Identification and Extraction of Chicken Egg Yolk Immunoglobulin from Egg by Polyethylene Glycol (PEG) Precipitation

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ABSTRACT

***Background*:** *Staphylococcus aureus* strains exhibiting multiple antibiotic resistances are isolated from most communities and hospital infections. Treatment of patients with these infections has been difficult. The aim of this study was to detect and extract, the egg yolk immunoglobulin Y as a potential source of anti- *S. aureus* antibody.

***Methods*:** Specific IgY was produced by immunizing hens with formalin-killed *S. aureus*. The specificity of serum`s antibody was confirmed by ELISA method. The antibodies were extracted from egg yolk by polyethylene glycol (PEG) precipitation. Proteins were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Results: Chicken egg yolk antibodies (IgY) were raised against *S. aureus* in the serum after injections. Up to 104 dilution specific antibodies were determined in serum.

***Conclusion*:** The results of the ELISA indicates the specificity of the immunoglobulin Y to the target antigen. In order to find a viable alternative to antibiotic treatments, more research must be done on the ability of these antibodies to inhibit the growth of *S. aureus*.

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Introduction

During the pre-antibiotic era, many staphylococcal invasive infections resulted in the death of the patient from the systemic effects of toxin or septicemia. This devastating effect was relieved to a great extent by introduction of penicillin in the 1940s. However, by the end of the 1950s, approximately 50% of hospital isolates of *Staphylococcus* were penicillin resistant. Within a few years, methicillin resistant *S. aureus* (MRSA) were found in the clinical setting. This has left vancomycin as the drug of choice to treat MRSA infections (1). However, strains of *S. aureus* that display intermediate or full resistance to vancomycin are increasingly being found (1, 2). *S. aureus* strains exhibiting multiple antibiotic resistance are isolated from most of communities and hospital infections (3). Treatment of patients with these infections has been difficult, though possible.

Passive immunization is another way for overcoming MRSA infections. The ability of antibodies to bind antigen with a high degree of affinity and specificity has led to their ubiquitous use in a variety of situations. However, the method has associated problems in terms of cost and productivity (3).

Recently, the utilization of antibodies from the eggs of chickens which were immunized with pathogens has been the focus of attention in immunotherapy and diagnosis (3). A major advantage of using birds is that the antibodies can be harvested from the egg yolk instead of serum. In addition, Purification of immunoglobulin from mammalian blood is time-consuming and expensive. Today, hens are recognized as a convenient and inexpensive source of antibodies. The chicken egg yolk antibodies (IgY) have been applied successfully for scientific, diagnostic, prophylactic and therapeutic purposes (4).

Here we studied a simple and efficient way of producing IgY antibodies against *S. aureus*.

Materials and Methods

Bacteria and Culture Conditions

S. aureus (ATCC 25923) were obtained from national center of genetic and biological reserves in Iran.

Experimental Animals

Four 50 weeks old, white highline chickens were obtained from aviculture and kept in animal house, School of Medicine, Alborz University of Medical Sciences, Karaj. They were used in the study for the production of anti-*S. aureus* antibodies (IgY).

Preparation of Immunogens

Formalin-inactivated *S. aureus* as immunogens for immunization and antigens for ELISA experiment were prepared. Bacteria were cultured in Brain Heart Infusion Broth overnight at 37 °C and harvested by centrifugation (15 minutes, 3000 rpm). Supernatant was discarded and the pellet was washed three times with phosphate buffer saline (pH 7.2, 3500 rpm, 15 min). The pellet was resuspended in PBS at a density of 108 cells/mL by comparing 0.5 McFarland and cells OD at 600 nm was recorded. Subsequently, bacteria were killed with 10% formalin (V/V), and suspension was hold at 4 °C for 16 hours. In order to remove the formaldehyde, bacterial suspension was washed twice with PBS and re-suspended in sterile PBS at same concentration. Complete killing of the bacteria was tested by culturing the S. aureus on MHA for 48 hours at 37 °C and the suspension was stored at -20 °C (5, 6).

Immunization and IgY induction

Chickens received four injections, one primary injection and three boosters. For the main injection, 500 µL of antigen was emulsified with an equal volume of Freund's complete adjuvant (FCA) using Herbert et al. procedure (7). Then the solution was injected intramuscularly at one site of breast muscle of chickens. Three booster injections of antigen with Freund's incomplete adjuvant (FIA) were given at 14 days interval by the same route of administration.

Collection of serum samples

Two mL blood sample was collected from chickens before immunization as preimmune sera. Then it was collected at 14 days interval frequently. Serum was separated by centrifugation for 10 minutes at 3000 rpm and stored at -20 °C until use.

