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Initiating an understanding of how ATP production and growth rate determine the strength of the inoculum effect in beta-lactamase expressing Escherichia coli.

Antibiotic resistant bacteria represent a significant cause of mortality; we must understand the mechanisms that bacteria use to resist antibiotics. One way that bacteria resist antibiotics is the inoculum effect (IE); for a given concentration of antibiotic, the bacterial population will resist the antibiotic if its density is sufficiently high. Bacteria that express beta-lactamases are associated with IE. Antibiotic failure and mortality have been observed as a result of IE with these bacteria. Despite the clinical significance of IE in beta-lactamase bacteria being first recognized in the 1970s, we lack approaches in the clinic to treat these bacteria. This is because we do not understand the where, when, and why IE arises. Recently, we have discovered that the relationship between ATP production and growth rate can account for IE in bacteria that lack beta-lactamases. This discovery is exciting; by manipulating ATP and growth, IE can be abolished, paving the way for novel interventions in the clinic. However, we do not know if ATP and growth can account for IE in beta-lactamase expressing bacteria. This is because the expression and use of beta-lactamases alters the relationship between ATP production and growth outside of any antibiotic treatment. To fulfill this need, we propose to investigate how ATP production, growth, and beta-lactamases expression rate determine IE in Escherichia coli. Our central hypothesis is: increasing beta-lactamase expression will affect ATP production, which will non-linearly impact growth rate, beta-lactamase-driven resistance, and antibiotic lethality; together this will determine the strength of IE. To address this hypothesis, we propose two Aims: 1) develop a flux balance analysis/whole genome modeling driven understanding of how the growth environment determines ATP production, growth rate, and beta-lactamase expression, and 2) quantify IE across multiple growth environments and beta-lactamase production rates to establish the relationship between ATP production, growth rate, beta-lactamase expression and IE. We will use of flux balance analysis, mathematical modeling, ATP assays, growth assays, and MIC assays. Our work will be performed by two experts in the field, Dr. Robert P. Smith and Dr. Allison J. Lopatkin, who will work alongside students to address a significant question in antibiotic resistance that will pave the way for the creation of novel intervention strategies in the clinic.