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ORIGINAL ARTICLE **OPEN ACCESS**

Comparative Study of IgA from Human Saliva against Skin Infection Causing *Staphylococcus aureus* Along with Commercially Available antibiotics

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ABSTRACT
Staphylococcus aureus is a gram-positive cocci that causes skin infections. Staphylococcus aureus is actually known as "the golden cluster seed" or "the seed gold". Saliva is an extracellular fluid produced and secreted via means of salivary gland within the mouth. Saliva plays an important role in wetting and allowing the initiation of swallowing. The application of ex situ method for the specificity of salivary immunoglobulin and microbial fraction that is being directly exposed to self and non self origins was investigated. The antibodies are being separated by magnetic bead for ex situ method. The magnetic bead separation indicates the relationship between the oral bacteria and salivary immunity, which reflects in the interactions of self and non-self- immunoglobulins in the study. Saliva plays as a natural defense in maintaining a healthy oral microbiota. Oral bacteria has the ability to coat or bind with the oral immunoglobulin A (IgA) in saliva. In future the IgA vaccination strategies can be developed for protection against various infections. The IgA produced in our body can be administered into our body through vaccination can prevent the infection. The study helps in the development of vaccines for immune system since the defense mechanism of IgA was being proved thus, IgA can be considered as an important therapeutic agent for various infection. The study indicates that the salivary immunoglobulin A (IgA) can possess antimicrobial activity. Further studies in the topic can provide a significant tool in medical science.
Keywords- *Staphylococcus aureus, immunoglobulins, IgA, extracellular fluid*

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INTRODUCTION

Antibodies are also known as immunoglobulin's, a protective protein produced by our immune system and those proteins will help our body to act against various foreign substances called antigen's that is, antibodies will help our body to defend against these foreign substances such as bacteria, fungi, virus, toxic compound etc. The antibodies produced in our body have the ability to recognize various invading foreign substances and by the help of those antibodies we can eliminate them. An important property of an antibody is antigen-antibody complex formation that is, each antibody has its own specific antibody to bind to forma complex, that will neutralize the antigen (i.e., foreign substances) or helps in its destruction and removed from the body i.e., there occurring phagocytosis and induce immune response. The structure allows antibody molecule to bind antigen and biological activity mediation. The domain shapes the paratope–the antigen binding site at the amino acid terminalend of the monomer. Fc region is the tail region of an antibody that interact with all cell surface receptors and some portion of complements. This property allows antibodies to activate the immune system.[1]

Antibodies are classified into 5 different classes based upon their constant region and each possess different functions. They are Immunoglobulin G (IgG), Immunoglobulin A (IgA), Immunoglobulin M (IgM), Immunoglobulin E (IgE), and Immunoglobulin D(IgD). Immunoglobulin A (IgA), found in mucosal areas and are also found in tears, saliva, breastmilk etc, they helps in colonization by pathogens [2]. It is also possible to distinguish forms of IgA based upon their location – serum IgA vs. secretory [2]. Secretory IgA (S IgA) is the principle immunoglobulin present on mucosal surface of human and other mammals. Secretory IgA, plays an important role in the immune function of mucosal membrane. The Secretory IgA

will prevent the entry of opportunistic pathogen and thus controls the symbiotic relationship between commensal's and host. Secretory IgA has the ability to block the microorganisms and toxin present on the epithelial cell and thus preventing surface damage colonization and massive invasion. Secretory IgA will protect immunoglobulin degrading from proteolytic enzyme and also inhibit inflammatory effect of other immunoglobulin's. The Secretory IgA has the ability to defeat against certain varieties of pathogens invaded through stomach, intestine, or nasal passage, at first they neutralized by binding to the pathogen or toxin produced by the pathogen that process called immune exclusion[3]

Amount of IgA produced in association with mucosal membranes is a greater thanall other types of antibody produced in our body[4]. Wound infection such as cut or graze might become infected with bacteria. Cellulitis is a spreading inflammation of the skin and the tissue directly beneath. Staphylococcus scalded skin syndrome (SSSS) is the most serious staph skin infection[5]. It usually infect babies and children under the age of 5 and occur when the staph releases a poison that can damage the skin. Impetigo also known as "school score" and is very contagious skin infection that infect children and infants [6].

