Title: Antibiotics and *Staphylococcus aureus* – more than meets the MIC.

Author: Benjamin P Howden

Affiliations:

[1]Austin Centre for Infection Research (ACIR), Infections Diseases Department, and Department of Microbiology, Austin Health, Heidelberg, Australia.

[2]Genomics Research Laboratory, Infectious Diseases Service, Geneva University Hospitals, Geneva, Switzerland

[3]Department of Microbiology and Immunology, The University of Melbourne, Parkville, Australia

[4]Department of Microbiology, Monash University, Clayton, Australia

The discovery of penicillin in the 1928, and its subsequent introduction into clinical practice in 1941, heralded a new era in the treatment of serious bacterial infections, including those caused by *Staphylococcus aureus*. This organism causes a wide range of infections in humans, with bloodstream infection and endocarditis being the most serious and potentially devastating. However, despite the discovery of antibiotics, *S. aureus* continues to cause a significant burden of illness around the world, with a high mortality rate for invasive disease [1]. One of the reasons for this is the ability of the organism to continually evolve and adapt to new environments, including antibiotic exposures, resulting in the ongoing accumulation of antibiotic resistance [2]. Remarkably, penicillin resistant *S. aureus* was detected within one year of the clinical use of penicillin, and the progressive acquisition of antibiotic-resistance in *S. aureus* since that time, particularly methicillin-resistant *S. aureus* (MRSA), has severely impacted the available antibiotic armamentarium. As an example of how serious this problem is, over the past ten years there have been more deaths from invasive MRSA infection than human immunodeficiency virus infection in the United States [3], yet MRSA comprises less than half of all *S. aureus* infections.

In day-to-day practice clinicians rely on susceptibility results from the diagnostic microbiology laboratory to guide appropriate antimicrobial therapy for their patients. Most susceptibility tests performed in these laboratories rely on *in vitro* phenotypic methods, where the test organism is exposed to different concentrations of antibiotic and the impact on growth of the organism is determined. Performance and interpretation guidelines for these assays are provided by regulatory authorities such as the Clinical and Laboratory Standards Institute (CLSI), and European Committee on Antimicrobial Susceptibility Testing (EUCAST). The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will

completely inhibit growth of an organism after a defined period of incubation, usually 18 to 24 hours, and is used by diagnostic microbiology laboratories to define antimicrobial susceptibility or resistance. All clinicians are aware, and medical students are taught, not to use an antibiotic to treat a bacterial infection if the report from the microbiology laboratory indicates it is resistant to that antibiotic. There is however a limitation to the current methods for antimicrobial susceptibility testing – they are performed *in vitro* and therefore do not measure other potentially important factors influencing the *in vivo* activity of the agent.

Aside from antibiotics, other significant contributors to the clearance of bacterial infections are the innate immune system factors including host defense peptides (HDPs) and neutrophils. Host defense peptides have received increasing attention in the scientific literature because of their antimicrobial activity against a wide range of pathogens, including drug resistant bacterial strains [4]. These amphipathic cationic peptides are naturally occurring antimicrobials produced by a variety of cell types such as epithelial cells and phagocytes [5], and while they have direct antimicrobial activity against human pathogens and cancer cells *in vitro*, they are increasingly recognized as important immunomodulators *in vivo* [6]. The cationic nature of HDPs is important for their activity, with the positive charge promoting attachment at anionic surfaces such as teichoic acids in the cell wall of Gram-positive bacteria [7]. Factors that alter cell surface charge therefore have the capacity to alter HDP function. While the direct antibacterial mechanism of action of HDPs is not completely understood, they act via inserting into the cytoplasmic membrane and appear to exert activity by either disrupting the physical integrity of the bilayer, or translocate across the membrane and act on internal targets [7].

The threat posed by increasing and emerging antimicrobial resistance in *S. aureus* is a significant public health problem. *Staphylococcus aureus* is one of the ESKAPE organisms, highlighted by The Infectious Diseases Society of America in their report 'Bad Bugs, No Drugs' policy report in 2004 and requiring the development of novel antibacterial agents [8]. The lack of new antimicrobials in the increasingly dry drug development pipeline, and increasingly common and novel resistance mechanisms continue to provide challenges for clinicians. For this reason clinicians and scientists, while continuing to hope for the arrival of new antibacterial agents, are now also looking backwards to explore opportunities to use previously discarded agents (for example the re-introduction of colistin for pan-resistant Gram-negative infections), or to use well established agents in a different way. In this issue of the journal, Sakoulas *et al* [9] have investigated the innate immune system impacts of a relatively old class of antibiotics (the β-lactams) against MRSA, and demonstrate that we still have a lot to learn about how some antimicrobial agents exert their full clinical effect. Their findings suggest that the standard antimicrobial susceptibility result that is delivered from the diagnostic microbiology laboratory may not always tell the whole truth.

