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Staphylococcus aureus) and its treatment regimen**

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Studies on community acquired MRSA (Methicillin resistant *Staphylococcus aureus*) and its treatment regimen

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Abstract

Community acquired Methicillin resistant *Staphylococcus aureus* (CA-MRSA) is a strain of *Staphylococcus aureus* that is resistant to a large group of β -lactam antibiotics and are most commonly isolated in a community and spreads as a silent killer pathogen. In this study the spread of MRSA among the school going children community was analysed and to prevent its spread an herbal handky was designed using the hot aqueous neem extract and as a novel treatment regimen the antibacterial protein (Bacteriocins) of *Staphylococcus aureus* was characterized immunologically and haematologically and is proven that at lower concentrations it can be used as a magic bullet molecule in the treatment of multidrug resistant superbugs.

Staphylococcus aureus also known as golden staph frequently living on the skin or nose of a person and causes a wide range of illness ranging from minor skin to major respiratory diseases which is now spreading as a silent killer pathogen among various communities and they are also found to be multidrug resistant¹ which is an alarm to the society to search for a novel preventive and treatment methodology. In this regard the school going children community was selected and the prevalence of CA-MRSA among the age group of 5-10 years of both sexes was statistically recorded and the strains were isolated, confirmed and were let through molecular profiling studies. As a preventive measure for

the spread of this deadly pathogen an herbal handkerchief was designed using the aqueous and methanol extract of neem and the handky's antibacterial activity too was assayed based on ATCC standard¹ so that it can be provided to school going children to prevent its spread among that community. A treatment method too was sought by exploiting the staphylococcal (Bacteriocin – antimicrobial peptide) producing nature of the MRSA strains which can be used as a novel magic bullet against the same species. The strain producing bacteriocin was identified, the bacteriocin molecule was extracted and its antibacterial nature, physiochemical property, molecular weight, Immuno-toxicological property was studied in detail and

in all these studies promising results were got which shows that the isolated protein can be used to design a drug which can be used to treat CA-MRSA.

Sample :

Thousand nasal swabs were collected from the anterior noses of school going children from various schools in and around Coimbatore. The age group was from 5-10 years and were of both sexes.

Sample processing and isolation of Staphylococcus aureus :

The swabs collected were transported to the laboratory in sterile peptone broth and it was incubated for one hour at 37°C. The broth was then used to inoculate mannitol salt agar plates after 24 hours of incubation the yellow colour mannitol fermenting colonies were selected and subcultured on to nutrient agar slants and these cultures were used for further screening².

Identification and confirmation of CA-MRSA :

The isolates were confirmed as *Staphylococcus aureus* based on standard biochemical test according to Bergy's manual of systemic bacteriology.

The confirmed *Staphylococcus aureus* were identified as methicillin resistant strains based on the oxacillin salts screening and oxacillin disc diffusion assay⁷. Molecular level confirmation of MRSA was done by detecting the *mecA* gene by PCR. Among the *MecA* gene positive MRSA strains; the community

acquired MRSA were screened by the molecular profiling of the PVL toxin gene⁸.

Prevention of CA- MRSA :

Herbal handky :

The medicinal herb neem was selected and the plant was got from TNAU, Coimbatore. The leaves of the plant were plucked and dried in shade and powdered. The powder was packed in the Soxhlet apparatus and the methanolic acid aqueous extracts were got, these extracts were tested for their antibacterial activity by agar well diffusion assay³ and the extract was coated on to cotton woven fabric by pad dry cure, direct application and by micro encapsulation methods¹² extract coated fabric too was assayed for its antibacterial activity by parallel streak method and agar diffusion method based on ATCC standards (ATCC Manual)¹.

Treatment of CA-MRSA :

Magic bullet :

For the treatment of CAMRSA, the bacteriocin producing nature of the isolates were identified by spot and lawn assay and the protein molecule staphylococcin (Bacteriocin) was precipitated by Ammonium Sulphate precipitation method and was purified by dialysis and by column chromatography. The purified protein molecular weight was analysed by SDS - PAGE and the protein's antibacterial activity was confirmed by agar well diffusion and agar disk diffusion methods⁹. To use the profile as a drug molecule, its toxicity and immunological nature was analysed by animal studies which was done on albino mice and it was found that the protein molecule was non

