The inoculum effect and band-pass bacterial response to periodic antibiotic treatment

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***Short Abstract* — The inoculum effect refers to the decreasing efficacy of an antibiotic with increasing bacterial density. It represents a unique strategy of antibiotic tolerance and it can complicate design of effective antibiotic treatment of bacterial infections. To gain insight into this phenomenon, we have analyzed responses of a lab strain of Escherichia coli and two bacterial pathogens to antibiotics that target ribosomal proteins. We show that the inoculum effect can be explained by bistable inhibition of bacterial growth. A critical requirement for this bistability is sufficiently fast degradation of ribosomal proteins, which can result from antibiotic-induced heat shock response. Furthermore, antibiotics that elicit the inoculum effect can lead to "band-pass" response of bacterial growth to periodic antibiotic treatment: the treatment efficacy drastically diminishes at intermediate frequencies of treatment. Our proposed mechanism for the inoculum effect may be generally applicable to other bacterial species treated with antibiotics targeting the ribosomal proteins.**

***Keywords* — antibiotic, inoculum effect, heat shock response, systems biology**

I. Introduction

Antibiotics are falling victim to their own success due to decades of overuse and misuse. Bacteria have developed resistance to all existing antibiotics and they are doing so at an alarming rate, considering the slow timescale at which new antibiotics are being developed. In addition to developing new antibiotics, there is a critical need to design better treatment protocols by using existing antibiotics. A major challenge facing the development of effective antibiotic treatment is the lack of understanding of how bacterial populations respond to treatment. As such, a better understanding of the myriad of ways which bacteria can resist or tolerate antibiotic treatment at the population level is required.

The efficacy of many antibiotics is known to decline with increasing inoculum of bacterial pathogens [1]. At intermediate concentrations of these antibiotics, a bacterial population can survive if its initial density is high, but can go extinct otherwise. Known as the inoculum effect (IE), this phenomenon has been observed for a wide range of antibiotic classes. In the clinical setting, the presence of IE is undesirable as it can cause overestimation of in vitro resistance, increased mortality rates of infected hosts due to administration of insufficient doses of antibiotics, and increases in the generation rate of resistant pathogens. To date, however, there is a general lack of mechanistic understanding of IE.

II. Results and Discussion

In this work, we show that, for an antibiotic targeting the ribosome, the IE can be explained by the bistable inhibition of bacterial growth. Our results demonstrate that a critical requirement for this bistability is the fast degradation of the ribosomal proteins induced by the antibiotics that elicit the heat shock response. We show that this mechanism is applicable to a wide range of antibiotics and occurs in different species of bacteria, including *ETEC* and *Salmonella typhimurium*. We also find that the fast degradation leads to delayed recovery of bacterial growth after transient antibiotic treatment, which has critical implications for the design of periodic antibiotic treatment. Indeed, the IE, combined with the delayed recovery kinetics, can lead to "band-pass" bacterial response to periodic antibiotic treatment. When this occurs, treatment efficacy drastically diminishes at intermediate frequencies of antibiotic treatment. We note that this property has neither been predicted nor demonstrated in the literature, despite extensive computational studies on the consequence of periodic antibiotic treatment.

III. Conclusion

In summary, we have defined a new mechanism by which the inoculum effect can arise for a wide variety of antibiotics, many of which are commonly used in the clinical setting. Importantly, we unravel the overlooked implication of this mechanism for the efficacy of periodic treatment. Taken together, our results contribute significantly to the understanding of how bacterial populations use non-genetic mechanisms to tolerate antibiotic treatment.

Acknowledgements: This work was partially supported by the National Science Foundation (BES-0625213), the National Institutes of Health (1P50GM081883), a DuPont Young Professorship (L.Y.), a David and Lucile Packard Fellowship (L.Y.), a Medtronic Fellowship (C.T.), and a Lane Postdoctoral Fellowship (C.T.).

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