In infectious diseases clinical practice combination antimicrobial therapy is often employed to treat life threatening bacterial infections including *S. aureus* bacteremia; however the clinical utility of this practice is not well established. Recently reports have emerged describing the use of β-lactams that the infecting organism is resistant to, in combinations with other antibacterials, to improve outcomes in difficult to treat infections caused by *S. aureus* and *Enterococcus faecium* [10, 11]. The rationale behind this has been that the β-lactam, despite the phenotypic resistance of the organism, has resulted in changes to the bacterial surface promoting enhanced

binding and activity of the other antibiotic, daptomycin. The addition of ampicillin to cultures of an ampicillin-resistant *E. faecium* strain enhanced binding and activity of daptomycin as well as host defense peptides tested *in vitro*, possibly mediated by alterations in cell surface charge [11]. Similarly, the addition of anti-staphylococcal penicillins to treatment of a small number of refractory MRSA bacteremia cases appeared to result in resolution of infection, and the addition of anti-staphylococcal penicillins to MRSA cultures reduced surface charge, enhanced daptomycin binding, and improved daptomycin activity [10]. In the article by Sakoulas *et al* [9], the potential mechanism resulting in the clinical efficacy of β-lactams against the resistant pathogen MRSA have been explored. The authors have uncovered an intriguing phenomenon where low doses of the antibacterial agent nafcillin enhanced killing of MRSA (*S. aureus* resistant to all β-lactams including nafcillin) through promoting the activity of HDPs and neutrophils.

The authors found that low doses of nafcillin (eg 20 μg/ml, when the MIC of the strains was >128 μg/ml) increased the *in vitro* activity of the cationic antimicrobial peptide LL-37 and other mammalian HDPs, and *ex vivo* increased killing of MRSA by neutrophils, keratinocytes and human whole blood (Figure 1). Other β-lactam antibiotics demonstrated a similar *in vitro* effect although not all to the same degree as nafcillin, while non-β-lactams had minimal effect. A number of experiments supported the conclusion that low dose nafcillin was promoting antibacterial activity of HDPs, with nafcillin promoting enhanced binding of LL-37 demonstrated on the surface of an MRSA strain. It may be that the reduction in cell surface charge noted after β-lactam exposure of MRSA in previous studies [10, 11] is responsible for the enhanced binding and activity of HDPs, however this requires further clarification. Daptomycin,

the antibiotic used clinically in combination with the β-lactams to successfully treat the reported cases is also positively charged *in vivo* after binding divalent calcium, and in fact has a mechanism of action reminiscent of HDPs, suggesting that the β-lactam could be promoting activity of innate HDPs and daptomycin concurrently.

Recent studies have suggested that the *in vivo* activity of HDPs may be linked more to immunomodulatory effects rather than direct antibacterial activity [6]. The most compelling result of the study by Sakoulas *et al* [9] however, was the attenuation of virulence of MRSA in the mouse skin infection model, upon pre-exposure to nafcillin, or upon treatment of mice with nafcillin alone, suggesting a direct impact on innate immune mediated killing in this model. Importantly, therefore, these results indicate that the nafcillin mediated effects observed *in vitro* and *ex vivo* may translate into important *in vivo* activity. Interestingly, a recent completed clinical study that assessed the benefit of adding a β-lactam to traditional therapy (vancomycin) for the treatment of MRSA bacteraemia cases has demonstrated an improvement in microbiological outcome with the addition of the β-lactam [12]. This was a retrospective study, and clearly large-scale prospective controlled clinical studies are now needed to formally evaluate the clinical benefit of adjunctive β-lactam therapy and the optimal combination therapies for MRSA bacteremia. While the authors have uncovered positive effects of nafcillin exposure against MRSA strains tested *in vitro* and in a mouse skin infection model, it remains possible that negative consequences of the use of such combinations could occur with the treatment of infections in humans, which will only be detected with appropriate prospective clinical studies. Human research ethics committees could have some difficulty justifying the use of an antimicrobial that the infecting strain has demonstrated *in vitro* resistance to. Here,

Sakoulas *et al* have provided biological plausibility to the role of adjuvant β-lactams in resistant MRSA bacteremia, that further support the preliminary data from clinical case series and retrospective studies.

There are still many unanswered questions from a mechanistic point of view. In particular, the cellular changes promoting nafcillin enhanced innate immune killing have not been defined. Clearly transcriptomic and comparative proteomic studies could provide some interesting insights here, and it appears that the authors are planning these. Further insights gleaned from these studies may provide novel avenues for treatment of MRSA strains in future.

