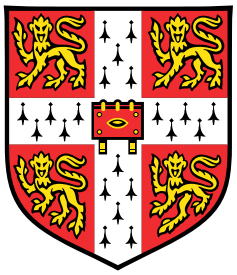
An integrated approach to ciprofloxacin susceptibility analysis and high-throughput bacterial phenotyping in *Salmonella*



Sushmita Sridhar

Wellcome Sanger Institute; Department of Medicine
University of Cambridge

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Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and Acknowledgements. This dissertation contains fewer than 60,000 words excluding appendices, bibliography, footnotes, and tables.

Sushmita Sridhar
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The world is full of obvious things which nobody by any chance ever observes.

- Sherlock Holmes, *The Hound of the Baskervilles*

Sherlock Holmes was no microbiologist, but this quote seems to ring true for biology. If I have learned anything during my PhD, it is to how to be a more critical observer of microbiological phenomena and follow those observations with hypotheses, experiments and analysis. It goes without saying that I have benefited from an incredible amount of support and encouragement during this process, first and foremost from an incredible set of supervisors: Gordon Dougan, Stephen Baker, and Nick Thomson. In particular, thanks to Doog for the conception of a challenging and stimulating project, always having an eye on the bigger picture, and being available anytime for a 'quick chat'. It has been a privilege to be in your group, and I cannot have asked for a better mentor. Thanks to Steve Baker for suggesting several of the (most time-consuming) experiments, all of which were incredibly useful. Thanks also for your boundless enthusiasm for discussing science and your unstinting support and leadership during the SARS-CoV-2 testing. Thanks to Nick Thomson for support at Sanger and critical high-level project advice and feedback at various intervals. I also owe an enormous thanks to Stanley Falkow, my first microbiology mentor.

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Abstract

An integrated approach to ciprofloxacin susceptibility analysis and high-throughput bacterial phenotyping in *Salmonella*

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Antimicrobial resistance is a growing threat across the world. *Salmonella* are Gram-negative, motile, rod-shaped bacteria that are transmitted through the faecal-oral route and invade the small intestine to cause self-limiting gastroenteritis or invasive, systemic disease. Invasive non-typhoidal *Salmonella* are a significant cause of bacterial infection globally, and the ST313 lineage of *Salmonella* Typhimurium are responsible for much of the burden of salmonellosis in sub-Saharan Africa. In recent years, there has been a drastic rise in multidrug resistance within this lineage, including fluoroquinolone resistance, a first line antimicrobial against invasive *Salmonella* species. In this thesis, I have explored the response of *Salmonella* Typhimurium (*S.* Typhimurium) to ciprofloxacin, a fluoroquinolone, using a combination of methodologies. In particular, this work was targeted at better understanding ciprofloxacin susceptibility in invasive non-typhoidal *S.* Typhimurium in sub-Saharan Africa. I began by assessing growth of *S.* Typhimurium in the presence of ciprofloxacin, finding that *S.* Typhimurium is capable of growth in concentrations of ciprofloxacin above the minimum inhibitory concentration (MIC). I have developed high-content imaging methodologies to screen *Salmonella* grown in the presence of ciprofloxacin. These morphological data suggest that there may be heterogeneous subpopulations with differential responses to ciprofloxacin, which was supported by studying the bacterial transcriptional response, and this may influence survival during ciprofloxacin treatment and interactions with host cells. Additionally, ciprofloxacin exposure triggers a bacterial stress response that appears to be distinct from responses generated by other stressors. Finally, I have investigated the genomic and phenotypic differences of a larger set of related *S.* Typhimurium ST313 isolates with

an array of susceptibilities to ciprofloxacin. High-content screening has shown that isolates appear to differ in their morphological signature depending on their genetic makeup. Together these data suggest that the study of the bacterial response to ciprofloxacin and integration of genotyping and phenotyping could significantly enhance our understanding of antimicrobial resistance and help guide appropriate antimicrobial usage.

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