



## Immunotoxicological studies of Staphylococcin of CA-MRSA

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**Abstract:** Methicillin-resistant *Staphylococcus aureus* isolated from the nares of school going children (CA-MRSA) 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 the mice immune system and the antibody titre was not found to be significant in the ouchterlony technique. 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 80µg/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.

### 1. 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 much 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, Immunogenicity of therapeutic proteins is a concern for clinicians, manufacturers and regulatory agencies. In order to assess immunogenicity of these molecules, appropriate detection quantitation and characterization of antibody response are necessary. So in this study, the antibacterial peptide staphylococcin was isolated and its

immunogenic nature was analyzed by immunodiffusion test and its effect on the hematological and biochemical parameters on 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 multidrug 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.

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## 2. Materials and Methods

### 2.1 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).

### 2.2 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 the agar well diffusion method and it was found to be effective in controlling the growth of MRSA.

### 2.3 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.

### 2.4 Toxic Studies in Rabbits

The animals were randomly divided into 5 groups of 4 animals, each. Group 1, animals served as controls and received normal saline (0.4). Group 2, 3 and 4 animals were treated with intravenous injections of 20, 40, 60 and 80µg/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 toxicity and 1 month for chronic toxicity 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 the end of the experiment (Schellens, 2002).

## 3. Results and Discussion

Staphylococcin produced by CA-MRSA was checked for its antibacterial activity and was found to be strongly active from the zone of inhibition produced. Animal models were used to predict the Immunogenic and toxigenic nature of the protein for humans reliably. In nonhuman primate studies, a variety of responses are noted including from little or no antibody response. Ouchterlony test of the bacteriocin was performed to check its antigenicity were no lines of precipitation were observed but when it reacted with mice antisera 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 were 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 biochemistry. 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 reservoirs of therapeutic interventions.

Table 1. Blood biochemistry of mice.

| S. No. | Group   | Dosage | Blood Glucose           | Total Lipid | Total Protein | A/G Ratio |
|--------|---------|--------|-------------------------|-------------|---------------|-----------|
| 1.     | Control | 0.4µl  | 86 mg mL <sup>-1</sup>  | 180         | 6.0           | 3.6       |
| 2.     | Group 1 | 20µl   | 126 mg mL <sup>-1</sup> | 180         | 6.5           | 3.6       |
| 3.     | Group 2 | 40µl   | 130 mg mL <sup>-1</sup> | 162         | 6.0           | 2.8       |
| 4.     | Group 3 | 60µl   | 135 mg mL <sup>-1</sup> | 172         | 6.8           | 2.5       |
| 5.     | Group 4 | 80µl   | 125 mg mL <sup>-1</sup> | 250         | 6.1           | 2.4       |

Table 2. Haematological parameters in mice.

| S. No. | Group   | Dosage | Hemoglobin | ESR  | Leukocyte Count | Erythrocyte Count | WBC |
|--------|---------|--------|------------|------|-----------------|-------------------|-----|
| 1.     | Control | 0.4µl  | 10.7       | 5.04 | 32              | 2                 | 7.0 |
| 2.     | Group 1 | 20µl   | 11.7       | 5.01 | 34              | 4                 | 8.8 |
| 3.     | Group 2 | 40µl   | 13.0       | 5.70 | 50              | 1                 | 5.5 |
| 4.     | Group 3 | 60µl   | 11.0       | 4.70 | 65              | 5                 | 6.7 |
| 5.     | Group 4 | 80µl   | 10.0       | 4.20 | 62              | 8                 | 6.6 |

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