

RESEARCH ARTICLE

MEDICAL MICROBIOLOGY

**ANTIOXIDANT NATURE OF ALLICIN TO CURE METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS.**



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**ABSTRACT**

Methicillin resistant *Staphylococcus aureus* (MRSA) continues to be one of the commonest pathogen encountered in clinical as well as laboratory practice. It has become a major health problem world wide. Newer antimicrobials/agents are urgently needed to combat this problem of MRSA resistance to various anti-staphylococcal agents. In the back drop of this difficult situation the main biological property of stabilized allixin (Diallyl thiosulfinate) and confirmed activity against MRSA is described. Allixin has been reported to have strong SH-modifying and antioxidant properties. The main antimicrobial effect of allixin may be due to its chemical reaction with thiol groups of various enzymes eg. alcohol dehydrogenase, thioredoxin reductase and RNA polymerase. In this study it was found that allixin is bactericidal against MRSA at concentrations of 130 to 138 $\mu$ g/ml.

## KEY WORDS

MRSA , Allicin , *Allium sativum*.

## INTRODUCTION

*Staphylococcus aureus* is a Gram positive bacterium responsible for severe morbidity and mortality worldwide. It is one of the leading cause of human infections in skin, soft tissues, bones and joints, abscesses and normal heart valves. The organism flourishes in the hospital setting producing bloodstream and surgical wound infections<sup>1,2</sup>. Methicillin was introduced 1959 to treat staphylococcal infections not responding to penicillin therapy. However only within a year some strains of *S. aureus* were reported to be resistant to it. These strains were named as 'Methicillin Resistant *Staphylococcus aureus*' (MRSA). During the past four decades MRSA has spread throughout the world and has become highly endemic in many geographic areas<sup>3</sup>. MRSA infections are difficult to treat because of their resistance to the commonly used anti staphylococcal antibiotics viz macrolides, tetracyclins, aminoglycosides etc. Some of these MRSA strains are resistant to even the most powerful antibiotics such as vancomycin<sup>4</sup>. WHO has been suggesting the need to find some new antibiotics or new approaches to overcome this problem.

In 1944 an Italian chemist, C.J. Cavallito, with his colleague J H Bailey<sup>5</sup> first isolated an unstable, odourous sulphur containing compound from extracts of fresh garlic and demonstrated its antibacterial properties. The substance was named allicin, after the generic name for the plant *Allium sativum*. Researchers Stoll and Seebeck, also working with garlic discovered an odourless sulphur- containing compound called alliin which they fully characterized some years later<sup>6</sup>. This they

found to be converted by a second garlic constituent, an enzyme called allinase, to form allicin. Before the advent of pharmaceutical antibiotic, crushed garlic extracts were used to treat a wide range of infectious disease including dysentery, typhoid, cholera, smallpox and tuberculosis. Then, in the 1920's, the first class of antibiotic drugs were invented, the sulphonamids<sup>7</sup>. The reason they were so successful was the presence of the reactive sulphur group- exactly the same group that allicin contains. Pharmacokinetic studies indicate that allicin will reach a maximum level in the blood after 30-60 minutes and may still be present 72 hours later with more than 85% clearance through urinary and faecal pathways<sup>8</sup>.

In the light of the above pharmacological properties exhibited by allicin, there became a need to investigate its efficacy against MRSA.

## MATERIALS AND METHODS

A total of 99 clinical isolates of MRSA were obtained from Kovai Medical Centre And Hospital. Identification was done on the basis of morphology, cultural characteristics, biochemical reactions and susceptibility to oxacillin discs (one micro gram) using Mueller-hinton agar supplemented with 4% NaCl. Garlic was procured from TNAU, they were freed from dust and crushed in a domestic grinder and then soaked in sterile distilled water for 24 hours at room temperature. They were filtered under UV light using filter paper. The filtrate was collected and stored in refrigerator till use.

MIC of garlic extracted was performed against all test strains.

### **AGAR WELL DIFFUSION TEST**

Mueller hinton agar medium was prepared and poured to petri dishes. The plates were allowed to solidify. The culture was swabbed on the surface of Mueller hinton agar plates using sterile cotton swabs. The well was made by using a well puncture. By using micropipette the garlic extract was added onto the well. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed.

