IMMUNOGLOBULIN A AS AN IMMUNOLOGICAL INDICATOR FOR RESTAURANT WORKERS INFECTED WITH VRSA AND MRSA BACTERIA AT THI-QAR PROVINCE

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ABSTRACT
The study aimed at finding out the relationship between the immunoglobulin A and the Vancomycin Resistance Staphylococcus aureus (VRSA) and Methicillin Resistance Staphylococcus aureus (MRSA) that infect restaurant workers. The blood was withdrawn from 50 restaurant workers infected with these bacteria and 30 non-infected restaurant workers for comparison. The blood was separated by Centrifuge to obtain the serum. And used the ELISA technique to determine the level of immunoglobulin A. Where it is shown through the results obtained that all of the samples showed that there was a significant increase in the level of the immunoglobulin of the infected workers when compared to healthy workers.

KEYWORDS: Immunoglobulin A, VRSA, MRSA, ELISA.

INTRODUCTION
Immune response against S. aureus include both activation the innate and adaptive immune system. As the first line of defense against infection, the innate immune response is activated rapidly through pattern recognition pathways which reveal non-specific markers of microbial infection. The main result of this is activation of phagocytic cells like macrophages and neutrophils. Neutrophils are recognized as a major component of the acute response and centrally important against S. aureus bacteria, as announced by the allergy of humans and mice with neutrophil inherited defects and acquired deep-seated infections. The adaptive immune response begins later in the infection, dependent on the presentation of bacterial antigens by cells presented to the antigen and affected by the cytokine environment generated by the innate response. (Hatice and Sandip 2017). The neutrophils use their oxidizing mechanisms to destroy the S. aureus and this has relied heavily on the MPO system "Myeloperoxidase: is a peroxidase enzyme that in humans is encoded by the MPO gene on chromosome 17. MPO is mostly expressed in neutrophil granulocytes and produces hypohalous acids to carry out their antimicrobial activity" (Klebanoff, 2005). ever since it has been shown to be faster with an active MPO (Hampton et al., 1996). It should be noted however, some S. aureus strains responses to major neutrophil oxidation on phagocytosis not seen with other types of staphylococcal (Nilsdotter et al., 2004). A large variety of proteins that secrete involved in immune evasion can be produced by S. aureus. Several proteins targeting immunoglobulin, supplementing or recruiting neutrophil, others against the effects of antimicrobial molecules like lysozyme and defensins "are small cysteine-rich cationic proteins". McCarthy and Lindsay (McCarthy and Lindsay, 2010.) found that when examining 13 genes with a distinct or assumed role in immune evasion, most of these were present in all the sequential S. aureus genomes, demonstrating the important role of immune evasion for S. aureus. "The first line of defense against inhaled bacteria is nasal secretions, a complex mixture of proteins, sugars and salts, containing such as lysozyme and immunoglobulins (IgA and IgG) (Kaliner, 1991), as well as defensins (Cole et al., 1999) and complement proteins (Casado et al., 2005)". S. aureus is resistant to lysozyme because the cell wall modification enzyme O-acetyltransferase (OatA) in collection with Wall teichoic acid (Bera et al., 2009) and iron-regulated determinant (Clarke et al., 2007) has been proven to make the surface of the S. aureus surface more hydrophilic, protection against the innate antimicrobial fatty acids for host that require hydrophobic reaction to be active. IgA considered is third fastest migrating group of serum globulins is Antibodies, the gamma globulins. Refers the term Immunoglobulin (Ig) to the portion that confers immunity from gamma globulin fraction (Sahingur and Cohen 2004). Based on physical and chemical antigenic differences, found five categories of immunoglobulins were identified —IgG, IgA, IgM, IgD and IgE (Ananthanarayan and Paniker 1990). The most abundant type of antibody is IgA and also is mostly

