RESEARCH ARTICLE

Extraction and Purification of Lipopolysaccharides from *Staphylococcus aureu*s and Their Use to Prepare New Technique

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ABSTRACT

In this study, we included the production of a new technique for diagnosing *Staphylococcus aureus*. This study included 16 blood samples from patients infected with *S. aureus*, in addition to 10 samples for a healthy individual (a control). Patients attended AL-Dowaly Private Hospital in Baghdad, Iraq from January 2021 to February 2021. All infections were diagnosed by consultant medical staff at the hospital using several tests, including Vitek.

Lipopolysaccharide **(**LPS) was extracted and purified from *S. aureus* using a chromatography device after measuring tubes with a spectrophotometer to obtain tubes containing large amounts of bacterial components (antigens). The samples were examined using the new technique and using the enzyme-linked immunosorbent assay (ELISA) technique. The results of the samples were compared with the use of the two techniques, and the results were close indicating infection with *S. aureus*. The two technologies were of high sensitivity and specialization. However, the new technique was of higher sensitivity than the ELISA technique. The reason is that light transmittance through the samples as the wavelength used in the method of working for the ELISA technology is 450 nanometers while the wavelength used in the new technique is 600 nm Also, it was easier, did not require a long time in the way of work, was inexpensive, and gave higher results than the ELISA technique.

Keywords: Diagnosis, Enzyme-linked immunosorbent assay (ELISA) technique, Lipopolysaccharides antigen, *Staphylococcus aureus.*

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INTRODUCTION

Immunological techniques are a wide variety of methods and specialized experimental protocols devised by immunologists to induce, measure, and characterize immune responses.[1] They allow the immunologists to alter the immune system through cellular, molecular and genetic manipulation. It is usually used to diagnose human diseases. Laboratory tests vary widely in clinical immunology; some are essential for diagnosis, while others are useful in sub-classifying disorders. Some are of research interest only but may add to our immunological armamentarium in the future. In this regard, it is important to understand that these tests do vary in their sensitivity and specificity. The test sensitivity is defined by the number of diseased individuals that are positive for the test compared with those who are negative. These techniques have been developed and used in the medical and biotechnology fields. And the immunological techniques used in the diagnosis, which relied on the principle of antigen reaction with antibodies (e.g. ELISA, Immune Fluorescent and Radioimmunoassay).[2-5]

Staphylococcus aureus is a gram-positive bacterium and causative agent of wide range of infectious diseases such as skin infections, bacteremia, endocarditis, pneumonia and food poisoning. The organism was initially a leading nosocomial pathogen, and afterward, epidemiologically distinct clones emerged in community settings. *S. aureus* expresses a number of virulence factors that help to establish infection by facilitating tissue attachment, tissue invasion, and evading from host immune response.[6,7]

MATERIALS AND METHODS

Subject

The case study included 16 blood samples from patients infected with *S. aureus*, in addition to 10 samples for a healthy individual (a control). Patients were attended to AL-Dowaly Private Hospital in Baghdad from January 2021 to February 2021. All infections were diagnosed by consultant medical staff at the hospital by using several tests, including Vitek.

Purification and Extraction of Lipopolysaccharide (LPS)

- A swab of *S. aureus* was taken by slide from the culture medium and mixed with 5 mL of distilled water to have a stuck of *S. aureus* bacteria.
  - (Para-film was placed at the end of the tube so that the stuck would not fall off when shaken).

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- The bacteria will be broken by boiling at a temperature of 100°C (the bacterial stuck is evacuation In a baker, the stuck heating is done using a burner, and then the heating process stops after observing the appearance of steam).

After that, the stuck is shaken using a shaker device for one minute, where the bacteria are broken down and the bacteria's cell wall separates from the cytoplasm.

- Putting the bacterial suspension in the chromatography device to separate the components of the bacterial cell from each other and obtain LPS (after opening the valve of the chromatography device, the distillation process is done, and 10 tubes are filled).
- After that, concentration is measured for each tube (tubes numbered 1–10, to which the chromatography device separated the bacterial components) by the spectrophotometer, and the highest value is the substance to be measured.
  - The first tube is neglected because it contains water only because water molecules are deposited faster.)
  - Before each tube is measured, shake a little to mix well-dissolved components and obtain a better result, each reading is recorded separately.
- After completing the measurement of sample's absorbance, a chart is made showing the absorbance values of each sample, which will be explained in the result.
- The tubes that contain the highest absorbance concentration of the sample (which is tube number 2,5,8) are taken. This indicates that they have the largest amount of bacterial substances.
  - The rest of the tubes are neglected.
- A special detector was placed in each of the following tubes (2/5/8) to detect LPS. After 5 minutes of placing the detector, we notice that tube. No. 5 had a change of color, indicating that the material LPS is present in this tube in large quantities.
  - Purify the LPS and obtain a concentrated substance through a dialysis bag (which consists of cellulose).