### **TUBE DILLUTION TEST**

A twofold serial dilution, each containing 1 to 2 of Antimicrobial agents was

prepared. A standardized suspension of test bacteria was inoculated to each dilution to obtain a final concentration of  $5 \times 10^5$  CFU/ml. Each dilution was dispensed into Mueller hinton broth separately. The tubes were incubated at 37°C for 24 to 48 hours. If the tubes showed the absence of turbidity, then OD was taken and spread plate was performed from each tube to each plate respectively.

### **RESULT**

MIC range of garlic extract on 99 MRSA strains is shown in table 1. MIC was in between 130- 138 microgram/ml at 130 microgram/ml.

**TABLE 1**  
**MINIMUM INHIBITORY CONCENTRATION OF ALLICIN AGAINST 99 STRAINS OF MRSA.**

MIC( $\mu$ /ml)	STRAINS INHIBITED
130	18
132	32
134	44
136	55
138	64

### **DISCUSSION**

Several case studies are reported to show how stabilised allacin of garlic can be used to resolve existing MRSA infections. In previous work it has been demonstrated that allacin is bactericidal against MRSA at concentration of 128- 256 micro gram/ml<sup>9,10,11</sup>. so our results too coincide with the authors and it is very important to identify the ingredient exactly which is responsible for the bactericidal activity by mass spectro photometric analysis.

### **CONCLUSIONS**

It may be concluded from this study that allacin of garlic which was extracted has antimicrobial activity against MRSA above 130 microgram/ml concentration. It is essential that research should continue to isolate and purify the allacin use in the experimental animals.

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## REFERENCE

1. Haddadin AS, Fappiano SA, Lipsett PA. Methicillin resistant *Staphylococcus aureus* (MRSA) in the intensive care unit Postgrad Med J.2002; 78: 385- 92.
2. Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, Nathwani D. Epidemiology and antibiotic susceptibility of bacteria casing askin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy, Int J Antimicrob Agents.2003; 22: 406-19.
3. Mark CE, Ashley RD, Gaynor R, Edward J, Brian G. The evolutionary history of methicillin- resistant *Staphylococcus aureus* (MRSA). PNAS.2002; 99: 7687-92.
4. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to Vancomycin and other Glycopeptides in *Staphylococcus aureus*. Emerg Infect Dis.2001; 7: 327-32.
5. Cavalitto CJ and Bailey JH Allicin , the Antibacterial principle of *Allium sativum*. Isolation ,Physical Properties and Antibacterial Action ,J.Am.Chem.Soc.Notes and Tips 1944(66)1950-51.
6. Stoll A ,Seebeck E ,Chemical Investigations on Alicin the specific Principle of Garlic, Advances in Enymology 1951(11),377-400.
7. Discoveries of anti – bacterial effects of penicillium moulds before Fleming LachmannG, Lorenz D,Radek W and Steiper M, Studies on the pharmacokinetics of the S35 labelled garlic constituents, allicin and vinylidithiine,s Arzneimittel Forschung 1994 44 (6),734 – 743.
8. Miron T ,Ra binkov A, Mirelman D ,Wilchek M ,Weiner L, The mode of action of allicin:its ready permeability through phospholipid membranes may contribute to its biological activity.Biochim Biophys Acta.2000 Jan 15 ;1463(1):20-30.
9. Cutler, RR, Townsend T , Sweeney D and Mukimbri, E.(1999)Antibacterial activity of garlic extracts against MRSA's and Gram Negative rods.Brit.J.Biomed.Sci.:57:43.
10. Cutler, RR (2002) A Stabilized, Natural, Allicin a Novel Phyto-chemical with the Potential to Combat Diseases Caused by Multiply Antibiotic Resistant *Staphylococcus aureus* (MRSA).Nursing Practice, 4: 64-66.
11. Gottleib RD, Shah MK, Perlman DC, Kimmelman CP , Community-acquired methicillin-resistant *Staphylococcus aureus* infections in otolaryngology.Otolaryngol Head Neck Surg. 1992 Sep;107(3):434-7.