About 20% of the populations are long term carries of S.aureus. S.aureus can cause a range of illnesses from minor skin infection, such as pimples, impetigo (may also caused by Streptococcus pyogenes), boils (furuncles), cellulitis folliculitis, crabuncles, scalded skin syndrome and abscesses, to life – threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacterimia and septicemia[7]. Methicillin-resistant Staphylococcus aureus (MRSA) infection has historically been associated with patients in hospitals and skilled nursing facilities. In recent years, there has been an increase in reports of community-associated MRSA infections (CA-MRSA)[8]. The level of SIgA in saliva vary in physical and psychological stress through interactions with the autonomic nervous system. SIgA level in saliva are affected by flow rates, with concentrations normally decreasing as flow rate increase[9]

MATERIALS AND METHODS:

The saliva was allowed to accumulate on the floor of the mouth and the saliva was split into a sterile container. The collected saliva was centrifuged for the separation of IgA and was partially purified by using different concentration (20%, 40%, 50% and 70%) of saturated ammonium sulphate precipitation method [10] and the pellets after precipitation were collected and resuspended in phosphate solubilizing buffer. For further purification dialysis method [11]. was performed & The supernatant was collected and stored at -20̊C. Protein Estimation by was done for the purified IgA.The absorbance of extracted IgA was compared with the absorbance of standard BSA solution.

The skin swabs were collected from the nearby microbiological laboratory All the swabs were inoculated onto an enriched alkaline peptone water and was incubated at 37^0C for 24 hours. Next day a loop full of broth was aseptically streaked into sterile nutrient agar plates [12].The colonies from the plate swere incubated in freshly prepared nutrient agar broth incubated at 37^0C for 24 hours in order to get pure culture of the isolates. This nutrient broth culture was used for further analysis. Various macroscopic and microscopic methods have been performed to confirm the isolated organism.

Antibacterial activity of purified SIgA was evaluated by disc diffusion method [13] and Well diffusion method [14]. Where, asceptically transferred the sterlised medium into each plates, and allowed it to solidification. A 0.5ml aliquot of 0.048 mol/L BaCL 2 is added to 99.5 ml of 0.18 mol/L H_2SO_4. Then OD value was taken at 650 nm for the preparation of McFarland Standard /Turbidity Standard by [14]. Place the resulting mixture in a foil and Store the covered McFarland standard at room temperature. Overnight bacterial culture with McFarland standard were swabbed over the solidified media. To those plates, along with the stored IgA antibody disc various antibiotic discs i.e.,Clyndamycin (2µg/disc),Chloramphenicol (30µg/disc), Ciprofloxacin (5µg/disc),Vancomycin (30µg/disc), Imipenam/cilastin (10/10µg/disc), Ampicillin (10µg/disc), Gentamycin (10µg/disc), Erythromycin (10µg/disc), were placed using flame sterilized forceps[15]. The plates were incubated at 37^0C for 24 hours and observe for the zone of inhibition. And for the well diffusion over night bacterial culture with McFarland standard were swabbed over the sterile solidified well cutted MH agar media [16]. To each well add the extracted sample in different concentration i.e., add 10µl, 20µl, 50µl and 100µl of different concentration of saturated sample i.e., 20%, 40%, 50% and 70% using micropipette. Plates were incubated for 24 hours and observe for the ozone.

