"Tolerance" of Misused Terminology? Enforcing Standardized Phenotypic Definitions

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The recent paper by Haaber and colleagues entitled "Reversible Antibiotic Tolerance Induced in *Staphylococcus aureus* by Concurrent Drug Exposure" (1) revealed a possible alternative mechanism by which pathogens become less susceptible to standard therapy by screening for inducible antibiotic resistance in *Staphylococcus aureus* USA300 strain FP3757. We agree with the sentiments expressed by Bean and Wigmore (2) about the timeliness of this article and the need to examine antibiotic combinations, especially with increasing multidrug-resistant pathogens. Furthermore, this article highlighted some of the potential pitfalls of combination therapy and stressed the need for further research in this area.

However, we have some concerns regarding the terminology and methods used in this study. First, the term "antibiotic tolerance" is used extensively throughout the article with no consideration of its official definition. The Clinical and Laboratory Standards Institute (CLSI) defines a vancomycin-tolerant strain as one for which the minimum bactericidal concentration (MBC)-to-MIC ratio is ≥32 after 24 h of incubation (3–7). The MIC, used as a measure of susceptibility, is the minimum concentration of an antibiotic that inhibits growth. In contrast, the MBC indicates the effectiveness of a bactericidal antibiotic, as it is the minimum concentration needed to kill an organism. Thus, although *S. aureus* FPR3757 showed an increased vancomycin MIC after pre-exposure to colistin, it did not display tolerance as per current CLSI definitions. As such, "reduced susceptibility" would be a more accurate description of the observed MIC changes. This observation is still of concern, however, as *S. aureus* infections with reduced antibiotic susceptibility are associated with increased patient mortality (8).

Second, several findings (such as reduced negative cell surface charge) led the authors to conclude, albeit incorrectly, that colistin induces a vancomycin-intermediate *S. aureus* (VISA)-like phenotype. VISA is defined either by an MIC between 4 and 8 μg/ml (9) (FPR3757 MICs were within the susceptible range, ≤2 μg/ml) or by population analysis profiling (10), which was not performed. In addition, increased cell wall thickness is a universal finding for VISA isolates but was not observed in this study. Finally, although *mprF* gene expression increased in *S. aureus* FPR3757 (after pre-exposure to colistin), growth was not evident in the presence of 2 μg/ml daptomycin. This may be a little surprising given the previously observed association between reduced negative cell membrane charge and daptomycin nonsusceptibility (11); however, this concentration (2 μg/ml) is greater than the clinical daptomycin breakpoint of ≤1 μg/ml. As such, the daptomycin MIC may have actually increased within the susceptible range, as has been observed for vancomycin, but this was not investigated.

In conclusion, Haaber's findings highlight stress responses that occur when bacteria are exposed to combination therapy. Despite an elevated yet susceptible vancomycin MIC being observed, it is important to note that this does not imply antibiotic tolerance, nor does it reveal antibiotic resistance development or VISA emergence; rather, it indicates reduced antimicrobial susceptibility. Regardless, the observed changes are of concern, as clinicians may be doing harm when treating patients with certain combination therapy regimens, and thus we would welcome further research in this area.

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