

Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*

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Introduction

Control of the spread of antibiotic-resistant bacteria and the treatment of infections caused by them is a major problem worldwide. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA) presents major infection control problems for patients and hospital staff, as its incidence in Europe has risen from 3% in 1992 to 37% in 1999.¹⁻⁴

Topical agents are important in controlling the carriage and spread of MRSA. Mupirocin (pseudomonic acid), a fermentation product produced by *Pseudomonas fluorescens* (NCIB 10586), is a standard product used to deal with MRSA carriage and to prevent its spread. It has also proved to be an effective treatment for skin infections and plays a crucial role in the control of MRSA outbreaks.

However, resistant strains were described soon after its introduction. Moreover, the increased use of mupirocin, especially for chronic infections, has led to an increased incidence of resistance. In a recent survey from Spain, levels of mupirocin resistance in clinical isolates was reported to have increased from 7.7% in 1998 to 19% in 2000, and some hospitals have reported incidences as high as 63%.² The continuing spread of MRSA and the increase in mupirocin-resistant strains⁵ highlight the need for alternative topical agents

Garlic and its extracts have been used to treat infections for thousands of years.⁶ Allicin (the name being derived from that of the garlic species *Allium sativum*) is considered to be the main biologically active antimicrobial phytochemical produced in garlic extracts, and was first recognised as such in 1944.⁷

Allicin is an oxygenated sulphur compound, formed when garlic cloves are crushed. Alliin is the stable precursor of allicin and is stored in compartments in the plant that separate it from the enzyme alliinase (also called alliin lyase). When crushed, they mix and alliin is converted rapidly to allicin by the action of this enzyme. The antibacterial activity of allicin was reviewed by Ankri and Mirelman in 1999.⁸

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ABSTRACT

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and the community has led to a demand for new agents that could be used to decrease the spread of these bacteria. Topical agents such as mupirocin have been used to reduce nasal carriage and spread and to treat skin infections; however, resistance to mupirocin in MRSA is increasing. Allicin is the main antibacterial agent isolated from garlic, but natural extracts can be unstable. In this study, a new, stable, aqueous extract of allicin (extracted from garlic) is tested on 30 clinical isolates of MRSA that show a range of susceptibilities to mupirocin. Strains were tested using agar diffusion tests, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Diffusion tests showed that allicin liquids produced zone diameters >33 mm when the proposed therapeutic concentration of 500 µg/mL (0.0005% w/v) was used. The selection of this concentration was based on evidence from the MIC, MBC and agar diffusion tests in this study. Of the strains tested, 88% had MICs for allicin liquids of 16 µg/mL, and all strains were inhibited at 32 µg/mL. Furthermore, 88% of clinical isolates had MBCs of 128 µg/mL, and all were killed at 256 µg/mL. Of these strains, 82% showed intermediate or full resistance to mupirocin; however, this study showed that a concentration of 500 µg/mL in an aqueous cream base was required to produce an activity equivalent to 256 µg/mL allicin liquid.

KEY WORDS: Allium.
Drug resistance, microbial.
Garlic.
Methicillin resistance.
Staphylococcus aureus.
Sulfinic acids.

Pure allicin (allyl 2-propenethiosulphinate)⁶ is said to be highly volatile, poorly miscible with water and has the odour of freshly crushed garlic.⁹ In order to produce a stable agent that can be used in topical formulations, an aqueous allicin extract is needed.

In this study, a purified aqueous extract of allicin, isolated from a natural source by a patented cold aqueous extraction method (Allicin International, Half House, Military Road, Rye, East Sussex, UK) is used. This extract is tested against mupirocin-resistant and mupirocin-susceptible strains of MRSA. Two formulations, liquid allicin and liquid allicin mixed in a cream formulation, are tested.

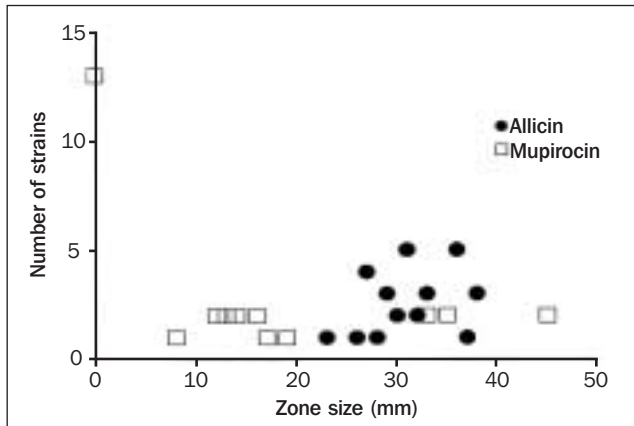


Fig. 1. Number of strains and the size of zones of inhibition with allicin (500 µg/mL) and mupirocin against *Staphylococcus aureus*.

