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Supporting The Development And Evaluation Of Novel Medical Countermeasures By Studying The Emergence Of Single And Multi-drug Resistance In Laboratory Passed Strains Of Bacillus Cereus

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Bacterial acquisition of antimicrobial resistance (AMR) and multi-drug resistance (MDR) remain one of our nation's top threats to our warfighters and to public health more broadly. Accelerated by overuse of antibiotics throughout the decades, AMR and MDR strains create significant challenges in the modern era, both on the battlefield and in hospital settings, as commonly prescribed antibiotics are becoming increasingly ineffective. While significant efforts are underway to repurpose existing antibiotics and discover novel antimicrobial compounds, our team seeks to understand the bacterial potential for multi drug resistance by tracking the acquisition of mutations in a controlled laboratory environment. Herein, we present our findings based on phenotypic and genomic characterizations of Bacillus cereus strain 03BB102 as it develops resistance to three antibiotics: ciprofloxacin, tetracycline, and vancomycin.

Bacillus cereus is a common food-borne pathogen, which occasionally exhibits naturally acquired resistance to one or more clinically relevant antibiotics. To better understand how the bacterium acquires multidrug resistance, we tracked B. cereus 03BB102 isolates as they developed resistance to elevated levels of three different antibiotics during passaging and identified key changes in their genomes that may inform the development of novel diagnostic tools and medical countermeasures. We will report these genomic findings in 18 derived mutant lineages of B. cereus 03BB102, exhibiting resistance to three different antibiotics. Illumina and PacBio sequencing revealed notable genomic changes when compared to the wild type genome, that accrued during the stepwise in vitro selection of single and multi-drug resistant isolates. In the first round of ciprofloxacin selection, we obtained an isolate that showed a deletion in a TetR-type transcriptional regulator that appears to confer increased expression of a multidrug efflux system. In the following rounds of tetracycline selection, we isolated two mutants having a highly replicated segment of the genome containing the TetO tetracycline-resistant ribosomal protection protein. In the final round of vancomycin selection, we also identified four independent SNPs in a specific histidine kinase gene that likely resulted in higher vancomycin resistance in the isolates.

This study investigated the innate capacity of AMR and MDR development in a single organism and identified notable genomic changes which can explain the increased resistance to multiple drugs. Understanding the mechanisms and pathways toward multi-drug resistance can help the development of countermeasures to prevent and/or counteract the clinical and environmental impact and emergence of such strains. Additionally, by accessioning these well characterized AMR/MDR variants into the DoD's Biodefense Reference Material repository, we are able to create a panel of drug resistant organisms against which to test and eveluate novel therapeutic compounds.
Disclaimer: Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army.

We are grateful to Maj. Jeffrey Havens and Dr. Amanda Horstman for their guidance and support of this project (Proj.#: R-00696-19-0).