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Original Research Paper

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DETECTION OF IgY ANTIBODIES IN CHICKEN SERUM AGAINST *STAPHYLOCOCCUS AUREUS* AND ITS NEUTRALIZATION ASSAY BY IN-VITRO

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ABSTRACT Production of Different Classes of immunoglobulin products in laboratories are a major class of avian origin. Egg lying chicken bring into being more amount of antibodies than any other animal models can produce. Most pleasingly the animal care and cost are lower the chicken when compare to other animal models. The objective of this study was to screen the development of IgY antibody production in serum of selected hens.

KEYWORDS : Chicken serum, antibodies, agglutination.

INTRODUCTION

Acne vulgaris is a disease of the pilosebaceous follicle characterized by non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules, and nodules). Its pathogenesis is multi-factorial or the interplay of hormonal, bacterial, and immunological (inflammatory) factors which results in the formation of acne lesions. The term acne is derived from Greek word acme, which means prime of life. It is a pleomorphic disorder, benign and self-limiting but may cause severe psychological problems or disfiguring scars that can persist for a lifetime. It can be manifested at any stage of life, but it is most commonly discerned between ages of 12-24, 85% of total population being affected globally (Muhammad Tahir, 2010). Though number of treatments is available to reduce the appearance of scars, it is important to reduce the duration and intensity of inflammation, thus stressing the importance of the acne treatment. The use of topical retinoid is effective in the prevention of acne scars but more than any other measure; the use of silicone gel has a proven efficacy in the prevention of hypertrophic scars and keloids (English and Shenefelt, 1999).

Antibodies currently available for research, diagnosis and therapies are mostly mammalian monoclonal or polyclonal antibodies. Recent research proved that antibodies from chicken will act as promising alternative for diagnosis and treatment of various pathogenic organisms. Although the fact that immunized hens transfer immune-globulins from the serum to the egg yolk has been known for over a hundred years, this alternative possibility of producing antibodies has attracted attention only in the last decade (Schade *et al.*, 1991). Chicken antibodies are cheaper and have potential as a diagnosis/therapeutic treatment for bacteriological diseases. Therefore, the aim of the present study was to develop antibodies from serum of chickens against Staphylococcus aureus and its neutralization assay by in vitro.

MATERIALS AND METHOD

Collection of Experimental Animals and Identification of Pathogen

Twenty four week old, white leghorn chickens obtained from the Poultry house, Department of Poultry management, Kerala Veterinary and animal Science University, Pookode, Wayanad. They were used for the immunization and generation of antibodies from serum. Pathogens were collected from DDRC Laboratory Kalpetta, Wayanad District, Kerala. Bacterial samples were isolated and identified on the basis of morphological, biochemical and cultural characteristics.

Preparation of Bacterial Antigen and development of antibodies in Chickens Staphylococcal cells were grown overnight in nutrient broth and the antigen was prepared by formaldehyde killed method. The cells were dissolved in PBS according to Mc-Farland standards and the antigen was injected intramuscularly at multiple sites of breast muscles of 24 week old white leg horn chickens with booster dose.

In-vitro neutralization assay of IgY antibodies

After four weeks of immunization, serum was separated and collected in fresh vials and direct agglutination assay was performed to detect the presence of anti-Staphylococcal antibodies in the serum.

RESULT

Pathogens were collected from DDRC and identification and isolation was done on the basis of morphological, cultural and biochemical characteristics. Gram staining and motility was done in order to study the morphological characteristics. (Table – 1) Taking place biochemical identification Methyl red, Voges–Proskauer (VP), catalase and citrate confer positive results.(Table – 2) The isolates were streaked on Mannitol salt agar and the isolates produced golden yellow colored colonies which were identified as Staphylococcus species.

Table- 1. Morphological characteristics:

METHOD/TEST	CHARACTERISTICS
Gram Staining	Gram positive , Cocci
Motility	Non-motile, Positive cocci

Table - 2 Biochemical characteristics:

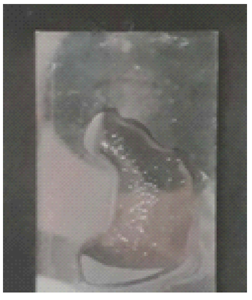
BIOCHEMICAL TEST	POSITIVE	NEGATVE
Indole		-

MR	+	
VP	+	
Catalase	+	
Citrate	+	

Generation of Staphylococcal Antibodies in Chicken

The preimmune sera and hyperimmune sera were collected at specified time intervals after various immunization schedules. The agglutinating antibodies were detected in serum two weeks after the primary immunization (Figure -1). Then specific antibodies were detected in serum after four weeks of immunization.

Figure – 1 Agglutinating Antibodies in Chicken Serum against Staphylococcal antigens



DISCUSSION

Acne vulgaris is the most common disorder of human skin (Nakatsuji, 2009) which has social and psychological impacts and are sometimes so complicated that they cause serious problems in patients' self-esteem and socialization (Safizadeh *et al.,* 2012). The development of acne is a multi-factorial process involving both endogenous and exogenous factors, (Davis and Callender , 2010) including excessive sebum secretion, ductal hypercornification, and changes in the microbial flora especially colonization with *Propionibacterium acnes.* (Hassanzadeh et al., 2008). *S. aureus* is the most common nosocomial pathogen, (Mertz *et al.,* 2009) with mortality rates ranging from 6% to 40 % (Frank et al., 2010). The organism is normally present in the nasal vestibule of about 35% of apparently healthy individuals (Adesida *et al.*, 2007).

In our present study we aimed to develop antibodies against Staphylococcal antigens from serum. Experimental animals white leghorn chickens obtained from the Poultry house, Department of Poultry management, Kerala Veterinary and animal Science University, Pookode, Wayanad. They were used for the immunization and generation of antibodies from serum. . On gram staining it showed gram positive cocci and biochemical tests showed as above. On cultural characterization on Manitol salt agar plates showed golden yellow colonies. Based on the morphological, biochemical and cultural characterization the isolates were identified as Staphylococcus aureus.

Formalin killed cells were dissolved in PBS in the concentration of 10^3cells/ml and injected intramuscularly at the multiple sites of breast muscles of 24-week-old white leghorn chickens. The booster dose of increasing concentration of antigen was given for every 7 days to raise the antibody level in egg yolk. The preimmune sera and hyperimmune sera were collected at specified time intervals during and after the various immunization schedules. The agglutinating antibodies were detected in serum two weeks after the primary immunization. Then specific antibodies were detected in serum after four weeks of immunization.

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