## Modelling Staphylococcus aureus-induced septicemia using NMR

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We present a novel NMR-based study of the molecular aspects of the "attack" on human red blood cells (RBCs) by growing bacteria. *Staphylococcus aureus* expresses virulence factors, including  $\alpha$ -haemolysin, which contribute to the clinical condition known as septic shock (Bhakdi & Tranum-Jensen, 1991).  $\alpha$ -Haemolysin is a pore-forming toxin and its secretion increases the permeability of a range of mammalian cell types infected with *S. aureus* (McEwen & Arion, 1985). <sup>31</sup>P NMR spectra of the probe molecules dimethyl methylphosphonate (DMMP) and hypophosphite (HPA) in RBC suspensions show separate intra- and extracellular resonances (Kirk & Kuchel, 1986). These resonances coalesced over time in RBC suspensions inoculated with *S. aureus* or pure  $\alpha$ -haemolysin, due to increasing permeability of the RBC membrane. Increased RBC permeability resulted in leakage of intracellular proteins, plus an increase in the exchange rates of the solutes between the intra- and extracellular compartments, both effects contributing to the coalescence of the split peaks (Plummer *et al.*, 2007). The addition of antibiotics prevented peak coalescence and enabled the minimal inhibitory concentration (MIC) for eight strains of *S. aureus* to be determined for oxacillin and erythromycin. The MIC values obtained by using <sup>31</sup>P NMR spectroscopy were within one dilution of the MICs obtained using the standard National Committee for Clinical Laboratory Standards (NCCLS) method (Jones, 1986). The results are encouraging for the use of NMR spectroscopy in clinical microbiology.

Bhakdi S & Tranum-Jensen J. (1991) *Microbiological Reveiews*, **55**: 733-51.

McEwen BF & Arion WJ. (1985) Journal of cell Biology, 100: 1922-9.

Kirk K & Kuchel PW. (1986) Journal of Magnetic Resonance, 68: 311-8.

Plummer R, Bodkin J, Yau TW, Power D, Pantarat N, Larkin TJ, Szekely D, Bubb WA, Sorrell TC & Kuchel PW. (2007) *Magnetic Resonance in Medicine*, **58**: 656-65.

Jones RN. (1986) Antimicrobic Newsletter, 3: 1-7.