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exported via mucus membranes. While IgG dominated by the overall immune system, IgA is immunoglobulin that dominates the humoral mucosal immunology (Lastra et al., 1998). IgA is synthesized and secreted on mucus surfaces that occur on the entire body. Although the digestive system is the most important location because of its area. This is a reasonable site for the majority of antigens that are ingested or inhalation will end up in the digestive tract. IgA can be found in two forms. IgA monomeric was mainly present in the bloodstream and dimeric in saliva, tears and other secretions. IgA is transferred across the epithelial boundary on mucous surfaces, and in the intestines (Launay et al., 2011). IgA can have different functional characteristics depending on location and molecular form (Lendvai et al., 2000). Intestinal IgA antibodies can stimulate immune responses through the association of high-affinity binding as well as low-affinity binding, provide effective protection (Lortan et al., 1992). IgA appear to lack ability to activate complement (Lastra et al., 1998). Vancomycin was the most reliable therapeutic agent against infections it caused by methicillin-resistant Staphylococcus aureus (MRSA). The most reliable therapeutic agent was vancomycin against infections that caused by methicillin-resistant Staphylococcus aureus (MRSA). So, the discovery first the MRSA in 1996 that acquire resistance to vancomycin, was isolated from a Japanese patient. The patient has been infected with the inflammation after surgery that appearance resistant for long-term treatment to vancomycin antibiotic. Subsequent, isolation of many vancomycin resistant S. aureus (VRSA) strains from USA, France, Korea, South Africa, and Brazil, that have confirmed a global problem is the emergence of resistance to vancomycin in S. aureus. Exposure to vancomycin often generate VRSA, is specific certain group of S. aureus, particular hetero-VRSA, and are linked to infections that is potentially resistant to heat of therapy for vancomycin antibiotic. Presence of hetero-VRSA may be an important indicator of malignant retraction of the clinical efficacy of vancomycin in hospitals. The occurrence of the mutation and thickening of cell wall because accumulation of excess amounts of peptidoglycan be the cause to vancomycin resistance. This appear to be a common resistance mechanism for all VRSA isolate in the world so far (Keiichi, 2001). MRSA has occurred in many countries since its discovery in 1961 (Jevons, 1961). However, in recent years, doctors were concerned about increased frequency of MRSA infections (Chambers, 2001). This MRSA resurgence problem appears to depend on the lack of potent therapeutic factors that have the effect of killing an unambiguous cell, and thus able to eliminate MRSA from the patient’s body. Increased use of vancomycin- weak drugs in killing cells against prevalent MRSA strain seems to have been developed mainly to select vancomycin resistance in MRSA. In 1997, we reported the first MRSA strains with low vancomycin susceptibility, which was isolated from patients who had vancomycin treatment was effective (Hiramatsu et al., 1997). MRSA are a type of staphylococcus or “staph” bacteria that resistance to many antibiotics. staphylococcus bacteria, such as other types of bacteria, which usually live on the skin and in the nose, usually without causing problems. MRSA is various from other types of staphylococcus because it cannot be treated with certain antibiotics like methicillin. Staphylococcus bacteria become a problem only when the infection is caused. For some people, especially those who are weak or sick, this infections can become serious. Treatment of MRSA infections is more difficult than ordinary staphylococcus infections. This is because the staphylococcus strains known as MRSA do not respond well to many of the common antibiotics used to kill bacteria (Martin and Hardy 1991).

**MATERIALS AND METHODS**

**Bacteria collection and Isolation:** Five hundred and seventy-six swab specimens were collected from restaurant workers who review the public health laboratory for a health certificate and include all workers throughout Thi-Qar province / Iraq from February to June, 2018. Swab collected were inoculated onto the mannitol salt agar which are selective and differential medium for the isolation, purification and identification of Staphylococci. All plates were incubated at 37°C for 24 h. After incubation period, yellow golden colonies will appear and then the single pure colonies will be transferred to the nutrient and blood agar for the purpose of diagnosing of these isolates. Diagnosis of S. aureus based on the colony's apparent characteristics on the petri, and then conducting the biochemical tests according to (Harley and Prescott, 2002). and finally conducting the confirmation test using Api Staph. System.

**Antimicrobial Susceptibility Test:** The antimicrobial susceptibility test has been done by disc diffusion method as described by Bauer et al. (1966). By using vancomycin and methicillin antibiotics to determine VRSA and MRSA.

**Blood Samples Collection and Preparation:** 50 blood samples were withdrawn from the restaurant workers, where approximately about 3 ml of each worker was taken. Samples should be collected into a serum separator tube (gel tube). After clotting formation, and then placed samples in the centrifuge at 3,000 rpm for 10 min at 4°C to remove clots. Immediately aliquot supernatants and store at -80°C. until used.

**Estimation of Human Immunoglobulin A (IgA) levels by ELISA technique:** Used the Human IgA ELISA Kit (KOMABIOSCHET) with washer device according to the procedure following: 

**- Reaction:** Add 100 µl of standard, blank and sample to each well in duplicate. Cover the plate with the plate Sealer. Incubate at room temperature for at least 1 h. 

**-Washing:** Aspirate the wells to remove liquid and wash the plate 4 times.

**-Detection:** Add 100 µl of the diluted detection antibody per well. Then cover the plate with the Plate Sealer.
Incubate at room temperature for 1 h. 

- **Washing:**
  Aspirate and wash plate 4 times.
- **Color Development:**
  Add 100 µl of TMB or pink-ONE TMB solution to each well. Incubate at room temperature for a proper color development. Add 100 µl of the stop solution to each well." After the colors appear in the wells, it is read using by reader device with measure observance at 450 nm.

