

## *In vitro* activity of an aqueous allicin extract and a novel allicin topical gel formulation against Lancefield group B streptococci

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**Background:** Studies have shown the efficacy of intra-partum antibiotics in preventing early-onset group B streptococcal sepsis. This approach results in a high intra-partum antibiotic use. Worryingly, the same antibiotics used in prophylaxis are also first-line treatment for neonatal sepsis, and antibiotic exposure in the peri-natal period has been shown to be a risk factor for late-onset serious bacterial infections and allergic disease. Antibiotic exposure in the peri-natal period is becoming a major public health issue; alternative strategies are needed. Garlic has been traditionally used to treat vaginal infections. Allicin is the main antibacterial agent isolated from garlic.

**Objectives:** The aim of the study was to investigate the *in vitro* activity of a novel allicin extract in aqueous and gel formulation against 76 clinical isolates of Lancefield group B streptococci (GBS).

**Methods:** MICs and MBCs of allicin were determined for 76 GBS isolates by agar dilution and microtitre plate methods. Killing kinetics were determined for a selected 16 of the 76 strains. Agar diffusion tests were compared for allicin liquid and gel (500 mg/L).

**Results and conclusions:** MICs and MBCs of allicin liquid were 35 to 95 mg/L and 75 to 315 mg/L, respectively. Time/dose kill curves produced a 2–3 log reduction in cfu/mL within 3 h and no detectable growth at 8 and 24 h. A novel 500 mg/L allicin gel produced an average zone size of  $23 \pm 6$  mm compared with  $21 \pm 6$  mm for allicin in water. Aqueous allicin is bactericidal against GBS isolates and maintains activity in a novel gel formulation.

Keywords: EOGBS, neonatal infections, garlic

### Introduction

Lancefield group B streptococci (GBS) colonize the human gastrointestinal and genital tracts and can cause a wide range of infections in neonates, pregnant women and non-pregnant adults. GBS sepsis is an important cause of maternal and neonatal morbidity and mortality. Before delivery, GBS can be transmitted to the fetus, resulting in intra-amniotic infection and stillbirth; it is also one of several bacteria known to enhance the risk of pre-term rupture of membranes in pregnant women.<sup>1</sup>

Cumulative carriage rates in pregnant women may be as high as 50% depending on the sensitivity of the detection method used.<sup>2</sup>

Various preventive strategies to reduce the incidence of early-onset group B streptococcal sepsis (EOGBS) have been proposed: intra-partum antibiotic prophylaxis; post-natal prophylaxis for newborn babies; vaginal disinfectants or vaccines. Garlic cloves have been used as a traditional treatment for vaginal yeast infections for many years. More recently, certain midwives have been recommending their use for treating *Candida* and GBS infections.<sup>3</sup> However, crude garlic extract can be irritating to tissue. Allicin, the main antimicrobial agent isolated from garlic, is formed when the garlic clove is crushed and the enzyme alliin lyase comes into contact with the substrate alliin.<sup>4</sup>

In this study, a purified extract of allicin, in water or in a novel gel formulation, is tested against clinical isolates of GBS.

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## Materials and methods

### Bacterial strains

Seventy-six non-duplicate clinical isolates of GBS, from vaginal swabs from Queen Elizabeth Hospital, Woolwich, were tested. Swabs were streaked on blood agar plates within 24 h of the samples being taken and plates incubated overnight at 37°C. Strain identification was based on colony appearance and  $\beta$ -haemolysis, followed by latex agglutination for Lancefield grouping (Prolex, Pro-Lab Diagnostics, UK).

### Aqueous extract of allicin (AEAllicin) and gel formulation

A 5000 mg/L solution of novel stabilized AEAllicin was provided by Allicin International. Purity and concentration were determined by HPLC. An allicin gel mixture was created from this by mixing AEAllicin (5000 mg/L) with a commercially available gel containing water, glycerine, *Aloe barbadensis*, dehydroxanthan gum, d- $\alpha$ -tocopherol, sodium ascorbyl phosphate, polysorbate 20, sodium hydroxymethylglycinate and geraniol (Faith Products Ltd, UK).

### MICs and MBCs of AEAllicin liquid and growth curves

Agar dilution methods were used to determine MICs for all 76 strains.<sup>4</sup>

Based on their MIC results, 16 strains were selected for further testing. MICs and MBCs were determined for these strains using a microtitre plate, liquid culture system. Growth in the presence of a range of concentrations of AEAllicin between 2500 and 2.5 mg/L and growth in Iso-Sensitest broth containing no antimicrobial agent (negative control) were assessed by spectroscopy using an ELx808™ Absorbance Microplate Reader (Bio Tek Ltd, USA) and a 490 nm filter to measure absorbance.

MIC was the concentration where no growth was detected by absorbance. Subcultures of these microtitre cultures were used to determine MBCs.<sup>4</sup>

### Time and dose kill curves

The 16 strains selected for MBC determinations were also used in killing curve experiments. Strains were incubated at 37°C in Iso-Sensitest broth in the presence of concentrations of AEAllicin equivalent to 0 (control), MBC, 2× MBC, 4× MBC and 8× MBC. Rates of killing were determined in duplicate by determining the bacterial counts (cfu/mL) in each culture hourly from 0 h up to 8 h and then at 24 h. The minimum detection level was 100 cfu/mL.

