Decreased susceptibility to biocides in bacteria has raised increased levels of attention in recent years due to a claimed hazard related to potential selection for antibiotic-resistant strains. In this context, Latimer et al. characterized *Staphylococcus aureus* ATCC 6538 mutants selected by the biocide triclosan for their *in vitro* phenotypes and virulence potential (3). Phenotypic characterization was carried out on one mutant (P10) that had been passaged 10 times in a triclosan gradient. Mutant P10 exhibited multiple phenotypes, including small colony size, slow planktonic growth, impaired biofilm formation, impaired hemolytic activity, impaired coagulase activity, and impaired virulence, in a model of *Galleria mellonella* infection. From the analysis of the P10 mutant, it was concluded that triclosan may select for reduced susceptibility to triclosan but that this reduced susceptibility may be associated with deficiencies in growth and virulence (3).

These data are in discordance with previous reports which describe clinical isolates of many species, including *S. aureus*, with reduced susceptibility to triclosan without reference to such profound impacts on virulence-associated phenotypes (5). Extensive work shows that mutation of *fabI*, encoding the enoyl-acyl carrier protein reductase FabI, a well-characterized target of triclosan, results in decreased MICs and minimal bactericidal concentrations (MBCs) to the biocide. The fact that these strains are readily detected in clinical isolates indicates that the *fabI* mutation does not confer a significant loss in virulence of *S. aureus* infecting humans.

We have recently performed a survey of biocide susceptibility in clinical isolates of *S. aureus* (2). This study identified in 1,385 clinical isolates 5.1% of strains with increased triclosan MICs and MBCs for which the EN1276 biocide test showed triclosan to be less active. These clinical isolates were compared to *in vitro*-selected mutants, obtained by single or multiple passages in triclosan. The *in vitro*-selected mutants showed none of the phenotypes listed by Latimer et al. (3). Likewise, none of the many previous publications describing *fabI* mutants in *S. aureus* except for two papers focusing just on small colony variants have noted such phenotypes (1, 4). This might be in part due to the intrinsic way in which strains are selected, a theory which is supported by the fact that wild-type *S. aureus* strains have a mode triclosan MIC of 0.03 mg/liter, most clinical isolates with reduced triclosan susceptibility have MICs between 1 and 4 mg/liter, most of the single and multistep mutants, published recently by us (Fig. 1), have a triclosan MIC of 4 mg/liter, and P10 has a triclosan MIC of 32 mg/liter (2, 3, 5). We have recently performed whole-genome sequencing of six of our *in vitro*-selected triclosan mutants, and no significant mutations outside the *fabI* locus were detected (data not shown). In the absence of a validated model for a globally valid fitness test, we have assayed in this work seven of these mutants in the same invertebrate model as that for P10. None of our triclosan-selected mutants showed significantly reduced killing capacity when assayed in wax moth larvae (Fig. 1A and B).

Combining (i) the observation that up to 5% of clinical isolates of *S. aureus* carry *fabI* mutations conferring reduced susceptibility to triclosan with (ii) the lack of virulence loss of triclosan-selected

**REFERENCES**


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For the author reply, see doi:10.1128/AAC.01515-12.

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doi:10.1128/AAC.01555-12


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