Preparation of Diagnostic Monovalent Antisera Against *Staphylococcus aureus*

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Abstract

Background: Monovalent antibodies have a great values in the diagnosis and monitoring of infectious diseases.

Objective: the present study an effort to prepare diagnostic monovalent antibodies against *Staph. aureus* in order to reduce time and cost of diagnosis in bacteriological and biochemical tests.

Methods: Diagnostic monovalent antibodies were prepared against *Staphylococcus aureus*. Hyper antibodies were obtained by rabbit immunization with whole antigen of *Staphylococcus aureus*. Anti-serum was checked for the presence of antibodies that detected by slide agglutination and ELISA tests. Sera were purified from cross-reacted antibodies by absorption with whole antigens of other bacterial isolates (*Staph. epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus*) that cross-reacted with *Staph. aureus* antibodies. Antibodies were purified by repeating absorption process, then checked by ELISA test to diagnose presence of cross-reacted antibodies.

Results: Specific monovalent antibodies against *Staph. aureus* were tested with different bacterial isolates, it was high sensitive and high specific diagnostic properties.

Conclusions: Monovalent antibodies could be used for rapid diagnosis instead of classical way of culture and biochemical tests.

Key Words: monovalent antibody, bacterial antigen, *Staphylococcus aureus*.

تحضيرأضداد تشخيصية أحادية التكافؤ ضد المكورات العنقودية الذهبية

الخلاصة

حضرت اضداد تشخيصية احادية التكافؤ لتشخيص المكورات العنقودية الذهبية. تم الحصول على الأضداد من خلال تمنيع الأرانب بمستضدات المكورات العنقودية الذهبية الجسمية. تم اختبار الأضداد عن طريق اختبارات التلازن البسيط والأليزا (معايرة الامتصاص المناعي المرتبط بالأنزيم).

تم تنقية المصول من الأضداد المتصالبة عن طريق تقنية الأمتصاص بمستضدات العزلات الجرثومية المتصالبة (المكورات العنقودية البيضاء، الزوائف الزنجارية، الأشريشيا القولونية والمتحولات) والتي اظهرت تأثيراً متصالباً مع اضداد المكورات العنقودية الذهبية.

تمت تنقية الأضداد بأعادة عملية الأمتصاص وتم التأكد من نقاوتها من الأضداد المتصالبة بأستخدام اختبار الأليزا.

اختبرت الأضداد احادية التكافؤ المحضرة ضد المكورات العنقودية الذهبية مع عزلات جرثومية مختلفة فأظهرت صفات عالية الحساسية والخصوصية.

Introduction

Staphylococcus aureus is a leading human pathogen in the hospitals and the community, that may cause a variety of diseases ranging from moderate to severe skin and soft tissue infections to very serious diseases such as septic shock, toxic shock syndrome,

endocarditis or necrotizing pneumonia [1, 2]. It is a primary cause of hospital acquired infections that increased the number of immune-compromised patients and thousands of deaths each year. The threat of the organism is compounded by its ability to gain resistance to conventional antibiotics therapy, that not confined in the hospitals, but also emerging in the community [3], the search for alternative strategies to efficiently combat staphylococcal infections is urgently demanded to decrease the enormous burden caused by pathogenic staphylococci. In particular, immunological strategies based on vaccine development or therapeutic antibodies may significantly enhance the efficiency of anti-staphylococcal therapy [4,5]. Companies have joined this quest for *Staph. aureus* vaccines, because the development of vaccines or antibodies is deemed easier and less costly than that of novel antibiotics.

Antibody – based immune diagnosis have returned as first-line for a variety of conditions because have significant advantages include versatility, specificity, and biological functions [6,7]. In order to specifically combat virulent strains, active and passive immunization efforts in clinical trials or pre-clinical investigation are often targeted at molecules involved in pathogenesis [8,9]. This situation is given that serum was one of the first effective treatments for microbial diseases and that specific antibodies have numerous diagnostic properties [10,11].

Methods

Bacterial isolates:- clinical isolates of *Staph. aureus, Staph. epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Proteus* that isolated from skin infection, burn infection, urinary tract infection, and diabetic foot ulcer respectively, were obtained from laboratory of research in College of Pharmacy/University of Basrah, Iraq.

The isolates were identified and diagnosed according to [12].

Antigen preparation:-

The following methods were employed according to [13, 14]. *Staph. aureus* was cultured onto nutrient agar medium, incubated at 37 ºC for 24 hrs. Pure colonies isolated and inoculated in BHI (Brain Heart Infusion) medium that dispensed in screw capped bottles, then incubated in shaker incubator (30 cycls/min) at 37ºC for 18 hrs.

The grown bacteria were harvested by centrifugation at 3000 rpm for 20 min. The supernatant was discarded while precipitate re-suspended in distilled water. The process were repeated 3 times.

The different bacterial isolates suspension adjusted to concentration about 4 X 10^6 cfu / ml, boiled in water bath for 1 hour to kill the bacteria, then the supernatant disrupted by ultra-sonicater for 10 min in an ice-water bath, then frozen at -20 ºC until use for injection.

