

Extraction Of Methicillin Resistant Staphylococcus Aureus (Mrsa) Golden Pigment And Study It Antioxidant Activity On Macrophage Killing Pathway

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Abstract: *Staphylococcus aureus* isolate was obtained from microbiology lab of biology department and re-identified as methicillin resistance *Staphylococcus aureus* (MRSA) by biochemical test and methicillin kit. Staphyloxanthin pigment was extracted by ethanol and centrifugation method. Effect of antioxidant activity of this pigment was studied on macrophage cell by using blood specimen and DPA. The result were showed the MRSA cell which treated with DPA and mixed with blood give no bacterial growth, while MRSA cell without treatment with DPA give bacterial growth. This means the DPA inhibit pigment synthesis and no antioxidant for macrophage happened and engulfment of bacterial cell happened and shows no growth for bacteria.

Key words: *Staphylococcus aureus*, Staphyloxanthin, Antioxidant, Macrophage

Date of Submission: 05-08-2018

Date of acceptance: 22-08-2018

I. Introduction

Staphylococcus aureus is a unique microorganism as compared with other clinically relevant bacteria as the organism expresses a variety of virulence factors. The bacterium continues to demonstrate the ability to develop resistance to include a broad array of antimicrobial classes (1,2). The species named *aureus*, refers to the fact that colonies often have a golden color when grown on solid media due to carotenoids and has been reported to be a virulence factor protecting the pathogen against oxidants produced by the immune system (3). General characterization of *S. aureus* is able to produce coagulase (4) and fermentation of mannitol and trehalose (5). Colonies appear smooth, convex and sharply defined on blood agar plates (6). Other characteristics for *S. aureus* secret proteases, phosphatases, phospholipase (7). Also secret Staphylokinase, lipase and deoxyribonuclease (DNase) (8). They grow most rapidly at 37 °C but form pigment best at room temperature (20 – 25 °C). They grow comparatively well under conditions of high osmotic pressure and low moisture, which partially explains why they can grow and survive in nasal secretions and on the skin (9). Staphylococci are susceptible to high temperatures, as well as to disinfectants and antiseptic solutions. The organisms can survive on dry surfaces for long periods (10). *S. aureus* is normal flora on skin and mucosal surfaces including the anterior nasal nares and nasopharynx (11). Other sites of colonization include intertriginous skin folds, perineum, the axillae and the vagina (12). They are well equipped to colonize the skin because they grow at high salt concentration and lipid concentration (13). Resistance of *S. aureus* to antibiotics has been observed very soon after the introduction of penicillin about sixty years ago. In the following years, the amazing ability of staphylococci to develop resistance to antibiotics has resulted in the emergence of methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE) strains. In fact, methicillin resistance was observed already in 1961 in a nosocomial isolate of *S. aureus*, one year after the introduction of methicillin (14). Nosocomial *S. epidermidis* isolates are characterized by their pronounced resistance against many of today's commonly used antibiotics including methicillin. Methicillin resistance is, just like in *S. aureus*, mediated by the *mecA* gene encoding a penicillin binding protein with reduced affinity to β -lactam antibiotics (15,16). However, in contrast to methicillin-resistant *S. aureus* (MRSA), very little attention is paid to methicillin-resistant *S. epidermidis* (MRSE) in hospital settings, and they are not dealt with by using intense hygienic measures as those for MRSA (17). As a result a high prevalence of resistant isolates is recorded worldwide. Thus, approximately 80% of *S. epidermidis* isolates from device-associated infections are resistant to methicillin, as well as being multi-resistant, whereas commensal strains obtained from the community are mostly susceptible to antibiotics. The pathogen *Staphylococcus aureus* is a gram-positive, gold-colored colony and all available β -lactam antibiotics (18). The yellow-to-orange colony color of *S. aureus* is one of the classical criteria for identification of this species (19). Staphyloxanthin is a membrane-bound carotenoid which plays a role in the environmental fitness of *S. aureus* (20,21). Membrane pigments have also been hypothesized to be virulence factors in *S. aureus*,

potentially by detoxifying reactive oxygen species produced by phagocytes(22). Carotenoids may also stabilize the S. aureus membrane during infection and pathogenesis(23). Staphyloxanthin is a typical secondary metabolite (24) it is not necessary for the growth and reproduction of S. aureus but might serve a role in survival in infected hosts and in combating the immune system , staphyloxanthin is mainly produced in stationary phase , it 's chemical formula (C₅₁H₇₈O₈) . Staphyloxanthin is a neutral molecule (25) and light had no effect on it 's synthesis \\ Consequently, the aim of the present study was to detect the role of staphyloxanthin pigment production from S. aureus isolates from different clinical sources as anti bacterial agent against some pathogenic bacteria used in this study

II. Materials And Methods:

1- Isolation Identification of Staphylococcus aureus

The isolation of MRSA was obtained from microbiology lab of biology department and reidentification by biochemical tests (26).

2-Extraction of Staphyloxanthin pigment

The specimen of MRSA had been streaked on (10) nutrient agar plates and incubated at 37 ° C for 24 hr. then add to it 5ml normal saline and harvest the growth in tubes . After centrifugation 3000rpm for 30 min we take the pellet and added to the pellet 8ml ethanol(99%) centrifuge at 3000rpm for 30 min.Discarded the supernatant and take the pellet incubate at 37 ° C for 20 min and added to it 3 ml ethanol and put in water bath 55 ° C for (15)min and add 8 ml ethyl acetate. After centrifugation (3) layers had been formed we took the pellet and the middle layers and added to it 3 ml normal saline. Measureit's optical density by spectrophotometer at 286 nm (Hammond and white,1970).After extraction of golden pigment from MRSA isolate, by ethanol method and centrifugation ,when measuring O.D at 286 nm the value of O.D was equal to 2.7564.