Where the hemodialysis bag is placed in distilled water until it grows in size, then put the bacteria components obtained from tube 5 inside the dialysis bag and close it tightly, then put it in sugar for half an hour in order to obtain a concentrated substance and get rid of the water,

- After that, the substance inside the dialysis bag (LPS, which are *S. aureus* antigens) is emptied into Eppendorf Tube, and then a freeze is placed for 24 hours.

Preparing the New Technique

A. Preparation of the ELISA plate, which takes place in several steps:

1. First step: Coating

- A prepared substance (LPS – *S. aureus* antigens) was added to the ELISA plate pits, but after diluting the substance with a 1000 to 10% concentration.
- To dilute the substance (LPS – *S. aureus* antigens), 10 mL of a substance are added to 100 mL of distilled water, to obtain a dilute solution.
- 0.08 mL of a 10% diluent solution is added to etch of the ELISA plate, and then the plate is covered with its cover. And leave the incubator at 37°C for 24 hours.

*The plate is washed twice

2- The Second Step: Blocking

- Add a solution of serum albumin and phosphate (an inhibitor) to fill the spaces between the walls (so that the antibody does not stick to the bottom of the pits) and leave the plate in the incubator a second time at 37°C for 24 hours.

*The plate is washed twice

B. Preparation of Conjugated Reagent

- 100 mL of stain solution added to 50 mL from Antibody-*S. aureus*.

Note: The concentration of the antibody is 10%.

- Shake the mixture for one min.
- The mixture incubated 37°C for (24 hours) was the optimization time to incubate the mixture and happened association between the staining antibody- *S. aureus* because 5, 10, 15, and 20 hours are not appropriate for a link to occur[6]
- Then kept at 4ºC until to use.

Preparation Procedures to Diagnosis the Samples

- Serum samples are prepared for persons infected with *S. aureus*
- 0.01 mL of serum is added to the pits of the ELISA plate that contains antigens for bacteria (*S. aureus*) (so that antibodies in the patient's serum are bound to the plate antigens)
  - Washing the plate is done to get rid of the rest of the antibodies and non-bound antigens.
- Conjugated (antibody- *S. aureus* + stain-UV-Light) to bind with bacterial antibodies.
  - A process of washing the plate is done to get rid of the remaining unconnected conjugated after half an hour
  - We notice that the color change does not occur as is usual in the methods of work of ELISA Kits, but when the ultraviolet rays are shed, we noticed the occurrence of glow, indicating the occurrence of a correlation between the antibodies of the bacteria with the conjugation (antibody + UV stain).
- After that, the plate is inserted into the ELISA device with a wavelength (405 nm). In order to obtain the controlled readings and concentrations of the samples to find out whether the infection rate of bacteria is high or low, by knowing the concentration of antibodies in the patient's serum. This method is known as the indirect method.

RESULT AND DISSECTION

Deduction in LPS Concentration of *S. aureus* Using a Spectrophotometer System

The method of measuring the concentration of all tubes (numbered 1–10) with a spectrophotometer is mentioned in "Material and Methods", where the components of the *S. aureus* were separated by a chromatography device and collected in 10 tubes, further, concentration of each tube was measured with a spectrophotometer. Table 1 shows the results

of each tube after measuring it with a spectrophotometer to obtain tubes containing the highest substance LPS in large quantities, As the readings of the tubes concentrations became clear. In succession 33, 360, 61, 20, 800, 55, 65, 600, 51, and 12. Where the highest value appeared in the following tubes, tubes No. tube-2,tube-5,tube-8, as shown in Figure 1, after using the LPS detector for bacteria *S. aureus*), it was confirmed that tube No. 5 contains the highest amount of substance LPS.

Using the New Technique

As mentioned in Chapter Three, after preparing the ELISA plate and using it as a procedure to find out the concentration of antibodies to *S. aureus*, the samples are inserted into the ELISA device and obtain the results. Table 2 illustrates the results of using a new technique for patient samples and control samples, as it showed that the concentration of samples infected with bacteria S, aureus is (0.1569) compared to control samples (0.0958), as shown in Figure 2, an indication that the new technique shows accurate results with knowledge of the concentration of bacterial antibodies in the patient's serum.