Titration of antibodies by Indirect ELISA

The antibodies titer was determined by an enzyme linked immunosorbent assay (ELISA) procedure as described by Sunwoo et al. (2000) (8).

Briefly, *S. aureus* antigens were coated onto microtiter plates in coating buffer (0.05 M carbonate bicarbonate buffer, pH 9.6) for overnight at 4 °C. After washing the plates three times with PBST and blocking non-specific sites with blocking buffer (1% BSA in PBST) test sera were serially diluted (in sample diluent) on the plate. Preimmune sera was used as control. The total Immunoglobulin titer was determined using rabbit anti-chicken immunoglobulin coupled to Horse Radish Peroxidase (diluted with buffer assay, 1:10000). The colorimetric detection was carried out using tetramethyl benzidine (TMB) as a chromogenic substrate of HRP, after washing the plate with PBST. The absorbance of each well was measured at 490 nm.

Extraction of total IgY from egg yolk

The antibodies were extracted from egg yolk by the method of Polson et al (9). Briefly the yolk (15 ml) was carefully separated from the white by "yolk spoon" in order to remove as much egg white as possible. Twice the egg yolk volume of PBS (pH=7.2) was mixed with the yolk, followed by the addition of polyethylene glycol 6000 (PEG 6000) up to 3.5 % (w/v) and mixing with magnetic stirrer for 20 min. After centrifugation for 20 min (10000 rpm, 4 °C), the supernatant was poured through a folded filter and 8.5 % PEG 6000/gram was added to the tube and the suspension was centrifuged. Finally, PEG 6000 up to 12% (w/v) was added to pellet and after centrifugation the pellet containing IgY was resuspended in 25 ml PBS.

Protein profile by SDS – PAGE

The chicken egg yolk antibodies and its molecular weight was determined by Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) using 10% polyacrylamide gel at 80 V according to the method of Laemmli (10).

Results

ELISA revealed the ability of serum IgY produced by immunized hens to bind with *S. aureus* and showed the pattern of the immune response by the hens. Chickens received four injections, one primary injection and three boosters. The IgY titre increased from initial immunization and the high titre (≥0.065) persisted after the first immunization (Figure 1).

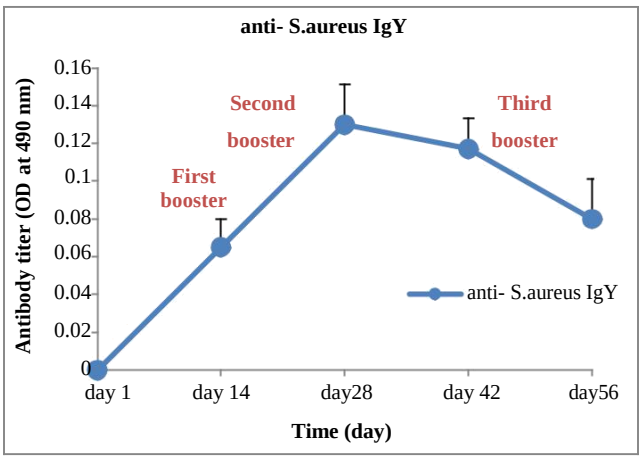

Figure 1. Titre of anti- *Staphylococcus aureus* immunoglobulin (IgY) in Serum (0.0001, mean ± STD).

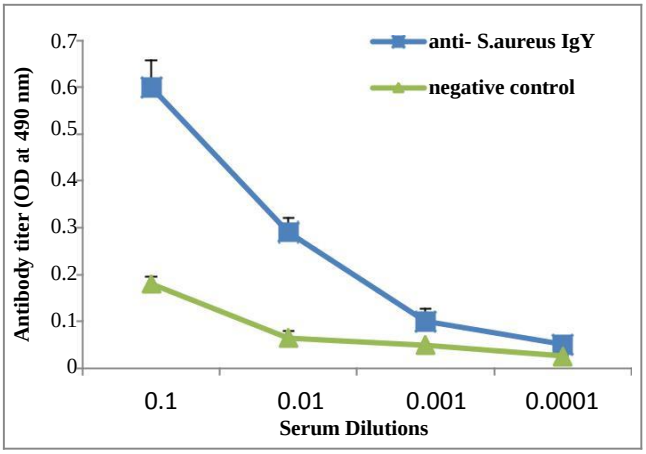

Figure 2. Titer of antibody against *S. aureus* in immunized chicken serum with the bacteria after injections, Preimmune sera was served as control (mean ± STD).

Furthermore comparing serum dilutions with control group (preimmune sera) indicate that up to 104 dilution antibodies were present in serum, suggesting high amount of antibodies production in the immunized chickens (Figures 2 and 3).

