

**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

This dissertation is submitted for the degree of  
*Doctor of Philosophy*

Downing College

September 2020



## **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  
September 2020



## Acknowledgements

*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.

I have been incredibly fortunate in my colleagues, many of whom have helped with various aspects of my project, from scientific discussions to protocol optimization. Thanks to Derek Pickard for helping generate mutant derivatives, optimize protocols, perform experiments, and data analysis support. I have learned so much from you, and it has been a pleasure to work with you in the lab. Thanks also to Sally Forrest, who has been indispensable in optimizing *Salmonella* imaging on the Opera Phenix and discussing data, and who is one of

---

the most thoughtful scientists I know. I am also grateful to Josefin Bartholdson Scott, Ben Warne, and Mailis Maes for considerable assistance in early project design and pioneering many of the high-content imaging protocols. A massive thank you to Sandra Van Puyvelde, who helped me develop my project, taught me phylogenetic analyses, and who has been instrumental in acquiring ST313 isolates for this work. It has been so much fun thinking through hypotheses and experiments with you, and your mentorship and friendship have been a highlight of my PhD. I am also thankful for the guidance and mentorship of Christine Boinett, who taught me TraDIS data analysis and provided considerable insight into my RNA-seq findings, as well as much laughter over Skype on Wednesday mornings.

Many of the bacterial isolates and methods used in this work were obtained or generated in collaboration with others. Special thanks to Jan Jacobs and Liselotte Hardy at the Institute of Tropical Medicine, Antwerp, and Octavie Lunguya at the National Institute for Biomedical Research, Democratic Republic of Congo, for providing many of the *S. Typhimurium* isolates. Additional thanks to Florian Marks, Hyonjin Jeon, and Ursula Panzner at the International Vaccine Institute and Rob Kingsley at the Quadram Institute for *S. Typhimurium* isolates. The *S. Typhimurium* D23580 TraDIS library used in this study was generated by Moataz Abd El Ghany (University of Sydney) and validated by Edna Ondari (Swiss Tropical and Public Health Institute). Organoid experiments were conducted by Emily Lees. Thanks to Dave Goulding and Claire Cormie for performing electron microscopy on various isolates. Thanks to Kirill Tsyganov and Rhys Dunstan at Monash University as well as Victoria Offord at Sanger for help with transcriptomic analyses. Thanks to Sally Kay for lab support and Pathogen Informatics for bioinformatic support at Sanger.

I am also thankful for an incredible group of friends, in Cambridge and across the world. In particular, Elsa Kentepozidou, Hannah Carrant, Harald Vöhringer, Gal Horesh, Tapoka Mkandawire, and Emily Hoyt. Thanks to Uttara Parthap and Sarah Kaewert for many laughs and adventures, and Emily Lees, who has been a friend, colleague, mentor, and biscuit co-conspirator, all wrapped into one. Thanks to Oliwia Baney, Natalie Rich, and Miguel Boluda, who have provided much motivation from abroad. Thanks to the Queens' College Grad Choir and Cambridge University Ceilidh Band for keeping me grounded.

Finally, I could not have done this without the unconditional love and support of my family. Thanks in particular to my dad for helping format this thesis. To my sister Sunita, brother-in-law Shavi, and parents Viji and Sridhar—you inspire me. Thank you for everything.

# Abstract

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

**Sushmita Sridhar**

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.



# Table of contents

<b>List of figures</b>	<b>xix</b>
<b>List of tables</b>	<b>xxv</b>
<b>1 Introduction</b>	<b>1</b>
1.1 The global burden of infectious diseases including <i>Salmonella</i> . . . . .	1
1.1.1 Definition of <i>Salmonella enterica</i> by classical phenotyping and genotyping using MultiLocus Sequence Typing (MLST) . . . . .	2
1.1.2 Burden of all <i>Salmonella</i> serovars . . . . .	4
1.1.3 Brief description of <i>S. Typhi</i> and <i>S. Paratyphi</i> . . . . .	5
1.1.4 Burden of non-typhoidal <i>Salmonella</i> . . . . .	7
1.1.5 Host distribution and transmission of <i>S. Typhimurium</i> . . . . .	8
1.1.6 Phylogeny of <i>S. Typhimurium</i> . . . . .	9
1.1.7 Disease presentation and treatment of <i>S. Typhimurium</i> in humans . . . . .	10

