COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

Understanding The Mechanisms Of High-level Ciprofloxacin Resistance In Bacillus Cereus Sensu Lato Group Of Bacteria

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Background: Bacillus anthracis is a pathogen of bioterrorism concern which has been used in the past in terrorism attacks. The potential consequences of using an antibiotic resistant B. anthracis in an attack of bioterrorism could be even more devastating. Although natural resistance to two commonly prescribed prophylactic- antibiotics, Doxycycline (DOX) and Ciprofloxacin (CIP) are rare, in vitro selection of resistance has been demonstrated either by plasmid transfer or serial passage in which case the underlying mechanisms are poorly understood. In previous work, mutants of attenuated B. anthracis with high-level CIP resistance were isolated using a three-step in vitro selection protocol. Step 1 mutants had a minimal inhibitory concentration (MIC) of 0.5 µg/ml and presented gyrA quinolone resistance-determining region (QRDR) mutations. Step 2 mutants had an MIC of 8-16 and showed parC QRDR mutations. Step 3 mutants were those with an MIC of 32-64, and it was found that isolates with an MIC of 64 had additional mutations within gyrA or gyrB QRDR. Mutants for which MICs were 32 had no additional target mutations but showed evidence of enhanced CIP efflux, and some had mutations in a TetR- type transcriptional regulator upstream of an efflux pump encoding gene. To better understand the genetic determininants of high-level CIP resistance phenotypes, we used B. anthracis near neighbor B. cereus, as a model organism to generate CIP resistant derivatives for whole genome sequencing.

Objective: To elucidate the genetic basis of ciprofloxacin resistance in B. cereus by comparing genomic data from evolved strains with varying ciprofloxacin resistance profiles to their ancestral parental strain.

Rationale: Stepwise isolation of increasing resistance to ciprofloxacin leads to accumulation of mutations, and mapping mutations at these evolutionary steps may elucidate the pathways resulting in higher level resistance.

Methods: AMR isolates were derived from a single colony of Bacillus cereus ATTC 14579 by serial selection using the microdilution assay. A 10^5 cfu/mL culture was incubated overnight with 0.125 – 32 µg/mL of ciprofloxacin or doxycycline in a 96-well plate. Bacteria in wells containing 0.5 x MIC of the antibiotic were collected and retreated with 0.125 – 32 µg/mL. After twenty passages, DNA was isolated from single colony isolates from each 0.5 x MIC passage culture and 100 CIP resistant strains of B. cereus, were sequenced using both Illumina and Nanopore technologies. Comparative genomic analysis was performed to identify single nucleotide polymorphisms (SNPs), insertions and deletions (INDELs), and structural variations (SVs) relative to the parent strain.

Preliminary Results: Analysis revealed a correlation between increased MIC and specific genomic variations. All isolates with an MIC greater than 16 exhibited an in-frame insertion in the transcriptional regulator codY gene, while those with an MIC over 32 were uniquely identified to contain a 252 bp deletion in a DEAD/DEAH box helicase. These mutations indicate the importance of these genes in B. cereus ciprofloxacin resistance. Significant differences were observed in the mutation profile from earlier results using B. anthracis and the current study. These results and their potential implications will be discussed.

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