THE RELATIONSHIP BETWEEN PRODUCTION OF PROTEIN A, CAPSULE STRUCTURE AND SOME ENZYMES OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM INFECTIONS OF HUMAN AND ANIMALS

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Summary

Thirty-five isolates of *Staphylococcus* were isolated out of 100 clinical specimens were collected from different infections of human and animals. These isolates were identified as *Staphylococcus aureus*, 15 of them isolated from human, 15 from cows and only 5 isolates from poultry. Twenty-five (71.4%) isolates of different sources revealed positive results for the detection of protein A, capsule formation and enzyme production of phosphatase, DNase and haemolysin. Seven (20 %) isolates showed negative results for both protein A and capsule as well as for the production of enzymes while only three (8.5%) isolates were not capsulated but able to produce protein A and the enzymes. During the detection of protein A, capsule structure and production of some enzymes, we found that the best value of pH used was the range of 7-7.5. The present study indicated that the presence of protein A and capsule structure is highly associated with the virulence of isolates, and loss of protein A doesn't necessary mean loss of capsule as well. The ability of the isolates to produce their essential enzymes to induct the infection is highly correlated with the ability to produce protein A, without considering the source of the isolates.

العلاقة بين إنتاج بروتين A والمحفظة وبعض الأنزيمات في عزلات العنقوديات الذهبية المعزولة من إصابات بشرية وحيوانية

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الخلاصة

تم عزل 35 عزلة من المكورات العنقودية من مجموع 100 عينة جمعت من إصابات مختلفة بشرية وحيوانية وتم تشخيصها على إنها *S. aureus* والتي كانت موزعة على النحو التالي: 15 عزلة بشرية و15 عزلة من الأبقار و5 عزلات فقط من الدواجن. أظهرت25 (71.4%) عزلة مختلفة المصدر إيجابيتها لبروتينA والمحفظة والأنزيمات الفوسفاتاز والديناز والأنزيم الحال للدم.و7 (20%) عزلات غير مكونة لكل من بروتينA والمحفظة مع فقدانها الأنزيمات

الفوسفاتاز والديناز والأنزيم الحال للدم، بينما3 (8.5%) عزلات فقط أظهرت فقدانها لتركيب المحفظة مع احتوائها لـــبروتين A والأنزيمات الأخرى.و من خلال التحري عن بروتينA ،المحفظة والأنزيمات الأخرى وجدنا أن أفضل القيم المســتخدمة للأس الهيدروجيني هي من بين المدى 7 – 7.5. لقد استدلت هذه الدراسة بأن وجود بروتينA وتركيب المحفظة له علاقــة كبيرة بضراوة العزلة وبأن فقدانها لإنتاج بروتينA لا يعني بالضرورة فقدانها لتركيب المحفظة.وان هنالك علاقة وثيقة بيـــن قابلية العزلات لإنتاج الأنزيمات الضرورية لأحداث الإصابة وبين قابليتها لإنتاج بروتينA وبغض النظر عن مصدر العزلة.

Introduction

Staphylococci are among the most important bacteria that cause disease in humans and animals; they can produce disease in almost every organ and tissue of the body [1]. Infections are primarily associated with *Staphylococcus aureus*; pathogenic strains are often responsible for the formation of localized pus-producing lesion [2].

The infection can be rapidly invasive spreading through the tissues and seeding the blood stream to produce a fulminate picture of septic shock [3]. The pathogenic capacity of a particular *S. aureus* strain is due to the combined effect of extra-cellular factors and toxins. Virulence factors include Enterotoxins, cytolytic toxins and cellular components such as protein A [4]. There is substantial strain to strain variation in pathogenicity among *S. aureus* strains virulence is probably related to the sum total of enzyme production that permits the organism to resist degradation by environmental substances [3]. Some of these enzymes are coagulase, which is an important factor in the localization of the infection [5]. Detection of microbial deoxyribonulease enzyme could used to identify pathogenicity of *S. aureus* especially when there is close correlation with coagulase production. Many strains of *S. aureus* produce one or more hemolytic toxins differ in their activity against red cells of animal species. In addition, leukocidin, hyaluronidase, etc---. Each of these enzymes may assist in establishing and disseminating infection [6]. Some strains produce a capsule or slime layer that enhances the virulence of organisms. And protein A though many researchers are using this protein as a useful immunosorbent in various fields of biological science. And due to the importance of protein A and capsule structure in the increasing of virulence of *S. aureus* and the important roles of enzymes in the induction of infection, we studied the relationship between producing of virulence factors and some enzymes produced by these pathogenic isolates and we also investigated the effect of pH on the detection work.

Materials and Methods

Thirty-five isolates of *S. aureus* were collected from different sources of human and animals: Fifteen isolates from blood samples of patients at Saddams Central Teaching Hospital for children in Baghdad City during the period from (June, 1997 to February 1998). Fifteen isolates from milk of cows infected with acute mastitis and five isolates from swabs of poultry infected with Staphylococcosis infection (Table 1,2).