Materials and methods

Bacterial strains

Thirty clinical isolates and one control strain (Oxford Strain, NCTC 6571) of *S. aureus* were tested. All 30 clinical isolates were obtained from the Royal London and St. Bartholomew's Hospitals and had been identified as showing multiple antibiotic resistance. All strains were resistant to methicillin and had no apparent epidemiological connection.

Allicin

A 5000 µg/mL solution of allicin in water was provided by Allicin International. As it was an extracted product, the purity of the allicin solution was tested and the concentration confirmed by Allicin International using the high-performance liquid chromatography (HPLC) method of Lawson, Wang and Hughes.¹⁰

Antimicrobial activity

The allicin liquid and allicin made up in aqueous cream were tested for antimicrobial activity against MRSA using an agar well diffusion method. Muller-Hinton agar plates were inoculated using the methods recommended by the British Society for Antimicrobial Chemotherapy (BSAC).¹¹

Circular wells (6 mm) were cut in the agar culture media and filled with 100 µL cream or liquid. The accuracy of the method used to deliver these volumes was confirmed by weighing the agar plates before and after the fluid and cream were added.

Mupirocin activity against the same MRSA strains was determined using a 5 µg paper disk (Oxoid, UK) on Muller-Hinton agar plates.

Minimum inhibitory and bactericidal concentrations of allicin liquid

Standard methods based on those of Lorian¹² were used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Strains were cultured overnight at 37°C in Muller-Hinton broth (Oxoid, CM 405). Concentrations of allicin between 1–1000 µg/mL and broth containing no antimicrobial agent (negative control) were assessed. Creams were not tested by this method because the opacity they produced in the liquid made determination of MIC impossible.

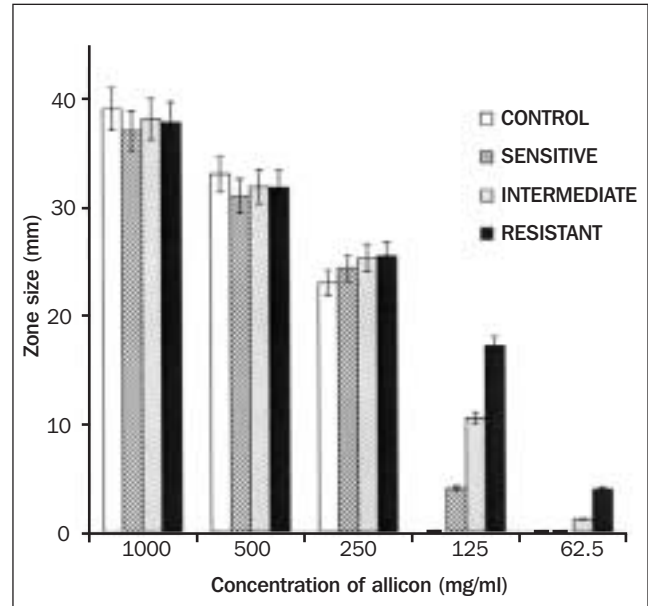


Fig. 2. Average diameter of zones of inhibition for allicin liquid against *Staphylococcus aureus* strains that showed resistance, intermediate resistance or sensitivity to mupirocin.

The bacterial inoculum used was approximately Log 6 colony-forming units (cfu)/mL. Cultures were incubated overnight at 37°C and examined for growth the following day. Subcultures of these were used to determine MBC.¹²

Results

Mupirocin activity

The control strain produced a 35-mm diameter zone of inhibition to mupirocin. Of the clinical isolates, five strains were identified as fully susceptible (zone sizes 33–45 mm), 12 showed intermediate susceptibility (zone sizes 12–23 mm) and 13 strains were resistant (no zone of inhibition). Variability of zone size was considerable, ranging from 0–45 mm. The most frequent result (13 strains) showed no zone of inhibition. The second most common group (11 strains) produced intermediate zone sizes (8–19 mm). Two strains were highly susceptible, producing zone sizes of 45 mm. (Fig. 1).

