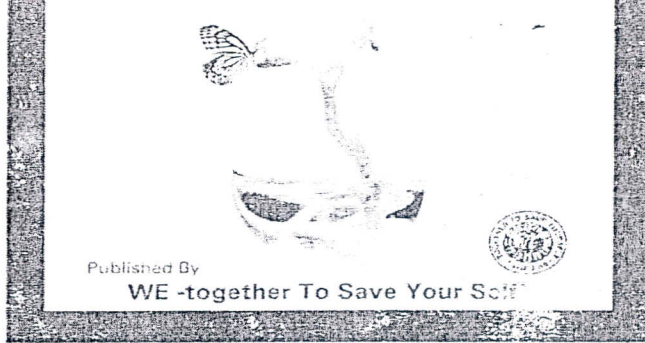
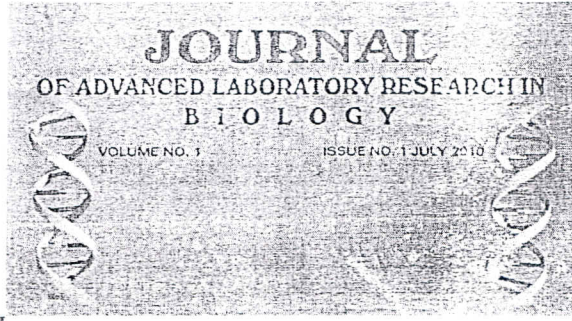


ISSN 0976-7614



JOURNAL OF ADVANCED LABORATORY RESEARCH IN BIOLOGY

ISSN 0976-7614

RESEARCH ARTICLE

IMMUNO-TOXICOLOGICAL STUDIES OF STAPHYLOCOCCIN OF CAMRSA

*J.VIMALIN HENA, **S.S.SUDHA

*Lecturer, Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore, India.
**Head, Department of Microbiology, Dr. N.G.P College of Arts and Science, India.

ABSTRACT

Methicillin resistant *Staphylococcus Aureus* isolated from the noses of school going children (C.AMRSA) has been found to produce bacteriocin like inhibitory substance tentatively termed Staphylococcin. It has a broad activity spectrum against many Gram Positive Bacteria including *Staphylococcus Aureus* which suggests that the protein molecule can be used as an alternative for antibiotics. In this regard, the Bacteriocin was studied for its immunological and toxicological properties in experimental mice. The Antigenicity/Immunogenicity of Bacteriocin was assayed by Ouchterlony technique. Accordingly, Staphylococcin is antigenic ally poor, in mice immune system and the antibody titre was not found to be significant in Ouchterlony test. The acute and chronic toxic studies do not show any toxic effects in laboratory mice when injected with different doses of Bacteriocin (20, 40, 60 and 80 µg/ml). Hematological studies with Staphylococcin showed a marginal increase in lymphocyte count in the test animal groups. Further, different doses of Staphylococcin showed no significant biochemical changes in the normal profile of mice blood biochemistry.

KEYWORDS: Staphylococcin, Immunogenicity, Hematology, Biochemistry.

INTRODUCTION

Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria. These proteins and peptides are generally active against species closely related to the producing microorganism (Saeed, 2004).

There are a large and increasing number of therapeutic proteins approved for clinical use and many more are undergoing preclinical studies and clinical trials in humans. Virtually, all therapeutic proteins elicit some level of antibody response which in some cases can lead to potentially serious side effects. Therefore, Immuno-genicity of therapeutic proteins is a concern for clinicians, manufacturers and regulatory agencies. In order to assess Immuno-genicity of these molecules, appropriate detection, quantitation and characterization of antibody response are necessary. So in this study, the antibacterial peptide *Staphylococci* was isolated and its immunogenic nature was analyzed by Immuno-diffusion test and its effect on the hematological and biochemical parameters of laboratory mice too was monitored for one month.

Staphylococcus Aureus has long been recognized as an important pathogen in human disease. Due to an increasing number of infections caused by MRSA strain, which are now often multi drug resistant therapy has become problematic so an alternative treatment methodology is the need of the hour where the Bacteriocins prove to be an effective alternatives and nasal carriage of *Staphylococcus Aureus* has been identified as a risk factor for community acquired infection (Creech, 2005). So, the *Staphylococcus Aureus* strains were got from the nares of school going children and their Bacteriocin production was assayed and further the protein molecule was studied by immunological and toxicological tests.

MATERIALS AND METHODS

Microbial Cultures

The Methicillin resistant *Staphylococcus Aureus* were isolated from the anterior nares of the school going children. Swabs were collected aseptically, transported to the laboratory using Peptone Broth as transport media and were plated on Mannitol Salt Agar (MSA) and mannitol fermenting colonies were selected and screened for *Staphylococcus Aureus* by performing various biochemical tests, the screened isolates were subjected to Oxacillin Salt screening and Oxacillin Disc Diffusion. PCR assay was done to confirm their resistant nature towards Methicillin (CLSI, 2006).