RESULTS AND DISCUSSION

The sample was being collected in a sterile container. The sample was then centrifuged approximately at 14000 rpm at 4^oc for 30 minutes. Supernatant was collected on a sterile container and stored at– 20^oc.the samples were taken for ammonium sulphate precipitation method. The 4 samples are being centrifuged at

10,000 rpm for 15 minutes and the pellets are being suspended in pbs buffer solution. The pellets were formed (Plate 1) after centrifugation at 10,000 rpm for 15 minutes because the proteins were precipitated out i.e., at higher salt concentration the protein solubility decreases.
Dialysis method was being carried out. The overnight kept solution was taken out using a sterile pipette. The centrifugation occurs at 13000 rpm for 4^{o}c for 20 minutes. Pellets were formed **(**Plate**2)** due to the removal of salt by moving from an area of high concentration to an area of low concentration, supernatant was collected and storedat-20^{o}c.



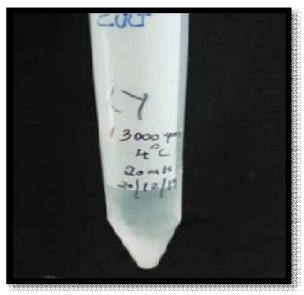


Plate 1: Pellet Formation after **Plate 2: Dialysis method Centrifugation**

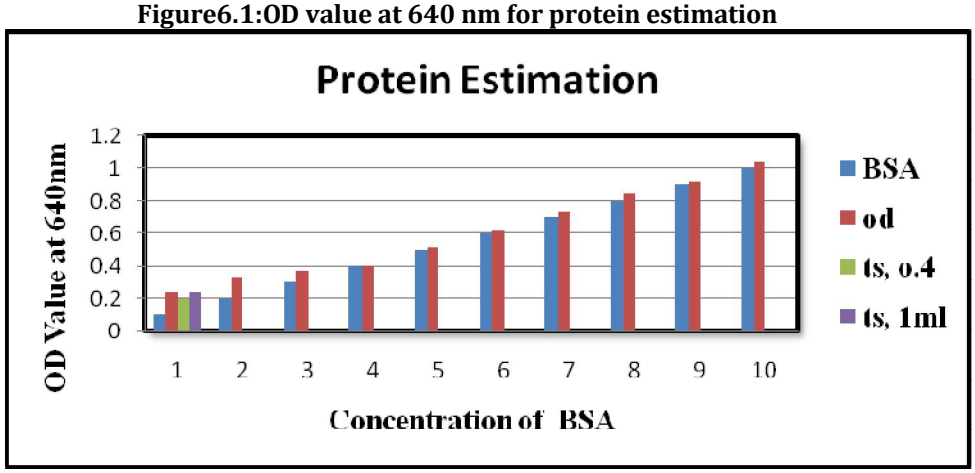
Standard BSA solution was being prepared.. To all samples add 5ml of reagent C and incubate for 10 minutes. Then add 0.5 ml of reagent D and incubate for 30 minutes in dark. Then absorbance was taken at 640nm and the reading is recorded. After the addition of reagent C and reagent D, the OD value of test sample 0.4mland 1ml was taken at 640 nm. The 0.4ml shows a reading of0.20 and 1ml shows a reading of 0.26.The readings were plotted in Table6.1 and also plotted inGraph6.1.

Table 1: Protein estimation

Concentration of BSA	OD valueat640nm
0.1	0.24
0.2	0.34
0.3	0.37
0.4	0.40
0.5	0.51
0.6	0.62
0.7	0.74
0.8	0.85
0.9	0.92
1ml	1.05
IgA,0.4ml	0.20
IgA1ml	0.26
Blank	0

The reading was noted as per the table 1 and the values were also represented as in graph. The unknown samples reading were also plotted according to the OD value at640nm, i.e., 0.4ml and 1mltest sample reading was taken. The results were tabulated in the Figure 6.1. The test sample 0.4ml and 1ml was plotted.