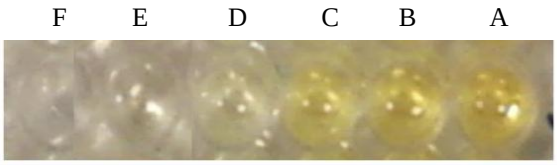

Figure 3. Titration of Antibody by ELISA in Dilutions of 1.10, 1.100, 1.1000, 1.10000. Preimmune sera was served as negative control. The positive reaction shows up to 1.10000 dilution. A: 1:10 Dilution, B: 1:100 Dilution, C-1:1000 Dilution, D: 1:10000 Dilution, E: Negative Control, F: Blank.

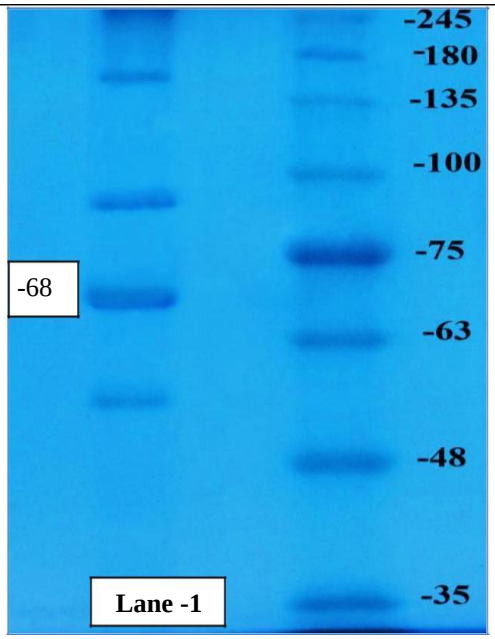

Figure 4. SDS-PAGE Profile of IgY. Lane-1: antibody extracted from egg yolk by polyethylene glycol precipitation with molecular weight about 68 kDa.

The antibodies were extracted from egg yolk by polyethylene glycol precipitation and proteins were analysed by SDS-PAGE using 3% (v/v) loading gel and 10% (v/v) separating gel. IgY contains two major bands, 68 kDa and 25 kDa as the heavy and light chains, respectively. The pattern of gel electro-phoresis (Figure 4) showed a single band with a molecular weight of ~68 KDa as heavy chain. Due to low molecular weight, light chain often could not be seen on the gel.

Discussion

Several studies have been carried out to explore the usefulness of avian immune-globulin Y in food, drug, microbial, and residual analyses (11), immunediagnostics (12), passive immunization and therapeutic functions (13). The advantages of using avians such as chicken and quails as a source of immunoglobulins have also been documented (14).

In the present study, the activity and titer of specific immunoglobulin Y in serum, determined by ELISA, showed the presence of antigen specific antibodies for the specific pathogenic bacteria. The titer of the specific antibody produced in immunized hens increased rapidly from the first injection and reached a peak after second booster injection at the fourth week which was maintained stable for two weeks. However, it decreased after last booster injection suggesting that in order to achieve the stability in antibody titer repeated booster injections are needed. Clifton-Hadley (2002) reported that specific IgY against *Salmonella typhimurium* has increased after booster injections and was stable for some weeks (15).

The extraction of antibodies from egg yolk was done by precipitation with polyethylene glycol. The presence antibody heavy chain in the gel electrophoresis pattern was an indication that the extraction method was appropriate. In addition, others bands were seen which may indicate they were impurities. This fact indicate that in order to achieving purified immunoglobulin Y, additional purification methods must be used. Pauly et al (2011) have reported that the IgY-sample that was obtained by PEG-precipitation, worked very well in a lot of different immunological assays (16). In another research that was done by Polson (1990), the chloroform-polyethylene glycol procedure was used which yielded 2.57 times more IgY than the conventional polyethylene glycol method (17).

After dialysis against a buffer, the IgY-extract can be stored at -20 °C for more than a year. As a result, the production of antibody in hens and the IgY-extraction by means of PEG-precipitation is very cost-effective and results in highly specific antibody with titer up to 104 or more. The purity of an IgY preparation can be increased by a combination of methods; for example, PEG precipitation can be combined with ion exchange chromatography.

The extraordinary amount of antibody obtained by IgY- technology opens the venues for using IgY in human and veterinary medicine for therapeutic and prophylactic purposes.

Conclusion

The results of the ELISA indicates the specificity of the immunoglobulin Y to the target antigen. In order to find a viable alternative to antibiotic treatments, more research must be done on the ability of these antibodies to inhibit the growth of bacteria and infections.

Conflict of Interest

None declared conflicts of interest.

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