Allicin liquid activity

Allicin liquid was active against *S. aureus* strains down to 62.5 µg/mL (equal to 6.25 µg of allicin in 100 µL [the volume added to each well]). No activity was detected below 62.5 µg/mL, and concentrations of 250 µg/mL and above were the most active.

The average zone sizes produced against these strains were 25 mm at 250 µg/mL, 32 mm at 500 µg/mL and 38 mm at 1000 µg/mL. The spread of zone size to allicin was smaller than that found with mupirocin (Fig. 1). Zone sizes ranged from 23–38 mm at 500 µg/mL, and from 31–44 mm at 1000 µg/mL (Figs. 2 and 3).

Allicin cream activity

The control strain produced a 20 (±1.5) mm zone of inhibition compared to 23 (±1.5) mm for mupirocin-susceptible MRSA

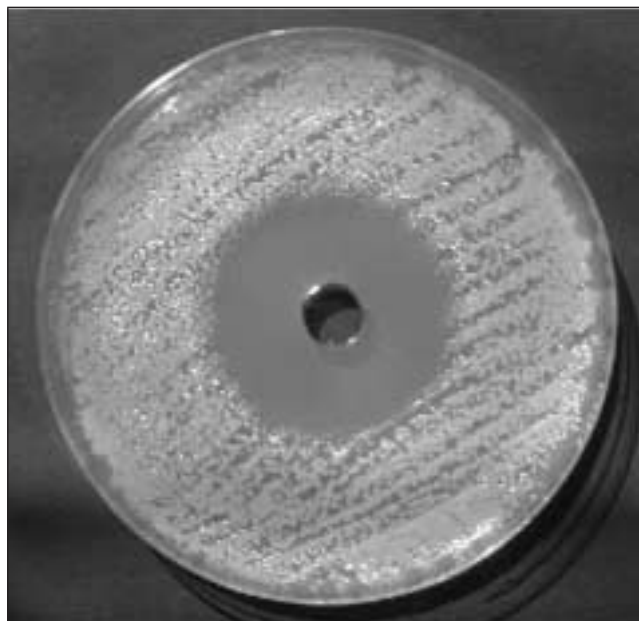


Fig. 3. A typical zone of inhibition produced by 500 µg/mL alliin against MRSA.

and 21 (±1.5) mm for mupirocin-resistant MRSA. When prepared in Boots' aqueous cream, alliin was found to be highly active against all strains at a concentration of 500 µg/mL. No activity was detected below 125µg/mL (Fig. 4).

Comparison of alliin activity in cream and liquid

The average zone size for 250 µg/mL alliin liquid was 25 (±1) mm. This was almost identical to the average zone size of 23.8 (±2) mm obtained with 500 µg/mL alliin in aqueous cream.

Minimum inhibitory and bactericidal concentrations

The control strain tested (Oxford *S. aureus*) gave an MIC of 32 µg/mL and an MBC of 256 µg/mL. The MICs for the clinical isolates tested were either 16 or 32 µg/mL, the MBCs were either 128 or 256 µg/mL. The majority of the clinical isolates had MICs of 16 µg/mL and MBCs of 128 µg/mL (Table 1).

Of the clinical isolates, 88% had MICs of 16 µg/mL and MBCs of 128 µg/mL, 18% were susceptible to mupirocin (as indicated by the disk diffusion test), 47% showed intermediate susceptibility and 35% were resistant. All mupirocin-resistant strains had MICs of 16 µg/mL, 23% had MBCs of 128 µg/mL and 12% had MBCs of 256 µg/mL.

Discussion

In a review of the subject, Schmitz and Jones¹³ considered the choices available in the battle against MRSA and concluded that only vancomycin as a systemic agent and mupirocin as a topical agent offered reliable treatment. They also pointed out that reports of mupirocin resistance had increased since 1990. The emergence of epidemic MRSA resistant to mupirocin has led many authors to suggest that the use of mupirocin should be controlled more strictly, especially as there is a lack of alternative agents. Consequently, alternative agents for topical use against MRSA would prove very popular and be in great demand