3- Study of antioxidant activity of Staphyloxanthin pigment on macrophage killing pathway

This means when DPA is found and bacterial cell treated with DPA the golden pigment at cell wall inhibited no antioxidant happened for macrophage activity thus phagocytosis process occur and engulfment .Thus no growth appeared while when bacterial cell not treated with DPA golden pigment synthesis and caused antioxidant for macrophage cells. Thus, no engulfment occurs for bacterial cell because macrophage lost activity by effect of staphyloxanthin pigment .Thus, showed bacterial growth. This means golden pigment have antioxidant for macrophage cells and prevent phagocytosis process and bacterial staph golden pigment effect on immune defense of the body.

III. Results and Discussion

1.Identification of Staphylococcus aureusisolation

The isolation of MRSA was obtained from microbiology lab of biology department and reidentification by biochemical tests (Bergers'.2000).The result was showed the isolate in give yellow colonies on manitol salt agar and when identification by biochemical tests the result were coagulase (+) and oxidase (-) as in (table 1).

Table 1: biochemical test and their results of (MRSA)

| Biochemical tests | Results |
|--------------------------|---------------------|
| Mannitol salt agar | Yellow colonies |
| Gram stain | Gram positive cocci |
| Coagulase test | (+) |
| Catalase test | (+) |
| Oxidase test | (-) |
| Motility test | (-) |
| Methicillin | (+) |

2.-Extraction of Staphyloxanthin pigment:

After extraction of golden pigment from MRSA isolate, by ethanol method and centrifugation ,when measuring O.D at 286 nm the value of O.D was equal to 2.7564.

3- Study of antioxidant activity of Staphyloxanthin pigment on macrophage killing pathway:

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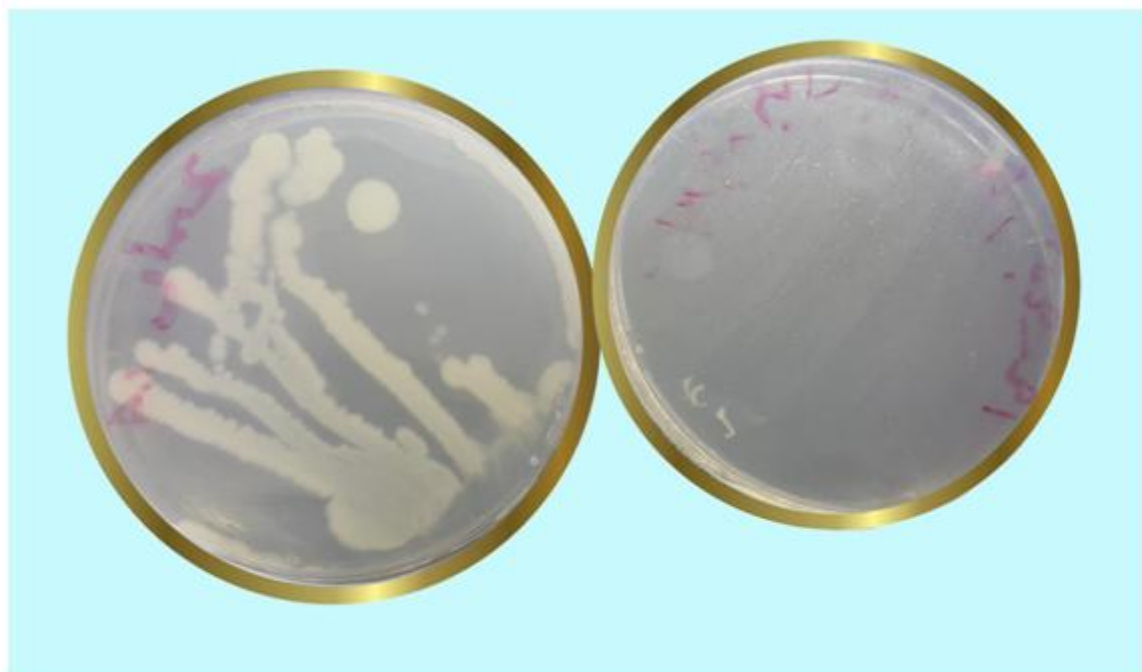


Fig. 1 : Methicillin resistant Staphylococcus aureus(MRSA) (without DPA). B:Methicillin resistant Staphylococcus aureus(MRSA)(with DPA)

Carotenoid which plays a role in the environmental fitness of *S.aureus* (27,28) Membrane pigments have also been hypothesized to be virulence factors in *S. aureus*, potentially by detoxifying reactive oxygen species produced by phagocytes(22). Carotenoids may also stabilize the *S. aureus* membrane during infection and pathogenesis. Staphyloxanthin is a typical secondary metabolite (29) it is not necessary for the growth and reproduction of *S. aureus* but might serve a role in survival in infected hosts and in combating the immune system , staphyloxanthin is mainly produced in stationary phase , it 's chemical formula ($C_{51}H_{78}O_8$) .

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