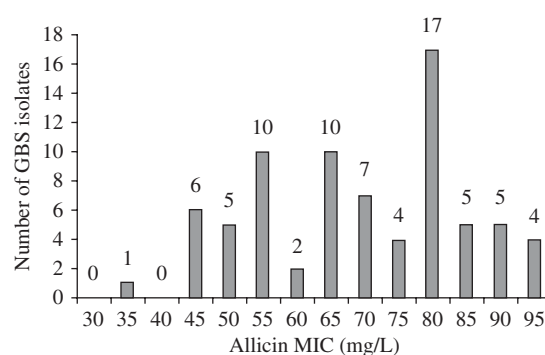
### Comparing the antimicrobial activity of AEAllicin liquid and gel

The antimicrobial activity of 500 mg/L solutions of AEAllicin liquid and AEAllicin gel was compared for the 76 strains using an agar diffusion method described previously with 6 mm wells cut in the agar and filled with 100  $\mu$ L of allicin fluid.<sup>4</sup>

## Results

### Susceptibility of strains to AEAllicin

Figure 1 shows that the MICs of AEAllicin for the 76 test strains varied from 35 to 95 mg/L. The most common MIC was



**Figure 1.** Numbers of *Streptococcus agalactiae* (GBS) strains with MICs between 30 and 95 mg/L.

80 mg/L; 22% of strains had an MIC of 80 mg/L. The majority of strains (66%) had MICs between 55 and 80 mg/L.

### Minimum bactericidal activity of AEAllicin

Sixteen strains were selected from the groups of strains with different MICs shown in Figure 1. These 16 strains were used for MBC and killing curve evaluations for AEAllicin.

Overall, the MBCs of allicin ranged from 1 to 5× the MIC. The MBCs ranged from 75 to 315 mg/L compared with the MIC range of 35 to 95 mg/L. The strains having the lowest MICs did not necessarily produce the lowest MBCs. The average MBC was twice the MIC; the mode MBC was 155 mg/L.

### Time and dose killing curves

Concentrations equal to the average MBC level were compared. For the test group, the average MIC was 70 mg/L, and the MBC was 155 mg/L. Figure 2 demonstrates that by 8 h there was a >6 log reduction in bacterial load in all cases compared with the growth control and there was no detectable growth (detection limit 100 cfu/mL) after 8 or 24 h of treatment.

### Susceptibility to AEAllicin gel formulation

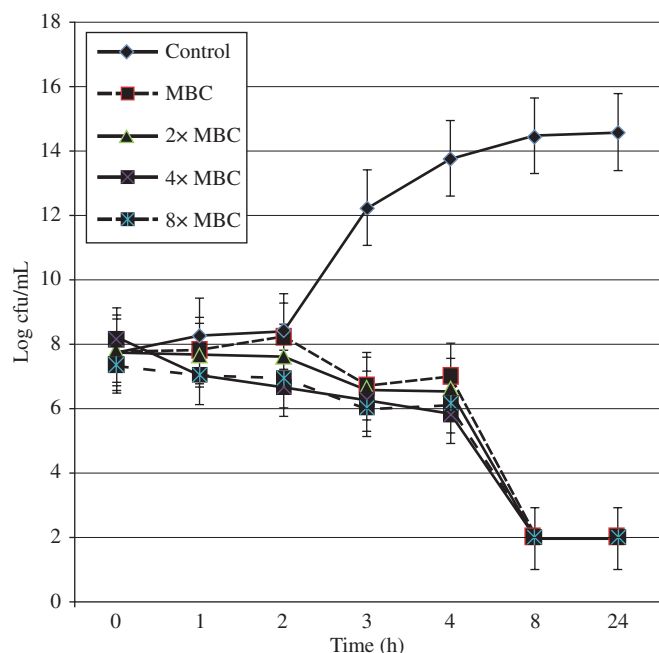
For the 76 isolates tested, the average zone size for the gel containing 500 mg/L AEAllicin was  $23 \pm 6$  mm; this was slightly greater than the equivalent activity for AEAllicin liquid in water ( $21 \pm 6$  mm). It was not possible to do microtitre-based spectrophotometric assays with the gel mixture.

## Discussion

The main finding of this study is that allicin extract, either in water or in a gel, is active against clinical isolates of GBS. Previously we have found that the incorporation of allicin extract into a cream such as Boots aqueous cream reduced the activity of the agent,<sup>4</sup> but this is not the case with the novel gel formulation.