Figure6.1:OD value at 640 nm for protein estimation



The skin infection causing organisms were isolated and the microscopic examination indicates that the isolated organisms are confirmed as" Gram positive cocci". After viewing through the microscope at 100x, the organism does not show any Brownian movement i.e., the organism remains calm in the medium. Thus the organism is confirmed as non-motile. The isolated organisms were confirmed as Coagulase negative *Staphylococcus aureus*, after serious of biochemical reactions.

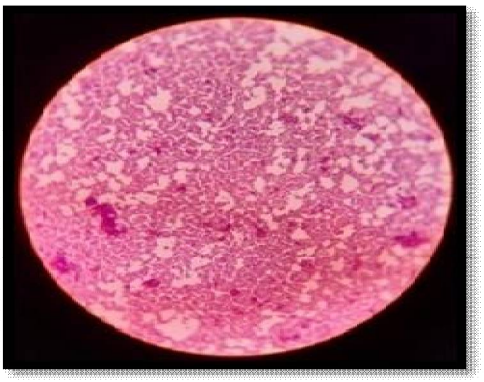


Plate 6.3 Gram Positive Cocci in clusters

For the characterization of the isolated pathogen, various media's were prepared and inoculated with isolated organisms. After incubation, the culture gave golden yellow pigmented colonies with smooth glistering surface in Nutrient agar (Plate 6.4), the plate showed beta hemolysis (complete lysis of the red blood cells around the colonies) on the blood agar shown in Plate 6.5

Plate6.4 Nutrient agar plate

Plate6.5 Blood agar plate

For Well Diffusion Method the MH agar was being prepared and inoculated with S*taphylococcus aureus* and 4 wells were cut using well cutter. In those wells various concentration of saturated solution of IgA was being added and kept for incubation. The result was shown in Fig 6.7and tabulated in Table 6.3as follows:

Plate6.7Antibiotic sensitivity method using secretory IgA Table

Table 6.3. Antibiotic susceptibility test result using SIgA

Concentration of ammonium sulphate (%)	10µl	20µl	50µl	100µl	Sensitivity
20%	1mm	2mm	3mm	4mm	Sensitive
40%	2mm	5mm	6mm	8mm	Sensitive
50%	2mm	6mm	10mm	15mm	Sensitive
70%	19mm	22mm	25mm	27mm	Resistant

After incubation of 24 hours, the resistant against *Staphylococcus aureus* was shown in this order i.e.,70% (100µl) >50%(100µl) >40% (100µl) >20% (100µl). The zone of inhibition was shown in this order.
The result shows that, IgA shows its antimicrobial action against S*taphylococcus aureus* compared to the antibiotic action shown by different antibody based on Table 2 and Table 3. From the tables it was confirmed that, more antibiotic action was shown by Clyndamycin and erythromycin antibiotics. Whereas 70% saturated IgA samples show more reaction compared to other saturations. Therefore it is confirmed that to a certain extent, IgA show resistant towards skin infection causing S*taphylococcus aureus*.

CONCLUSION

The biochemical analysis and presence of infections associated with wound or burn infections indicates their resistance due to the environment present in the tissues created a suitable environment for the growth of organism that cause infections.By the microscopic, biochemical and characteristic analysis, the organism was confirmed as *Staphylococcus aureus*, whose commercial application of antibiotics canalsobe administered by blocking the replication of organism this was proved by the formation of zone of inhibition. The secretory immunoglobulin A (SIgA) could be used as controlling agent to resist the infection and thus enhance the prevention of growth in the infected area of a tissues. Thus, the study proves that the secretory IgA might also be a choice of an agent to control the growth of skin infection causing organism to a certain level.
Secretory IgA serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms. SIgA constitutes the main specific immune defense mechanism in saliva and may play an important role in homeostasis of the oral microbiota. Naturally occurring antibodies that are reactive against a variety of indigenous bacteria that are detectable in saliva. The importance of secretory immune system in controlling allergic symptoms.
Our aim of the of the study was to isolate the *Staphylococcus aureus* causing skin infection to compare its antibacterial activity against the SIgA isolated from saliva. The SIgA was bring isolated from saliva by various process. The partial purification was done by ammonium sulphate and precipitation method. The result shows that a certain level the growth of organism can be controlled by SIgA. Thus it is proved that IgA provide the primary defense mechanism against local infection.
A major break through which provided a way of producing mouse antibodies in definitely i.e.,the development of hybridomatechnology.But mouse antibodies are limited for development of human immunity, thus development of human antibodies for therapeutic activity was improved. So, our study proves that the IgA produced in ourbodyshowssuchdefenseactivityagainstvariouspathogenicandnon-pathogenicorganisms. Since the antibodies produced in our body itself, so those antibody does not damage our immune system.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

