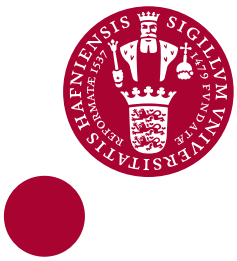

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Enforcing standardized phenotypic definitions"

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Reply to "'Tolerance' of Misused Terminology? Enforcing Standardized Phenotypic Definitions"

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We thank Dimitrijovski and co-authors for their interest in our recent paper "Reversible antibiotic tolerance induced in *Staphylococcus aureus* by concurrent drug exposure" (2), but we also wish to respond here to their comments about the terminology and methods we've used.

Regarding terminology, Dimitrijovski and colleagues (1) express concern that our use of the term "tolerance" is not in accord with what they state is the "official definition" by the Clinical and Laboratory Standards Institute (CLSI) of "a vancomycin-tolerant strain as one for which the minimum bactericidal concentration (MBC)-to-MIC ratio is >32 after 24 h of incubation." We certainly agree to the importance of having uniform terminology; however, we are not aware that the testing standards developed by CLSI to accomplish their stated mission of facilitating comparison of data from different laboratories have been adopted as part of an "official" nomenclature, and in fact not all references cited by Dimitrijovski et al. adhere to what they have termed the official definition. In any case, as explained in the text of our paper (2), our use of the term "tolerance" was intended to distinguish noninherited, reduced antibiotic susceptibility from resistance, which commonly is used to indicate inherited reduced susceptibility that is mutational in origin (for example, also see reference 3).

Dimitrijovski et al. also question our conclusion that colistin induces a VISA-like phenotype, noting that "VISA is defined either by an MIC between 4 and 8 μg/ml (FPR3757 MICs were within the susceptible range, ≤2 μg/ml) or by population analysis profiling." This concern is puzzling, as our paper states that the characteristics of colistin-induced bacteria are not identical to those of VISA strains but rather that exposure to colistin results in some of the phenotypic changes common to VISA, and also that the genes induced by colistin have very significant overlap with those reported previously to be altered in VISA strains. Therefore, we used the term "VISA-like" rather than "VISA." As we reported, the MIC of vancomycin for bacteria exposed to colistin is not in the range observed for VISA strains. We also showed (see Fig. 4 of our paper [2]) and noted explicitly in the text that thickening of the cell wall, which as correctly stated by Dimitrijovski et al. is a feature of VISA strains, was not observed in the colistin-induced bacteria showing other phenotypic characteristics in common with VISA.

The additional notion of Dimitrijovski et al. that our paper concludes that colistin induces development of resistance or the emergence of VISA is especially puzzling, as no such conclusions were in fact made. We sincerely regret an apparent misinterpretation of our conclusions by at least some readers. Finally, Dimitrijovski et al. raise the concern that we used 2 μg/ml daptomycin instead of the official MIC breakpoint of 1 μg/ml to conclude that colistin exposure does not affect bacterial susceptibility to daptomycin. However, the MIC breakpoint seems to us to not be relevant to the test (i.e., colony formation) that we employed in this experiment. The mechanisms leading to reduced susceptibility to daptomycin are complex and may be induced by multiple processes (4), which may explain why the colistin exposure leads to reduced negative cell surface charge without affecting daptomycin susceptibility.

Dimitrijovski et al. share the concerns raised in our paper about the possible induction of reversible reduced antimicrobial susceptibility by certain combination therapy regimens, and agree with the need for further investigations of reversible resistance. Hopefully, our findings will help to encourage such investigations.

REFERENCES

1. **Dimitrijovski B, Jensen SO, Espedido BA, van Hal SJ.** 2015. Tolerance of misused terminology? Enforcing standardized phenotypic definitions. mBio **6:**e00446-15. http://dx.doi.org/10.1128/mBio.00446-15.
2. **Haaber J, Friberg C, McCreary M, Lin R, Cohen SN, Ingmer H.** 2015. Reversible antibiotic tolerance induced in *Staphylococcus aureus* by concurrent drug exposure. mBio **6:**e02268-14. http://dx.doi.org/10.1128/mBio.02268-14.
3. **Wiuff C, Zappala RM, Regoes RR, Garner KN, Baquero F, Levin BR.** 2005. Phenotypic tolerance: antibiotic enrichment of noninherited resistance in bacterial populations. Antimicrob Agents Chemother **49:** 1483–1494. http://dx.doi.org/10.1128/AAC.49.4.1483-1494.2005.
4. **Bayer AS, Schneider T, Sahl HG.** 2013. Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. Ann N Y Acad Sci **1277:**139–158. http://dx.doi.org/10.1111/j.1749-6632.2012.06819.x.

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