Partial Purification of Bacteriocin

The MRSA stains were used for the production of Bacteriocin. It was grown on Brain heart infusion broth at 37°C for 24 hrs. The bacterial cells were removed by centrifugation at 10,000 rpm for 10 Minutes at 4°C. The supernatant was filtered and precipitated by using 80% Ammonium Sulphate and the system was held overnight at 4°C and the precipitate was recovered by centrifugation at 15,000 rpm for 30 Minutes at 4°C and the pellet was dissolved in Phosphate Buffer and is designated as crude Bacteriocin. The antibacterial activity was assayed by Agar Well Diffusion method and it was found to be effective in controlling the growth of MRSA.

Ouchterlony

Plate Ouchterlony was performed in 1% Agarose in Sodium Barbital Buffer (ph 7.0) control wells contained BSA and anti BSA. Test wells, central wells contain first dose of antigen, while slide wells contain pre-immune serum and first, second and third sera, respectively.

Toxic Studies in Rabbits

The animals were randomly divided into 5 groups of 4 animals, each. Group 1, animals served as control and received normal Saline (0.4). Group 2, 3 and 4 animals were treated with intravenous injections of 20, 40, 60 and 80 $\mu\text{g}/\text{kg}$ body weight of the Bacteriocin. Blood samples were drawn from each member of all groups. Toxic manifestations like abnormal motor activity alteration in water or food intake, respiration body temperature, diarrhea and mortality were observed for (1, 2, 6 and 2 hours) for acute toxic and 1 month for chronic toxic studies. Before the first and last treatment, blood samples were collected from each animal in syringes containing Sodium Citrate as Anticoagulant and Hematological and Biochemical tests were performed at the beginning and end of experiment (Schellens, 2002).

S.NO	GROUP	DOSAGE	BLOOD GLUCOSE	TOTAL LIPID	TOTAL PROTEIN	A/G RATIO
1.	Control	0.4 μl	86 mg mL^{-1}	180	6.0	3.6
2.	Group 1	20 μl	126 mg mL^{-1}	180	6.5	3.6
3.	Group 2	40 μl	130 mg mL^{-1}	162	6.0	2.8
4.	Group 3	60 μl	135 mg mL^{-1}	172	6.8	2.5
5.	Group 4	80 μl	125 mg mL^{-1}	250	6.1	2.4

Table 1: Blood biochemistry of mice.

S.NO	GROUP	DOSAGE	HEMOGLOBIN	ESR	LEUKOCYTE CC/UNT	ERYTHROCYTE COUNT	WBC
1.	Control	0.4 μl	10.7	5.04	32	2	7.0
2.	Group 1	20 μl	11.7	5.01	34	4	8.8
3.	Group 2	40 μl	13.0	5.70	50	1	5.5
4.	Group 3	60 μl	11.0	4.70	65	5	6.7
5.	Group 4	80 μl	10.0	4.20	62	8	6.6

Table 2: Haematological parameters in mice.

RESULT AND DISCUSSION

Staphylococcin produced by CAMRSA was checked for its antibacterial activity and was found to be strongly active from zone of inhibition produced.

Animal models were used to predict the Immunogenic and Toxigenic nature of the protein for humans reliably. In non human primate studies, variety of responses is noted including from little or no antibody response. Ouchterlony test of the Bacteriocin was performed to check its antigenicity where by no lines of precipitation were observed but when it reacted with mice Anti-sera taken after immunization with different doses of Bacteriocin. However, normal precipitation was seen in control but further more sensitive ELISA test has to be performed to exactly analyze the grade of Antigenicity and Immunogenicity (Bussiere, 2003).

For the toxicological studies of Bacteriocin when mice was injected with varying concentration no abnormal effects (i.e.) abnormal motor activity Sedation, Hyphosis, changes in respiration and body weight was found in both controls and test groups. Food and water intake was also found to be normal. The Biochemical Parameters & Hematological Parameters too were not affected (table: 1 and 2) which shows that the Bacteriocin has no adverse side effects on mice Blood Bio-chemistry. Hence, this study demonstrates the evidence of Toxicological; Immunological and Biochemical studies of *Staphylococcal*

Bacteriocin (*Staphylococcin*) concluded *Staphylococcin* did not show any toxicological effects. On that ground it seems plausible that the Hypothetical *Staphylococcin* could act as potential reservoir of therapeutic interventions.

REFERENCES

- 1) Clinical laboratory standards institute, (2006): Performance standards for Antimicrobial Susceptibility testing; 16th Informational Supplement. M100-S16 Wayne, Pa.
- 2) Creech, C. B, Kernolde, D.S., Alsentzer, A., *et al.*, (2005): Increasing rates of nasal carriage of Methicillin-resistant *Staphylococcus Aureus* in healthy children. *Pediatr. Infect. Dis. J.*, 24, 617-621.
- 3) Saeed, S., S. Ahmad and S.A. Rasool, 2004. Antimicrobial Spectrum, production and mode of action of *Staphylococcin* 188 produced by *Staphylococcus Aureus* 188. *Pak. J. Pharmac. Sci.*, 17:1-8.
- 4) Schellekens, H., 2002. Immunogenicity of Therapeutic Proteins: Clinical implications and future prospects. *Clin Ther.*, 24; 1720-1740.
- 5) Bussiere, J, L., 2003. Animal models as indicators of Immunogenicity of Therapeutic Proteins in Humans. *Dev. Boil.*, 112:135.

*Corresponding Author: Dr. J.Vimalin Hena, Lecturer, Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore, India