REFERENCES

1. Dryden, M. S. (2010) Complicated skin and soft tissue infection. J. Antimicrob. Chemother, 10.1093.302
2. Brandtzaeg, P., Fjellanger, I., and Gjeruldsen, S. T. (1968) Adsorption of immunolgobulin A onto oral bacteria in vivo. J. Bacteriol,10.1128/jb.96.1.242-249.
3. Fishman, J. B., and Berg,E. A. (2018) Ammonium sulfate fractionation of antibodies. Cold Spring Harb. Protoc,10.1101pdb.prot099119
4. Acharya,T.(2016)Preparation of McFarl and Turbidity Standards. Microbe Online.

5. Balouiri,M.,Sadiki,M.,andIbnsouda,S.K.(2015)Methodsforinvitroevaluating antimicrobial activity : A review Author ' s Accepted Manuscript. J.Pharm.Anal., 10.1016/j.jpha.2015.11.005.
6. Turula, H., and Wobus, C. E. (2018) The role of the polymeric immunoglobulinreceptor and secretory immunoglobulins during mucosal infection and immunity.Viruses,10.3390/v10050237
7. Dowd,S.E.,Halonen,M.J.,andMaier,R.M.(2009) ImmunologicalMethods.In
8. EnvironmentalMicrobiology,10.1016/B978-0-12-370519-8.00012-2
9. Hung, A. F. H., Chen, J. B., and Chang, T. W. (2008) Alleles and isoforms ofhumanmembrane-boundIgA1.Mol.Immunol.,10.1016
10. Sweeney, R. E., Budiman, E., and Yoon, J. Y. (2017) Mie scatter spectra-based device for instant, contact-free, and specific diagnosis of bacterial skin infection. Sci. Rep. ,10.1038 s41598-017-05061-1
11. Karmakar, A., Dua, P., and Ghosh, C. (2016) Biochemical and Molecular Analysisof Staphylococcus aureus Clinical Isolates from Hospitalized Patients. Can. J.Infect.Dis. Med.Microbiol.,10.1155/2016/9041636
12. LOWRY,O.H.,Rosebrough,N.J.,FARR,A.L.,and RANDALL,R.J.(1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem.10.1016/0922-338X(96)89160-4.
13. Lovrien, R., and Matulis, D. (2005) Assays for Total Protein. In Current Protocols in Microbiology, 10.1002/9780471729259.
14. Ogai, K., Nagase, S., Mukai, K., Iuchi, T., Mori, Y., Matsue, M., Sugitani, K.,Sugama, J., and Okamoto, S. (2018) A comparison of techniques for collecting skinmicrobiome samples: Swabbing versus tape-stripping. Front. Microbiol,10.3389/fmicb.2018.02362
15. Centers for Disease Control and Prevention (CDC) (2001). Methicillin-resistant Staphylococcus aureus skin or soft tissue infections in a state prison--Mississippi, 2000. MMWR. Morbidity and mortality weekly report, 50(42), 919–922.
16. Wingfield, P. T. (2016) Protein precipitation using ammonium sulfate. Curr.Protoc.Protein Sci.,10.1002/047114086

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