It is not uncommon in clinical infectious diseases practice to encounter a patient with MRSA bacteremia that persists despite apparent appropriate antibiotic therapy. So what will I do in this situation in future if the patient is receiving vancomyin or daptomycin and remains bacteraemic? I will be increasingly inclined to add a β-lactam antibiotic to the regimen, with increasing clinical experience demonstrating the potential efficacy of this decision, and the study by Sakoulas *et al* providing insights into the potential basis for the clinical efficacy of the additional agent. How then will I answer the medical student when they ask why I am adding an agent to which the infecting pathogen is resistant? I will tell them that "there appears to be more to antibacterial therapy than meets the eye (or MIC)".

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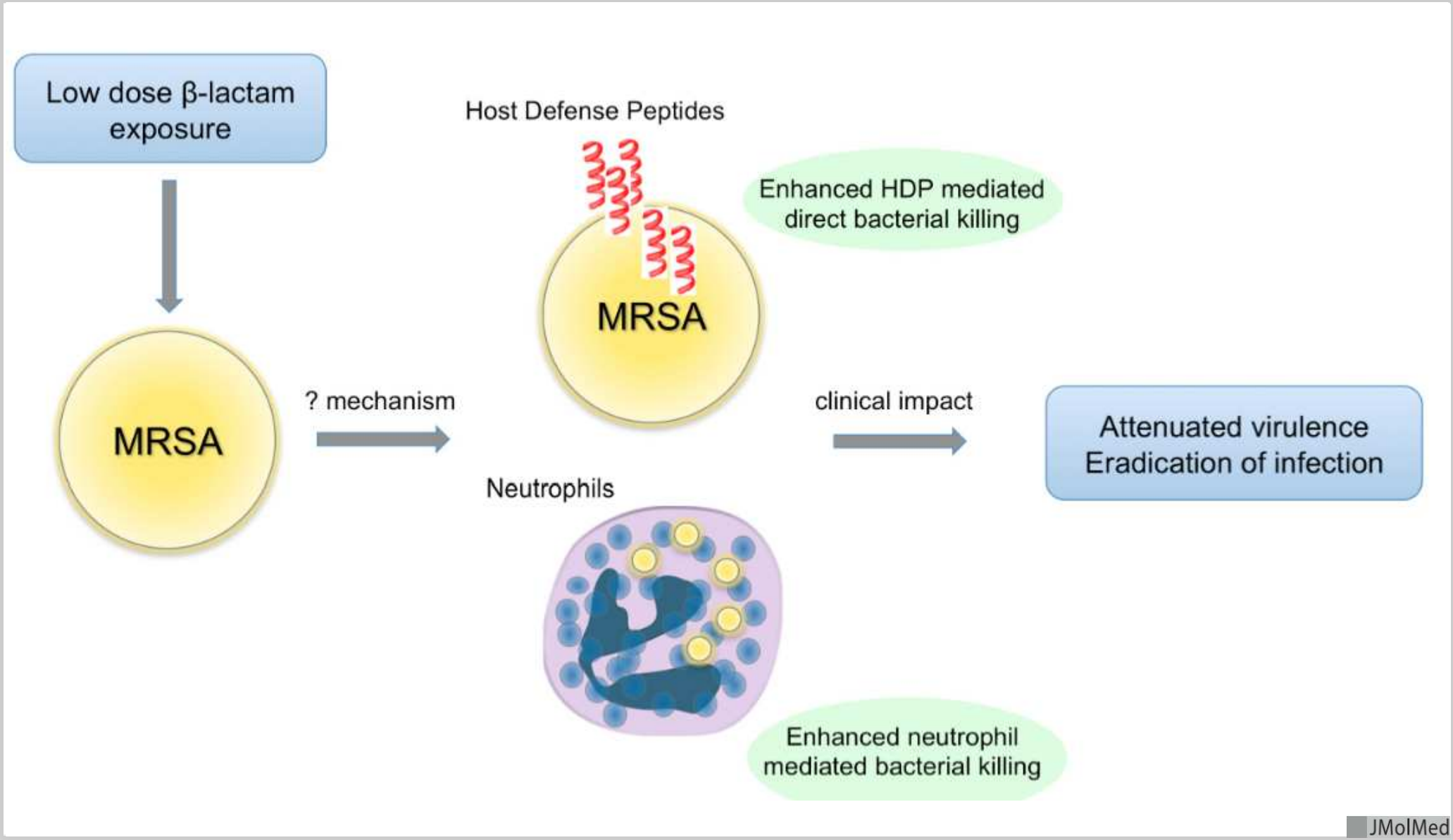
Figure Legend

Figure 1. Tentative model illustrating the impact of β-lactam antibiotics on innate immune mediated killing of methicillin-resistant *Staphylococcus aureus* (MRSA), based on the data from Sakoulas *et al* [9].


Low dose β-lactam exposure
MRSA
? mechanism
Host Defense Peptides
MRSA
Enhanced HDP mediated direct bacterial killing
Neutrophils
Enhanced neutrophil mediated bacterial killing
clinical impact
Attenuated virulence
Eradication of infection
JMolMed

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