---

1.1.8	Human infection by <i>S. Typhimurium</i> . . . . .	11
1.2	Invasive non-typhoidal <i>Salmonella</i> compared to other non-typhoidal strains, an overview . . . . .	12
1.2.1	Global distribution of iNTS . . . . .	14
1.2.2	Disease presentation and treatment of iNTS . . . . .	16
1.2.3	Risk factors for iNTS disease . . . . .	16
1.2.4	Genetic factors characterizing/influencing <i>S. Typhimurium</i> iNTS . . . . .	18
1.2.5	Phylogeny of iNTS . . . . .	19
1.3	iNTS in sub-Saharan Africa . . . . .	19
1.3.1	<i>S. Typhimurium</i> ST313 as major contributors to iNTS . . . . .	20
1.3.2	Brief description of <i>S. Enteritidis</i> . . . . .	20
1.3.3	iNTS surveillance in Africa . . . . .	21
1.3.4	Phylogeny of <i>S. Typhimurium</i> ST313s . . . . .	22
1.3.5	Genetic and phenotypic characteristics of ST313 reference strain D23580 . . . . .	24
1.4	AMR in <i>Salmonella</i> . . . . .	26
1.4.1	WHO R&D ‘directive’ on FQR <i>Salmonella</i> . . . . .	27
1.4.2	How AMR is assessed in clinical settings . . . . .	28
1.4.3	Distribution of fluoroquinolone usage . . . . .	29

Table of contents

---

1.4.4	FQR in <i>S. Typhi</i> and <i>Paratyphi</i> . . . . .	30
1.4.5	FQR in <i>S. Typhimurium</i> . . . . .	32
1.4.6	Other AMR profiles in invasive <i>S. Typhimurium</i> . . . . .	33
1.4.7	iNTS vaccines . . . . .	35
1.5	Fluoroquinolone mechanism of action and resistance . . . . .	36
1.5.1	Mechanism of action of fluoroquinolones in Gram-negative bacteria	37
1.5.2	Chromosomal resistance to ciprofloxacin in Gram-negative bacteria	38
1.5.3	Plasmid-mediated resistance by <i>qnr</i> to ciprofloxacin in Gram-negative organisms . . . . .	39
1.5.4	Other genes involved in resistance . . . . .	40
1.5.5	Mechanisms of resistance found in African <i>S. Typhimurium</i> ST313	41
1.5.6	Pharmacokinetics/pharmacodynamics of ciprofloxacin . . . . .	42
1.6	Observation of disconnect between phenotypic and genotypic resistance . .	42
1.6.1	Observation of phenotypic heterogeneity in ST313s . . . . .	43
1.6.2	‘Adaptive resistance’ and desensitization to antimicrobials . . . . .	47
1.6.3	Lack of understanding of additional factors involved in FQR in <i>S. Typhimurium</i> . . . . .	49
1.7	Summary . . . . .	53

---

<b>2</b>	<b>Materials and Methods</b>	<b>55</b>
2.1	Bacterial isolates . . . . .	55
2.1.1	Growth medium and growth conditions . . . . .	55
2.1.2	Ciprofloxacin susceptibility testing by MIC ETEST . . . . .	55
2.2	Time kill curves . . . . .	56
2.2.1	Ciprofloxacin-degradation kill curves . . . . .	56
2.3	Spontaneous <i>gyrA</i> mutant generation and validation . . . . .	57
2.4	<i>S. Typhimurium</i> D23580 bacteria grown for 24 h in ciprofloxacin medium for whole genome sequencing . . . . .	57
2.5	Whole genome sequencing: library creation and sequencing . . . . .	58
2.6	Read mapping, variant detection, and SNP analysis . . . . .	58
2.7	Opera Phenix confocal phenotyping of bacteria . . . . .	59
2.7.1	Opera Phenix microscopy phenotyping of <i>S. Typhimurium</i> bacteria during ciprofloxacin exposure . . . . .	59
2.7.2	Opera Phenix phenotyping of <i>S. Typhimurium</i> ST313 isolates after ciprofloxacin exposure . . . . .	60
2.7.3	Opera Phenix image analysis . . . . .	60
2.8	RNA extractions and RNA sequencing . . . . .	62
2.8.1	RNA extractions from 4 ‘pilot’ isolates under 2x MIC ciprofloxacin exposure . . . . .	63

