly after β-lactamases was introduced into clinical practice in 1960, (Bush and Smith, 1987). The resistance of *S. aureus* to penicillin and penicillin-like antimicrobials is via penicillin-binding protein 2a (PBP2a) that decrease binding affinity to β-lactam antibiotics (Ravazishzili *et al.,* 2006).The high sensitivity of *S. aureus* isolates toward vancomycin is due to that vancomycin and other closely related glycopeptides inhibit peptidoglycan biosynthesis of the bacterial cell wall of gram

positive bacteria (Reynolds and Somner, 1990). Vancomycin do not penetrate into the cytoplasm of the cell but form complexes with the D-ala –D-ala carboxyl termini of peptidoglycan precursors outside the cell membrane (Barna and Williams, 1984; Perkins, 1969).

Table (2). Results of antibiotic sensitivity of different bacterial isolates.

Sample	Amp	CTX	fox	Aml	Te	Va	PB	PRL	Ra	fa
1	R	S	S	R	R	S	S	S	R	S
2	R	S	S	R	S	S	S	S	S	S
3	R	S	R	R	S	S	S	S	S	S
4	R	S	R	R	R	S	S	S	S	S
5	R	R	S	R	S	S	S	S	S	R
6	R	S	S	R	S	S	S	S	S	S
7	R	S	S	R	S	S	R	R	S	S
8	R	S	S	R	R	S	S	S	R	S
9	R	R	S	R	R	S	S	S	S	S
10	R	S	S	R	S	S	R	S	S	S
11	R	S	S	R	S	S	S	S	S	S
12	R	S	S	R	S	S	S	S	S	S
13	R	S	R	S	S	S	S	S	S	S
14	R	R	S	R	S	S	S	S	S	S
15	R	S	S	R	S	S	R	S	S	S
16	R	R	S	R	S	S	R	S	S	S
17	R	R	S	R	S	S	R	R	S	S
18	R	S	R	R	R	S	R	S	S	S
19	R	S	S	R	S	S	R	R	S	S
20	R	S	S	R	S	S	R	R	S	S

Table (3). Represent antibiotic resistance pattern of *S.aureus* isolates isolated from Healthy persons as normal flora.

Antibiotics	Resistant Isolates	
	No.	(%)
Amoxicillin	19	95
Ampicillin	20	100
Cefotaxime	5	25
Cefoxitin	4	20
Fusidic acid	1	5
Piperacillin	4	20
Poly mxin B	8	40
Rifampicin	2	10
Tetracycline	5	25
Vancomycin	0	0

4.3. Detection of β-lactamase Enzyme by Rapid Iodometric Method

Results from table (1) revealed that all 20 isolates (100%) of *S. aureus* were positive for yhe production of β-lactamase. And the colure changed from blue to white.

This method depends on the principle of the reaction of iodine with starch to form dark-blue complex. Bacteria that produce this enzyme break down the β-lactam ring and alter the penicillin to pnicilloic acid which reduces the iodine to iodide, and the latter is disabling to react with starch this result in the changing of the color directly to white (Sykes and Matthew, 1976; Muftin, 2000).

Results obtained from different studies showed that iodine reduction to iodide depends essentially on the concentration of the enzyme; in addition both temperature and pH play a role in increase or decrease of the activity of the enzyme (Catlin, 1975; Folly and Perret, 1962; Perret, 1954).

All *S. aureus* (coagulase positive) isolates (20) had a β-lactamase activity (100%). This incidence was in accordance with those reported by other researchers (Paul *et al.,* 1982; Jones, 1996; Al-Douri, 1999; Al-Jeboury, 2000; Salih, 2007).

The genes for β-lactamase synthesis may be harbored on a plasmid or may be carried on the bacterial chromosome; either of these can result in the production of enzymes leading to the resistance to β-lactam antibiotics (Bush, 1988, Bush and Jacoby, 1995).

The iodometric method characterized by its short time requirement and the availability of materials needed (Livermore, 1995).

Penicilline G used in the test acts as a substrate for both types of β-lactamase: Pencillinase and cephalosporinase, so the test represents a comprehensive basic survey for all β-lactamase enzymes present (Bush *et al,* 1995; Lucas, 2007).

A previous study mentioned that this method has some disadvantages:

It may give false positive result when the antibiotic solution is not freshly prepared (Sykes and Mathew, 1976).

It has also been emphasized by a study performed by Skinner and Wise (1977) that starch solution should be prepared freshly.

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