Comparison Between New Technique and ELIZA Technique

Table 3 shows a comparison between a new technique and the ELISA technique, using the same samples of patients infected with *S. aureus.* The difference between the two techniques was that a new technique was used only on the congregate (Ab-*S. aureus*/UV-Stain) and inserted it into the ELIZA device with a wavelength (405um), unlike the old technique where the preparation method was used in congregate (Ab-*S. aureus*/ Enzyme) with the substance TMP and the stop solution and entered into the ELISA device with a wavelength of (450 um). But the use of the new technique was easier and faster and gave higher results than the results of the ELISA technique, as shown in Figure 3.

* Optical density (O.D- patient) The values of the 8-samples infected with S. aureus measured by the ELISA device.
* Optical density (O.D- control) The values of the 8-samples non- infected with *S. aureus* measured by the ELISA device.

Table 1: LPS concentration of *S. aureus* using a spectrophotometer system

Test tube	*LPS concentration of S. aureus using a spectrophotometer system*
1-Tube	33
2-Tube	360
3-Tube	61
4-Tube	20
5-Tube	800
6-Tube	55
7-Tube	65
8-Tube	600
9-Tube	51
10-Tube	12

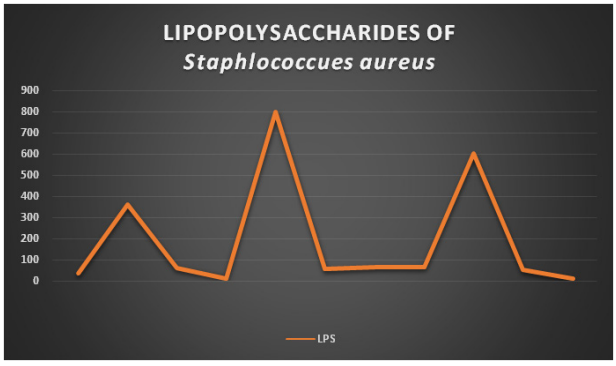

Figure 1: LPS concentration of *S. aureus* using a spectrophotometer system

Table 2: The different in the value of the asthmatic mean sample of plaints infected with *S. aureus* and the asthmatic mean of the comrade sample using the new technique

New technique	
Patient$^{O.D*}$	Controle$^{O.D*}$
0.1569	0.0958

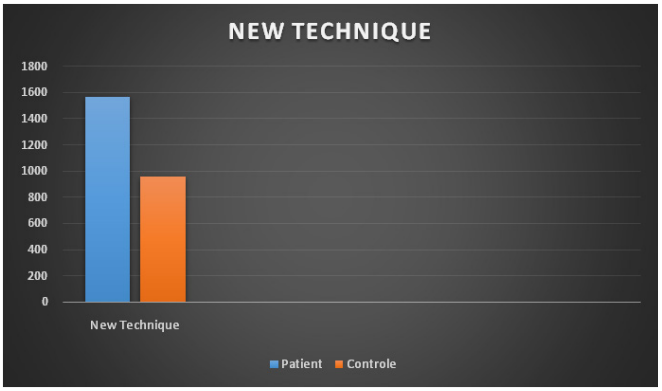

Figure 2: The difference in the value of the asthmatic mean sample of plaints infected with *S.aureus* and the asthmatic mean of the comrade sample using the new technique

Tabel 3: Comparison between new t echnique and ELIZA technique

Comparison between new technique and ELIZA technique	
New technique	*ELIZA technique*
0.1569	0.1026

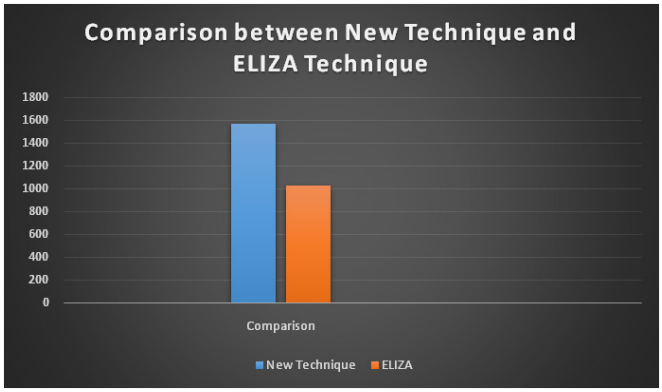

Figure 3: Comparison between new technique and ELIZA technique

Table 1 showing the results of the concentrations of the tubes containing the components of bacteria that were separated from each other using the chromatography device, after measuring the tubes with a spectrophotometer to obtain tubes containing large quantities of bacterial components, this study agrees with,[9] an Figure 1 shows the top three tubes containing The components of bacteria (2, 5, 8), and to ensure the presence of LPS material in these tubes, and LPS reagent for *S. aureus* was added. After adding the reagent to three tubes, a color change occurred in a tube. No. 5, indicating that it contains large quantities of LPS. A hemodialysis bag was used to purify the material and obtain a concentrated LPS of 1000, this study agrees with Schindler *et al.*[9]