Table of contents

---

2.8.2	RNA extractions of <i>S. Typhimurium</i> D23580 under parallel treatment conditions . . . . .	64
2.8.3	Sucrose gradient separation of ciprofloxacin-treated D23580 . . . . .	64
2.8.4	RNA extractions from <i>S. Typhimurium</i> D23580 after sucrose gradient separation . . . . .	66
2.9	RNA sequencing analysis . . . . .	66
2.9.1	RNA sequencing analysis of gradient-separated bacteria . . . . .	66
2.10	Light microscopy of gradient-separated <i>S. Typhimurium</i> D23580 bacteria . . . . .	67
2.11	Generation of <i>S. Typhimurium</i> D23580 single-gene knockout derivatives . . . . .	67
2.12	TraDIS screen on time kill curves of ciprofloxacin-exposed <i>S. Typhimurium</i> D23580 . . . . .	68
2.13	TraDIS screen on <i>S. Typhimurium</i> D23580 injected into intestinal organoids . . . . .	69
2.14	Phylogenetic analysis . . . . .	70
2.14.1	SNP analysis of <i>S. Typhimurium</i> ST313 isolates . . . . .	70
2.15	Pangenome analysis . . . . .	71
2.16	<i>In silico</i> AMR analysis . . . . .	72
2.17	Biofilm growth conditions . . . . .	72
2.18	Scanning electron microscopy (SEM) of bacteria grown under stress conditions . . . . .	73
<b>3</b>	<b>Development of high-content imaging of individual bacteria</b>	<b>75</b>

---

3.1	Introduction . . . . .	75
3.2	Optimization of bacterial adhesion for imaging . . . . .	77
3.3	Optimization of staining for <i>S. Typhimurium</i> . . . . .	80
3.4	Development of an <i>S. Typhimurium</i> analysis pipeline . . . . .	82
3.5	Imaging of antimicrobial-treated bacteria . . . . .	84
3.6	Discussion . . . . .	88
<b>4</b>	<b>Characterization of growth dynamics of <i>S. Typhimurium</i> following ciprofloxacin exposure</b>	<b>91</b>
4.1	Introduction . . . . .	91
4.2	Ciprofloxacin susceptibilities of the <i>S. Typhimurium</i> isolates . . . . .	93
4.3	Phenotypic assessment of ciprofloxacin MIC and growth dynamics . . . . .	94
4.4	Microscopic visualization of isolates exposed to ciprofloxacin . . . . .	100
4.5	Generation of spontaneous <i>gyrA</i> mutations in D23580 and SL1344 . . . . .	107
4.6	Investigation of SNPs involved in desensitization to ciprofloxacin . . . . .	109
4.7	Discussion . . . . .	112
<b>5</b>	<b>Investigation of transcriptional response of <i>Salmonella Typhimurium</i> under ciprofloxacin exposure</b>	<b>115</b>
5.1	Introduction . . . . .	115