**CALCULATION OF RESULT**
Create a standard curve by reducing the data using ELISA reader's computer software capable of generating Standard curve-fit. A standard curve should be generated for each set of samples.

**Figure (1): Standard curve for Human Immunoglobulin A (IgA)**

![Standard curve for Human Immunoglobulin A (IgA)](image)

**Statistical analysis:** In order to compare numerical variables, we used the Student’s t-test, version 23 according to SPSS program and values of p < 0.05 were considered statistically significant.

**RESULT**

**Isolation and Identification of Bacteria:** Five hundred and seventy-six nasal swabs were taken from the restaurant workers with ages ranging from 18 to 40 years from males. only 50 (8.7%) were mannitol fermenter and showed as golden yellow colonies on the mannitol salt agar and identified as S. aureus, 30 (5.2) were growth on mannitol but no fermenter and showed white colonies, others were mannitol fermenter and showed as yellow colonies but identified as other species, which comprised 3 (0.5%). While the rest of the swabs when cultured on mannitol salt agar did not grow.

**The enzyme-linked Immunosorbent assay:** ELISA technique was used to determine the level (Titer) of immunoglobulin A in the serum of restaurant workers who carry bacteria. After the testing and reading the results through the Reader device, the results showed a clear increase in the level of immunoglobulin A in the workers who are carriers of bacteria compared to healthy workers, as shown in the table (1) and in the figure (2). Therefore, immunoglobulin A can be considered as a guide for people who have *staphylococcus aureus*.

<table>
<thead>
<tr>
<th>Samples</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>0.295 ± 0.039 a</td>
</tr>
<tr>
<td>Healthy persons</td>
<td>0.250 ± 0.014 b</td>
</tr>
</tbody>
</table>

* P-value = 0.001

**Table (1): Explained Titer of Immunoglobulin A for infected and healthy workers.**

![IgA level for carriers workers and healthy](image)

**Figure (2): The level of IgA for carriers workers and healthy.**
DISCUSSION

The nasal swabs of *S. aureus* among restaurant workers in Thi-Qar province was investigated. Only 50 (8.7%) of the restaurant workers screened carried *S. aureus* in their noses. This results was low rate to infection of *S. aureus* compared with restaurant workers in Kuwait City (Albustan and Chugh, 1996). On the other hand, the indicator of post process contamination is *S. aureus* that cause by food workers, which most likely occurred in our case. The main reservoir for *staphylococcus aureus* they humans. The predominant sites of colonization of this bacteria are hands and the mucous membranes of the nasopharynx. High rates of carrier of bacteria and large numbers of cells are two major factors that explain the important role of workers in the field of food in the epidemiology of outbreaks for staphylococcal food poisoning (Soriano et al., 2002). The vast majority of the staphylococci inhabit the nasal fossa of food workers belong to the species of *E. epidermidis* and *S. aureus* (Vora et al., 2003).

In the present study, all of the strains were resistance to Methicillin antibiotic While only 13 isolates showed resistance to vancomycin antibiotic In our study, the prevalence of *Staphylococcus aureus* was found among restaurant workers (8.7%) different to other research reports conducted by Lamaro-Cardoso et al. In Brazil and Davoodabadi et al. In Isfahan (Lamaro-Cardoso et al., 2009; Davoodabadi et al., 2016).

The generation of an adequate protective response in the infectious process is the main aspect that will determine whether the host will advance to healing or developing infection. In this study we investigated the immunoglobulin A with *staphylococcus aureus* in restaurant workers. We showed a significant rise in levels in the IgA of all infected restaurant workers during the time of diagnosis. after recognition of immunity, according to measure by antibody responses ,they can be very different in distinct individuals of human population. In this regard, our study showed that healthy individuals can have levels of circulating antistaphylococcal antibodies, as indicated by the results of previously studies (Colque et al., 2000).

*S. aureus* bacteria use at least three different ways to stimulate adaptive immune system and the induction of special antibodies: (i) Exposure to mucous surfaces not produced in colonization, (ii) colonization of the mucosal surfaces "nasopharynx and intestines" and special anatomical niches "anterior nares and anorectic locations", (iii) Infections are latent or clinically clear from otherwise sterile body locations (wounds, blood, bones, etc.) (Vernachio et al., 2003).

Not characterized the role of antibodies in the serum IgA in antistaphylococcal immunity well. A previous study obtained significant results for our observation that both secretory and serum IgA which has been shown to stimulate opsonophagocytosis of *S. aureus* by polymorphonuclear leukocytes in vitro (Gorter et al., 1987).

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