Preventive strategies based on the use of intra-partum antibiotics have led to a significant decline in the incidence of early-onset neonatal GBS sepsis since their introduction in the USA and Europe. With this approach, however, it has been estimated that between 25% and 30% of women giving birth in

## Activity of allicin against GBS



**Figure 2.** Average killing curves determined from 16 strains of *Streptococcus agalactiae*, GBS, treated with an aqueous extract of allicin at concentrations from 1 to 8× the MBC for each strain. Killing curves are compared with the growth of a control in antibiotic-free medium (Control). The lowest detection limit was 100 cfu/mL. Each strain was tested in duplicate. The error bars represent the standard errors.

some countries will receive antibiotics. Not surprisingly, antibiotic resistance rates have also increased, not only in GBS but also in other organisms causing sepsis in newborns. Ampicillin and penicillin are used for intra-partum prophylaxis; worryingly, they are also first-line agents for the treatment of neonatal sepsis.

### *New strategies are needed: are topical agents a solution?*

Chlorhexidine is a topical agent whose potential role in reducing carriage and preventing neonatal infection has been studied, with mixed results.<sup>5</sup> Other agents, like intra-vaginal clindamycin, have also been investigated, but fulminant pseudomembranous colitis caused by clindamycin phosphate vaginal cream has been described.<sup>6</sup>

Garlic bulbs have been used historically for the treatment of vaginal infections, including thrush.<sup>7</sup> The problem with using this natural system for the delivery of allicin is the lack of control over the dose given and the possibility of mucosal irritation due to release of breakdown products that occur in the presence of raw plant material.

Our data show that a purified allicin extract was active against all GBS strains tested, with MICs ranging between 35 and 95 mg/L. These MICs may seem high compared with those of conventional antibiotics, but topical antibiotics can achieve high local levels and such levels in topical preparations are usually acceptable because of the poor systemic absorption. The concentrations reported previously for a topical allicin cream, 500 mg/L, are lower than those commonly used for topical agents such as mupirocin (2%).<sup>4</sup> The zone sizes around GBS for allicin liquid were comparable but smaller than those reported previously for

methicillin-resistant *Staphylococcus aureus* (MRSA) ( $31 \pm 5$  mm), but the zone sizes for the gel were greater than those reported for a cream formulation against MRSA ( $22 \pm 2$  mm),<sup>4</sup> and at 500 mg/L allicin creams and liquids have been successfully used to treat chronic MRSA wounds.<sup>8</sup>

Allicin has been shown to penetrate rapidly through membranes and has a broad mode of action; a rapid reaction with thiol-containing proteins and enzymes.<sup>9</sup>

Vaginal infection is closely linked with premature rupture of membranes, in turn strongly associated with EOGBS. The prevalence of maternal GBS colonization is twice as high in pre-term as in term deliveries.<sup>10</sup> Allicin shows a potent activity against vaginal microbiota, here against GBS and against *Candida albicans*.<sup>3</sup> Strategies based on the administration of antibiotics during labour will have no impact on reducing the rate of premature rupture of membranes. Our aqueous allicin gel could be used in preventive strategies to reduce the risk of premature rupture of membranes and of chorioamnionitis. Further studies will, however, be needed before allicin can be proposed as part of a preventive strategy against EOGBS disease.

To our knowledge, this is the first report that demonstrates that allicin is bactericidal against GBS, and this activity is maintained when it is incorporated into a gel, unlike with previously reported formulations.<sup>4</sup> While a vaccine against GBS is still under investigation, we believe allicin has the potential to become an effective agent in strategies aimed at reducing the risk of EOGBS disease, without jeopardizing our antibiotic arsenal.

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## Transparency declarations

N. J. B. and P. D. J. are both directors of Allicin International Ltd and own shares in the company. All other authors: none to declare.

## References

1. Heath PT, Schuchat A. Perinatal group B streptococcal disease. *Best Pract Res Clin Obstet Gynaecol* 2007; **21**: 411–24.
2. Hansen S, Uldbjerg N, Kilian M *et al.* Dynamics of *Streptococcus agalactiae* colonization in women during and after pregnancy and in their infants. *J Clin Microbiol* 2004; **42**: 83–9.
3. Cohain JS. GBS, pregnancy and garlic: be a part of the solution. *Midwifery Today Int Midwife* 2004; **72**: 24–5.
4. Cutler RR, Wilson P. Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*. *Br J Biomed Sci* 2004; **61**: 71–4.
5. McClure EM, Goldenberg RL, Brandes N *et al.* The use of chlorhexidine to reduce maternal and neonatal mortality and morbidity in low-resource settings. *Int J Gynaecol Obstet* 2007; **97**: 89–94.
6. Trexler MF, Fraser TG, Jones MP. Fulminant pseudomembranous colitis caused by clindamycin phosphate vaginal cream. *Am J Gastroenterol* 1997; **92**: 2112–3.

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7. Carlson KJ, Eisenstat SA, Ziporyn T. *The Harvard Guide to Women's Health*. Cambridge, MA: Harvard University Press, 1996.

8. Cutler RR, Josling PD, Bennett NJ. Treatment of chronic MRSA infections using a novel aqueous extract of allicin (AB1000). *Clin Microbiol Infect* 2005; **11** Suppl 2: 515–6.

9. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes Infect* 1999; **1**: 125–9.

10. Borchardt SM, DeBusscher JH, Tallman PA *et al.* Frequency of antimicrobial resistance among invasive and colonizing group B streptococcal isolates. *BMC Infect Dis* 2006; **6**: 57.