Immunization

Twelve male rabbits, weight 1.5kg-2kg, (duplicate for each isolates) were adapted in animal house for 10 days before immunization. Ten rabbits were tested and two rabbits were control. Each one of tested rabbits were injected with 1 ml of whole bacterial antigen that related to the studied bacterial isolates (*Staph. aureus, Staph. epidermidis, P. aeruginosa, E. coli* and *Proteus*). The injection were done between the shoulder blades, and it was one week interval. A booster injection containing 2

ml from soluble antigens was administrated one week after the second injection at marginal ear vein. Anti-sera were collected 3 days after the last injection.

Anti-serum preparation:

The blood was collected from the marginal ear vein of rabbits. The collected blood left 1 hour in room temperature for clotting, then centrifuged at 2000 rpm for 10 min. Sodium Azide was added to the serum as preservative with concentration of 0.1%, then stored at 4°C .

Agglutination test:

Pure colony of *Staph. aureus* of 24 hrs growth from nutrient agar medium was emulsified with one drop of normal saline, then mixed with prepared *Staph. aureus* antiserum. Agglutination an indicator of positive results.

ELISA test:

ELISA test were dependent on modification of hepatitis C ELISA kit.

cut - off value:

a. The mean of the measured absorbance value for the 4 positive control serum:-

Mean optical density (OD) = total optical density / 3

b. Calculation of cut-off (CO) value:-

CO = mean OD of 3 samples / 4

Antibody purification:

Antibody purification depend on absorbance of cross reacted antibodies with diagnostic specific antibodies.

Multiple agglutination tests were done to check cross reacted bacteria with *Staph. aureus.* Cross reacted bacteria were added to the prepared antiserum with ratio 1:1(*Staph*. antiserum : whole bacterial antigen), simple shaking and incubation for 10 min in water bath at 37°C followed by centrifugation at 3000 rpm for 20 min.

The above step was repeated till the prepared anti-serum have no residual of cross reacted antibodies.

Results and Discussion

Bacterial isolates

Clinical isolates of *Staph. aureus, Staph. epidermidis, P. aeruginosa, E. coli* and *Proteus* were diagnosed by Gram's stain and biochemical tests.

Slide agglutination and ELISA tests

The staphylococcal antiserum was tested with *Staph. aureus, Staph. epidermidis, P. aeruginosa, E. coli* and *Proteus* antigens separately by simple slide agglutination to detect cross reacted antibodies , the results were summarized in table (1).

Table 1 Slide agglutination and ELISA results after two stages of absorption

Bacterial antigen	Staphylococcal anti-sera agglutination				
	Before absorption	1st absorbance		2nd absorbance	
		Slide agglutination	ELISA	Slide agglutination	ELISA
Staph. aureus	+	+	+	+	+
Staph. epidermidis	+	–	+	–	–
P. aeruginosa	–	–	+	–	–
E. coli	+	–	+	–	–
Proteus	–	–	+	–	–

According to results of slide agglutination test (before absorption), *Staph. epidermidis* and *E. coli* showed cross reacted antibodies with *Staph. aureus*, while *Proteus* and *P. aeruginosa* have no cross reacted antibodies that appear on slide agglutination. The explanation of cross reacted antibodies in the *Staph. aureus* antiserum, may be related to the identical antibodies among *Staph. aureus* and other bacterial isolates that have similar antigens epitops as a results of cross reacted antibodies have the ability to agglutinate more than semi identical antigens where each bacterial surface may be contain different antigens like lipopolysaccharides, capsules, and pilli antigens [15,16]. Also, the cross reacted antibodies could be as a result of rabbit contact with other isolates like *Staph. epidermidis*, *E. coli*, and *P. aeruginosa* that stimulate antibodies immune response [17,18] .

After first absorption stage, cross reacted antibodies were negative by simple slide agglutination, while by ELISA it was gave positive results, ELISA considered the highest sensitive test to detect positive or cross reacted antibodies in comparison with simple slide agglutination. In the second absorption stage, cross reacted antibodies were negative by both slide agglutination test and ELISA, the presence or absence of cross reacted antibodies to different bacterial isolates is determined by comparing for each sample the recorded absorbance with that of the calculated cut - off value.

The prepared monovalent antibodies were tested with many isolates of *Staph. aureus* were gave positive results (agglutination), while it gave negative results with other bacterial isolates (no agglutination). These results were indicate that the prepared anti-sera are high sensitive and high specific and compatible with the studies of [19-24].

Continued success in the development of antibody-based diagnosis will require extensive clinical research to learn how to use these compounds and basic

immunological research to define the basic mechanisms of antibodies action, so the present study was an attempt to prepare monovalent antibodies that could be used for:

1. Rapid diagnosis instead of classical way of culture and biochemical tests that cost much time and money.
2. Passive immunization against different infectious diseases.
3. Monovalent antibodies could be used as solutions for direct neutralization to many bacteria and toxins.

Acknowledgment

This work was supported by technical assistance from the workers in the Primary Health Care / Health Ministry.

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