Table 2 show that the new technique gave results that the patients were infected with *S. aureus*, the concentration of antibodies to the bacteria was measured, and the result patients infected with it was (0.1569) compared to control samples (0.0958). Figure 3 shows a comparison between the new technique and the ELISA technique, the difference between the two techniques is that A new technique that uses only conjugated (Ab +UV-stain) to detect bacterial antibodies, and we can know that the patient samples are infected with bacteria by shining UV-Light. We notice the occurrence of a glow indicating that the conjugate has been linked to the antibodies against the bacteria (patient samples). These results correspond,[10] after which the samples (found on the ELISA plate) are entered into the ELISA device. With a wavelength of 405 nm (note that no color change occurs when adding ultraviolet rays, a glow occurs if antibodies to the disease are present). While the ELISA technique requires a congregate (Ab +Enzyme), TMP substance that changes to a blue color, and stop solution changes to a yellow color, the samples are entered into the ELISA device with a wavelength of 450 nm is taken. The results of the two techniques were almost close, as shown in Table 3 were the result of the new technology (0.1569) and the result of the ELISA technique (0.1026) that is, an indication of the disease infection with *S. aureus*, The two technologies were of high sensitivity and specialization, but the new technique was of higher sensitivity than the ELISA technique, and the reason is that (light transmittance through the samples as the wavelength used in the method of working for the ELISA technology is 450 nanometers while the wavelength used in the new technique is 600 nm).[11] Also, it was easier, did not require a long time in the way of work, was inexpensive, and gave higher results than the ELISA technique.

CONCLUSIONS

1. The new technique is easy to use, does not require a long time, and is inexpensive.
2. The new technique is more sensitive than the ELISA technique because of the transmittance of light through the samples, as the wavelength used in the method of working for the ELISA technique is 450 nm, while the wavelength used in the new technique is 600 nm.
3. The conjugate (Ab-*S. aureus* -UV-stain) is a specific bound with Ab-*S. aureus* patient, it has been proven the correlation is the occurrence of a glow while shining UV-light.

REFERENCES

1. Van Emon JM. (2016). Immunoassay and other bioanalytical techniques. CRC Press.
2. Carpenter PL (1975) Immunology and serology (No. QR181. C37 1960.). Philadelphia: Saunders.
3. Dunbar BS. (2012). Two-dimensional electrophoresis and immunological techniques. Springer Science & Business Media.
4. Schultz J (2009) Immunological techniques: a different approach for the analysis of proteins in cultural heritage. Part 1: the basics explained. Zeitschriftfür Kunsttechnologie und Konservierung: ZKK 23(1):129–239.
5. Zabriskie JB (ed.). (2009) Essential clinical immunology. Cambridge University Press.
6. Kourtis AP, Hatfield K, Baggs J., Mu Y, See I., Epson E,... & Cardo D. (2019). Vital signs: epidemiology and recent trends in methicillin-resistant and in methicillin–susceptible Staphylococcus aureus bloodstream infections-United States. Morbidity and Mortality Weekly Report, 68(9):214.
7. McCaig LF, McDonald LC, Mandal S., & Jernigan DB. (2006). Staphylococcus aureus–associated skin and soft tissue infections in ambulatory care. Emerging infectious diseases, 12(11), 1715.
8. Russul AH. 2019. New Immunological Technique for Diagnosis of Candida albicans Infection. University of Baghdad, College of Education for Pure Science (Ibn Al-Haitham). pp. 1-69.
9. Schindler R, Ertl T, Beck W, Lepenies J, Boenisch O, Oppermann M, Frei U. (2006). Reduced cytokine induction and removal of complement products with synthetic hemodialysis membranes. Blood purification, 24(2):203-211.
10. Hassan RA, Al-Abassi, HM, & Mahdi AAAH. New Immunological Technique for Diagnosis of Candida Albicans in Different Sites.
11. SJÖHOLM, I. (1975). Protein A from Staphylococcus aureus: spectropolarimetric and spectrophotometric studies. European journal of biochemistry, 51(1):55-61.