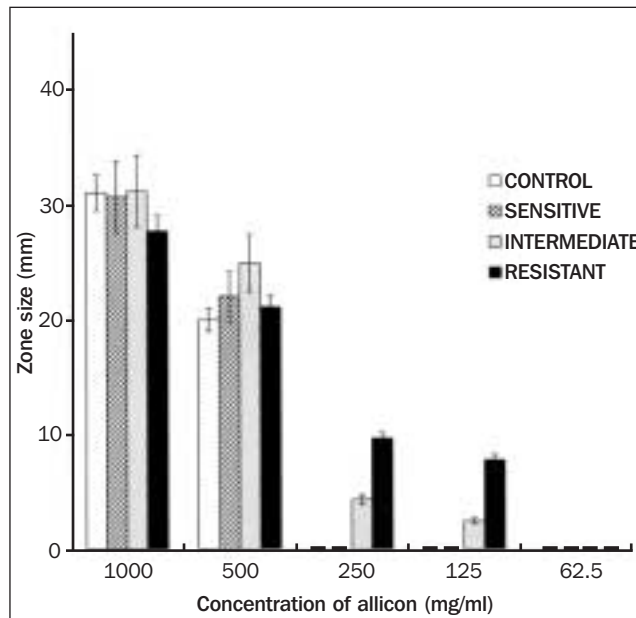


Fig. 4. Average diameter of zones of inhibition for alliin cream against *Staphylococcus aureus* strains that showed resistance, intermediate resistance or sensitivity to mupirocin.

Table 1. Susceptibility of MRSA clinical isolates to a stabilised alliin extract, shown as the percentage of strains with different minimal inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC).

	MIC 16	MIC 32	Totals
MBC 128	76%	12%	88%
MBC 256	12%	0	12%
Totals	88%	12%	100%

Alliin is considered to be the most potent antibacterial agent in crushed garlic extracts, but it can be unstable, breaking down within 16 h at 23°C. However, the use of a water-based extract of alliin stabilises the alliin molecule. This may be due to two factors: the hydrogen bonding of water to the reactive oxygen atom in alliin can reduce its instability; and/or there may be water-soluble components in crushed garlic that destabilise the molecule.¹⁴

The disadvantage to this approach is that alliin can react with water to form diallyl disulphide,^{15,16} which does not exhibit the same level of antibacterial activity as does alliin. In 1991, Hughes and Lawson¹⁷ reported tests on a single strain of *S. aureus* and noted that pure alliin had an MIC of 27 µg/mL compared with 900 µg/mL for diallyl disulphide.

In the present study, 88% of strains had MICs for alliin of 16 µg/mL, and all strains were inhibited by alliin at 32 µg/mL. This compares well with Lawson's results with purified alliin on a single isolate.

There is always concern that antibiotic resistance to new agents may develop rapidly when they have similar modes of action to established agents, and can be a particular problem with topical agents. This, however, is not the case with alliin and mupirocin. The S(=O)S thiosulphinate group in alliin is thought to react with a variety of SH-containing enzymes within the bacterial cell, and alliin has

been reported to have a range of potential targets. It is reported to inhibit the acetyl CoA forming system, to inhibit DNA and protein synthesis, and to target RNA polymerase;¹⁸⁻²⁰ and these are responsible for the agents antibacterial effect.

More general proposals about the broad-spectrum activity of allicin were provided by Rabinkov *et al.* in 1998. This group compared the importance of its antioxidant properties with its thiol disulphide exchange activity and suggested that activity is related to allicin's rapid reaction with thiol-containing proteins. In contrast, mupirocin (pseudomonic acid) inhibits protein synthesis by slowing the activity of RNA synthetase.¹³

For a topical agent to produce maximum benefit, its strength in any formulation should not only be sufficient to inhibit growth but also to be bactericidal. The present study demonstrated that the majority (88%) of strains had MBCs for allicin of 128 µg/mL and all the strains were killed by allicin at 256 µg/mL.

Allicin liquid extracts were highly active against clinical isolates of multiple antibiotic resistant *S. aureus*, including those strains that were identified as mupirocin-resistant. Although agar diffusion tests showed that the activity of allicin was reduced in the cream formulation, comparison of zone sizes achieved with 250 µg/mL in liquid showed similarity to those achieved with 500 µg/mL in cream (average zone sizes of 25 mm and 23.7 mm, respectively).

When made up in an aqueous cream base, 500 µg/mL allicin produced bactericidal levels high enough to support the testing of allicin cream as a topical agent against *S. aureus*, including MRSA.

In conclusion, the present study demonstrated that liquid and cream formulations containing allicin are active against *S. aureus*, including MRSA strains, showing both high and low levels of resistance to mupirocin. The aqueous cream formulation showed reduced activity compared with allicin in water. At 500 µg/mL, however, the cream was active against all the organisms tested, suggesting that this therapeutic concentration compares well with the 20,000 µg/mL mupirocin currently used for topical application.²¹ □

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