Table-1. Sample size

S.No.	Age group	Sex		MRSA strains		CAMRSA strains	
		Male	Female	Male	Female	Male	Female
1	5 – 6	300	170	222	83	150	20
2.	6 – 7	175	80	98	43	52	12
3	7 – 8	150	65	63	23	33	12
4	8 – 9	25	14	8	2	2	0
5	9- 10	2	12	5	3	1	0

Table-2. Antibacterial activity of *Neem*

S.No.	Test organism	<i>Neem</i> extract	
		Methanolic	Aqueous
1.	Community acquired MRSA	+	-

+ -> Zone of inhibition seen
 - -> No Zone of inhibition

Table-3. Antibacterial activity of neem coated fabric

S.No.	Test organism	Activity spectrum	
		Agar Diffusion	Parallel streak
1.	Community acquired MRSA	+	-

+ -> Zone of inhibition seen
 - -> No Zone of inhibition

Table-4. Activity spectrum of staphylococci

S.No.	Test organism	Activity spectrum	
		Agar well Diffusion	Agar disk Diffusion
1.	Community acquired MRSA	+	+

toxic at lower concentration¹⁰.

Table-1 shows the total number of samples collected from the different age group of children of both sexes and the number of methicillin resistant strains among the isolates and finally the number of community acquired methicillin resistant strains which were also

resistant to methicillin.

Table-2 shows the antimicrobial activity of methanolic extract of neem by agar well diffusion method and in table three is antimicrobial activity of neem coated fabric.

Table-4 shows the activity spectrum

of the staphylococcin (Bacteriocin) of the isolated 282 strains of community acquired methicillin resistant strains and only 132 strains produced bacteriocin and had antibacterial activity against community acquired methicillin resistant *Staphylococcus aureus*.

The staphylococcin (Bacteriocin) activity was similar to the reports of Hsu and wiseman on *Staphylococci*.⁵ The bacteriocin activity was also found to be similar to be works of Jetton and Vogels⁶.

The molecular weight of the bacteriocin was 10 dalton which is quite smaller when compared to the earlier reported molecular weight of staphylococcin¹¹. Albino mice was used as the animal model to predict the immunogenic and toxigenic nature of bacteriocin for human use. Ouchterlony test of the bacteriocin was performed to check its antigenicity where by no lines of precipitation was observed but it reacted with mice antisera taken after immunization with different dozes of bacteriocin. For the toxological studies of bacteriocin when mice was injected with varying concentration no abnormal effects i.e. abnormal motor activity, sedation, hypnosis, changes in respiration, body weight was found in both controls and test groups. The biochemical parameter and the hematological parameter too were not affected which shows that the hypothetical staphylococcin could act as an potential reservoir of therapeutic interventions.

References :

1. ATTCC (1981). Manual test method 124,

- Antibacterial finishes on fabrics, North Carolina.
2. Creech C.B. Kernol D.S. Alsentaer A *et al.*, (2005). *Pediatr infect disease J*; 24: 614-621.
 3. Ekpendu to, Akshomejee A, Okogunje (1994). Antiinflammatory Antimicrobial activity. *Lett Appl microbial* 30: 379 – 384
 4. Foster. T. (1996). *Staphylococcus* Barron's medical microbiology (Barron Setal eds) 4th ed, university of Texas, medical Branch ISBN0-963172-1-1.
 5. HSU Cy and G.M. Wiseman (1967). *Can. J. microbial* 947-955.
 6. Jetton A.M. and G. D. Vogels (1972). *J. Bacteriol*, 112 : 243-250.
 7. Kloos, W.E. and P.B. Smith (1980). Aerobic bacterial *Staphylococci* in Lunette, E.H Baloo, A Hausler W.J and Truant, J.P. (eds) manual of clinical microbiology ed American society for microbiology Washington DC 83-88.
 8. Langec, Cardoso and Scenczek D., Schuarzs (1999). *Vet Microbial* 67: 127-141.
 9. Patnakar C.V. and L. M. Joshi (1985). *J. Postgrad, Med* 31: 46-57.
 10. Saeed, S., S Ahmed and S.A. Rasool (2004). *Pak. J. Pharmac Sci*, 17: 1-8.
 11. Shal, H.G. and H. Brandis (1981). *J. Gen Microbio* 127: 377-384.
 12. Sousa M. Pinhario. C., Maatos Meo, Matos F.J., Laorda M.I. and Raverio A. (1991). *Constituintes quimicos de plantas medicinas brasilerias, universidade federal*, P. 385-388.