Powerful Genetic Resource for the Study of Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT In "A Genetic resource for Rapid and Comprehensive Phenotype Screening of Nonessential *Staphylococcus aureus* Genes" (mBio 4(2):e00537-12, doi: 10.1128/mBio.00537-12, 2013), Fey et al. describe the creation and application of a defined transposon mutant library of methicillin-resistant *S. aureus*. This library is well organized and made accessible to the research community through an easily navigable central repository. The mutant library promises to be a significant resource for researchers seeking a greater understanding of this pathogen.

The Gram-positive pathogen *Staphylococcus aureus* is a significant cause of morbidity and mortality worldwide. The success of this pathogen can be partially attributed to its remarkable ability to infect nearly every vertebrate organ. Several virulence traits endow *S. aureus* with the capacity to manipulate and survive within the host environment. These include, but are not limited to, the production of hemolysins, the secretion of proteases, and the synthesis of the organism's clinically distinguishing golden pigment, staphyloxanthin (1). Treatment of *S. aureus* infections are confounded by the incidence of antibiotic resistance strains. In fact, methicillin-resistant *S. aureus* (MRSA) is now the predominant cause of staphylococcal infection in the United States (2), and mortality due to MRSA infection in the United States is greater than that of AIDS and tuberculosis combined (2, 3). Once thought to be confined to the clinical setting, MRSA has been increasingly isolated from community-acquired infections (4). These facts highlight the need to understand the physiology and virulence strategies of this organism in an effort to uncover novel therapeutic targets for the treatment of MRSA. Therefore, a library of defined genetic MRSA mutants would represent a valuable resource for the community of researchers focused on understanding the pathogenesis of *S. aureus* infections.

Constructing mutants of *S. aureus* in a laboratory involves, primarily, random-transposition and allelic-replacement strategies, which can be tedious and cause secondary mutations that confound results (5). A defined transposon library was previously created with the methicillin-sensitive strain *S. aureus* Newman, enabling a number of exciting discoveries (6–9). This resource made clear the value that an openly accessible, defined transposon library could have on the staphylococcal research community. Fey et al. have taken on this challenge by creating a defined transposon library of *S. aureus* that is distributed through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) (10). This library was constructed with the MRSA strain JE2, a USA300 derivative which represents the predominant clonal lineage of MRSA isolated from infected patients in the United States (11). A *mariner*-based *bursa* transposon mutagenesis approach was used to create random insertion mutations throughout the genome (6). Erythromycin resistance was used to select for strains containing a transposon insertion, and the sites of mutation were then mapped in order to determine the site of insertion. Strains containing a transposon insertion within the first 90% of a coding sequence (CDS) were prioritized and included in the Nebraska Transposon Mutant Library (NTML). This selection resulted in the final collection of the 1,952 mutants that comprise the NTML, representing 76% coverage of the predicted CDSs within the JE2 genome. Interestingly, 579 putative CDSs were not identified in the screening process for the NTML, raising the intriguing possibility that these represent the suite of essential genes within *S. aureus* which could include novel targets for therapeutic intervention.

As a demonstration of the utility and comprehensiveness of the NTML, Fey et al. screened for mutants altered in four phenotypes that clinically distinguish *S. aureus* from other bacteria: the production of hemolysin, the ability to produce the golden pigment staphyloxanthin, the production of proteases, and the ability to utilize mannitol as the sole carbon source. The power of the NMTL is indicated by the fact that the authors identified mutants known to be impaired for these respective pathways. These mutants include the alpha hemolysin involved in lysing red blood cells, the known staphyloxanthin biosynthesis genes, two secreted proteases, and all of the genes in the mannitol utilization operons (12–15). In total, 71 mutants with altered hemolytic activity, 62 mutants with altered protease production, and 38 mutants affected for pigment production were identified. Some of these mutants have been identified in previous screens, while others have not been associated with these processes (7, 16–20). These findings further refine our knowledge of the genetic networks involved in these pathways and underscore the effectiveness of using the NTML in a phenotypic screening approach.

Individual mutants or the entire NTML can be ordered free of charge by members of the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). A list of the mutants can be found at the following URL: http://app1.unmc.edu/fgx/. Fey et al. are also in the process of making additional tools available for the NARSA community, including codon-optimized transcription reporters that can be utilized in conjunction with the NTML for further study. Thus far, 2,588 individual mutants have been sent to independent investigators, and 20 laboratories throughout the

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world have received the entire NTML, highlighting the demand for this valuable resource (personal communication).

Due to the fact that the NTML was created using a protocol that required changing incubation temperatures, a procedure known to cause off-target mutations in major *S. aureus* virulence two-component systems (9), researchers should be cautious when interpreting results from a phenotypic screen using the NTML. In keeping with this, investigators should validate any observed phenotypes of interest by backcrossing insertions into clean strain backgrounds, inactivating associated genes through traditional allelic-replacement strategies, and/or complementing phenotypes of interest by providing a full-length copy of the gene in *trans*. As the NARSA community begins to make use of the powerful genetic tools provided by Fey and his collaborators, research into *S. aureus* is poised to make a significant leap forward.

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