Each specimen was collected and inoculated directly on to brain heart infusion broth, and then transported to the Microbiology lab, College of Vet. Med., Univ. of Baghdad.

Each sample was cultured onto blood agar, nutrient agar, and Baired Parker agar and mannitol salt agar. Isolates were examined hemolysin production, pigment production and mannitol fermentation. The isolates were further checked to confirm the identification by some other tests such as catalase, coagulase, phosphatase, DNase and Acetoin tests, as well as using the gram staining for microscopically examination [7].

1. Detection of the presence of protein A was determined depending on qualitative test using dog-serum. The result showed appearance of a halo zone around the colonies on Brain Heart Infusion Agar-containing dog-serum [8].
2. Detection of capsule was done by using wet India ink method[9].
3. Study the effect of pH on the detection tests. pH of values 5.5-9.5 was tested[10].

Results

Among 100 samples collected from different sources of humans and animals, thirty-five isolates were identified as *S. aureus* on the basis of Gram-staining reaction and some typical biochemical tests like coagulase test, Mannitol fermentation, acetone production and pigment production. As shown in Table 1and 2, thirty-five isolates of *S. aureus* were tested for detection of protein A, capsule formation and production of important enzymes.

The results showed that twenty-five (71.4%) isolates of *S. aureus* out of thirty-five isolates of humans and animals sources were positive in α-Double, β-hemolysin production, DNase, phosphatase and protein A detection. These isolates were also able to be capsulated while seven (20%) isolates lack both protein A and capsule structure as well as loss the ability to produce either α,β-hemolysin or DNase, phosphatase.

Only three (8.5%) isolates showed the ability to produce either protein A or enzymes, bot not the capsule. The pH value of 7-7.5 appeared to be the best value for testing the presence of protein A, capsule and enzyme production.

Table 1: Results of detection tests of *S. aureus* isolated from different human infections.

No. of isolate	source (isolate)	mannitol fermentation	hemolysis	pigment	DNase	phosphatase	coagulase	protein A	capsule
1 – 5	Septicemia	+	α	+	+	+	+	+	+
6 - 10	Endocarditis	+	α	+	+	+	+	+	+
11	Otitis	+	–	+	–	–	+	–	–
12	Skin	+	–	+	–	–	+	–	–
13-15	Tonsillitis	+	α	+	+	+	+	+	–

Table 2: Results of detection tests of *S. aureus* isolated from different infections of animals

No. of isolate	source (isolate)	mannitol fermentation	hemolysis	pigment	DNase	phosphatase	coagulase	protein A	capsule
1	acute mastitis (Bovine)	+	B-double	+	+	+	+	+	+
2	=	+	=	+	+	+	+	+	+
3	=	+	=	+	+	+	+	+	+
4	=	+	=	+	+	+	+	+	+
5	=	+	=	+	+	+	+	+	+
6	=	+	=	+	+	+	+	+	+
7	=	+	=	+	+	+	+	+	+
8	=	+	=	+	+	+	+	+	+
9	=	+	=	+	+	+	+	+	+
10	=	+	=	+	+	+	+	+	+
11	=	+	=	+	+	+	+	+	+

No. of isolate	source (isolate)	mannitol fermentation	hemolysis	pigment	DNase	phosphatase	coagulase	protein A	capsule
12	=	+	=	+	+	+	+	+	+
13	=	+	=	+	+	+	+	+	+
14	=	+	=	+	+	+	+	+	+
15	=	+	=	+	+	+	+	+	+
16	different infections from poultry	+	-	+	-	-	+	-	-
17	=	+	-	+	-	-	+	-	-
18	=	+	-	+	-	-	+	-	-
19	=	+	-	+	-	-	+	-	-
20	=	+	-	+	-	-	+	-	-

Discussion

The results of the present study showed that all the isolates of *S. aureus* collected either from human or animal have the same main characteristics but the ability of these isolates to produce their essential enzymes is highly associated with the virulence of these bacteria, we found that 25 isolates which were positive in all detection tests used, were collected from acute cases of infections like septicemia in man and mastitis in cows. While the 3 isolates which were positive for all the tests except capsule formation, were collected from moderate cases of infections and we also observed that the 7 isolates which were negative for all the tests of detection used, were isolated from minor cases of infections, the conclusion of that is most of isolates which lack the virulence factors like protein A and capsule, do not produce the enzymes tested (haemolysin, phosphatase and DNase) without considering the source of the isolates. These results are agreed with ours [11], which resulted from detection of protein A of human isolates, while they are disagreed with the other work done by[12], which was about the relation between the production of protein A and hemolysin, who observed that the isolates which produce Protein A has low production of this enzyme.

In this study, it was also found that the best value of pH used in culturing and detection of the virulence factors and production of some enzymes the range

of 7-7.5, while the results of previous work done by [10] was that the use of pH 6.5-7.5 is the best during the study of enzymes production of *S. aureus*.

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