

Antibiotics and *Staphylococcus aureus*—more than meets the MIC

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The discovery of penicillin in 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 1 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 10 years, there have been more deaths from invasive MRSA infection than human

immunodeficiency virus infection in the USA [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, and European Committee on Antimicrobial Susceptibility Testing. The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will completely inhibit the growth of an organism after a defined period of incubation, usually 18 to 24 h, 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].

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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. *S. aureus* is one of the ESKAPE organisms, highlighted by The Infectious Diseases Society of America in their “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 the treatment of a small number of refractory MRSA bacteremia cases appeared to result in the 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.

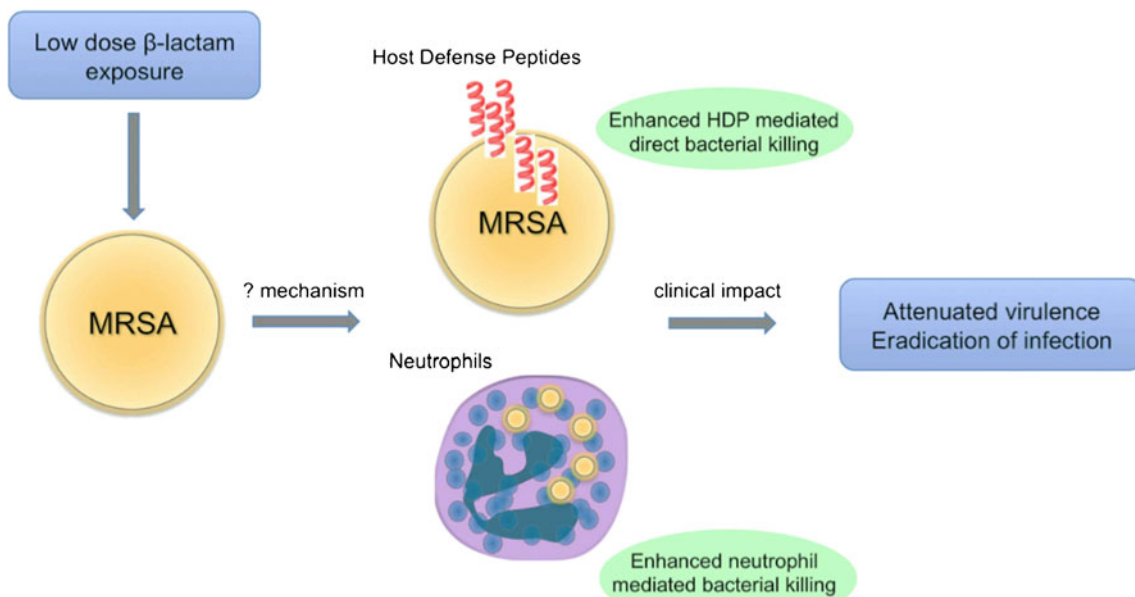


Fig. 1 Tentative model illustrating the impact of β -lactam antibiotics on innate immune mediated killing of methicillin-resistant *Staphylococcus aureus* (MRSA), based on the data from Sakoulas et al. [9]

The authors found that low doses of nafcillin (e.g., 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 (Fig. 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 the 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 bacteremia 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 vancomycin 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).”

References

1. Lowy FD (1998) *Staphylococcus aureus* infections. *NEJM* 339:520–532
2. Lindsay JA (2010) Genomic variation and evolution of *Staphylococcus aureus*. *Int J Med Microbiol: IJMM* 300:98–103
3. Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, Lessa FC, Lynfield R, Nadle J, Petit S et al (2013) National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. *JAMA Int Med* 173:1970–1978
4. Peschel A, Sahl HG (2006) The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat Rev Microbiol* 4: 529–536
5. Nijnik A, Hancock R (2009) Host defence peptides: antimicrobial and immunomodulatory activity and potential applications for tackling antibiotic-resistant infections. *Emerg Health Threats J* 2:e1
6. Hilchie AL, Wuert K, Hancock RE (2013) Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. *Nat Chem Biol* 9:761–768
7. Hancock RE, Sahl HG (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 24: 1551–1557
8. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis: Off Publ Infect Dis Soc Am* 48:1–12
9. Sakoulas GOC, Thienphrapa W, Olson J, Nonejuie P, Dam Q, Dhand A, Pogliano J, Yeaman MR, Hensler ME, Bayer AS et al (2013) Nafcillin enhances innate immune-mediated killing of methicillin resistant *Staphylococcus aureus*. *J Mol Med*. doi:10.1007/s00109-013-1100-7
10. Dhand A, Bayer AS, Pogliano J, Yang SJ, Bolaris M, Nizet V, Wang G, Sakoulas G (2011) Use of antistaphylococcal beta-lactams to

- increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant *Staphylococcus aureus*: role of enhanced daptomycin binding. *Clin Infect Dis* 53:158–163
11. Sakoulas G, Bayer AS, Pogliano J, Tsuji BT, Yang SJ, Mishra NN, Nizet V, Yeaman MR, Moise PA (2012) Ampicillin enhances daptomycin—and cationic host defense peptide-mediated killing of ampicillin—and vancomycin-resistant *Enterococcus faecium*. *Antimicrob Agents Chemother* 56:838–844
 12. Dilworth TJ, Ibrahim O, Hall P, Sliwinski J, Walraven C, Mercier RC (2013) Beta-lactams enhance vancomycin activity against methicillin-resistant *Staphylococcus aureus* bacteremia compared to vancomycin alone. *Antimicrob Agents Chemother*. Epub. In Press