---

Table of contents

---

5.2	Comparative transcriptomics of four <i>S. Typhimurium</i> isolates . . . . .	117
5.3	Investigation of specificity of D23580 transcriptional response to ciprofloxacin	124
5.4	Investigation of transcriptional effect of <i>gyrA</i> mutation in <i>S. Typhimurium</i> D23580 . . . . .	129
5.5	Transcriptional profile of density-separated D23580 upon ciprofloxacin ex- posure . . . . .	131
5.6	Discussion . . . . .	137
<b>6</b>	<b>TraDIS analysis of <i>S. Typhimurium</i> D23580 during ciprofloxacin exposure and infection of intestinal organoids</b>	<b>141</b>
6.1	Introduction . . . . .	141
6.2	<i>S. Typhimurium</i> D23580 transposon mutant library quality control . . . . .	145
6.3	Required genes in <i>S. Typhimurium</i> D23580 after exposure to ciprofloxacin .	147
6.4	<i>S. Typhimurium</i> D23580 genes required for the invasion of intestinal organoids	155
6.5	Discussion . . . . .	156
<b>7</b>	<b>Investigation of genomic and phenotypic characteristics of selected <i>S. Typhimurium</i> ST313 isolates with a range of ciprofloxacin susceptibilities</b>	<b>161</b>
7.1	Introduction . . . . .	161
7.2	Genomic analyses of 108 selected African <i>S. Typhimurium</i> isolates . . . . .	165
7.3	Small-scale high content imaging screen of ciprofloxacin-exposed <i>S. Ty-</i> <i>phimurium</i> ST313 isolates . . . . .	172

---

7.4	Assessment of two distinct biofilm morphotypes in <i>S. Typhimurium</i> ST313 lineages. . . . .	183
7.5	Discussion . . . . .	187
<b>8</b>	<b>Developing and implementing a SARS-CoV-2 testing workflow in a CL2 research laboratory for screening and viral sequencing</b>	<b>191</b>
8.1	Introduction . . . . .	191
8.2	A blueprint for the implementation of a validated approach for the detection of SARS-CoV-2 in clinical samples in academic facilities. . . . .	193
8.3	Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission. . . . .	194
8.4	Effective control of SARS-CoV-2 transmission between healthcare workers during a period of diminished community prevalence of COVID-19. . . . .	194
8.5	Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study.	195
8.6	Secondary pneumonia in critically ill ventilated patients with COVID-19. . . . .	195
<b>9</b>	<b>Future directions</b>	<b>197</b>
9.1	Further development of genotype-phenotype investigations . . . . .	197
9.2	Effects of ciprofloxacin on <i>Salmonella</i> invasion of host cells . . . . .	199
9.3	Interaction of <i>S. Typhimurium</i> with additional antimicrobials . . . . .	201
	<b>References</b>	<b>203</b>

---



Table of contents

---

<b>References for COVID-19</b>	<b>263</b>
<b>Appendix A Supplementary Materials and Methods</b>	<b>267</b>
<b>Appendix B Supplementary Information to Chapter 3</b>	<b>277</b>



# List of figures

1.1	Radial phylogeny of <i>S. Typhimurium</i> . . . . .	10
1.2	<i>Salmonella</i> invasion strategies of the intestine. . . . .	13
1.3	iNTS disability-adjusted life years (DALYs)/100k. . . . .	15
1.4	African <i>S. Typhimurium</i> ST313 sub-lineage II.1 in context of other African invasive <i>S. Typhimurium</i> . . . . .	25
1.5	Structure of the fluoroquinolone ciprofloxacin. . . . .	36
1.6	Mechanisms of fluoroquinolone resistance. . . . .	39
2.1	Assembly of sucrose gradient columns. . . . .	65
3.1	High content bacterial imaging and analysis workflow. . . . .	76
3.2	Comparison of Opera Phenix plates for <i>S. Typhimurium</i> imaging. . . . .	78
3.3	Optimization of fixation and temperature conditions for <i>S. Typhimurium</i> adhesion. . . . .	79

---

3.4	Comparison of <i>S. Typhimurium</i> SL1344 adhesion on 11 coatings and uncoated wells. . . . .	80
3.5	Quantification of bacterial single cells adhered to coated plates. . . . .	81
3.6	Optimization of staining for <i>S. Typhimurium</i> . . . . .	82
3.7	Analysis pipeline steps to identify and segment bacterial objects. . . . .	83
3.8	Linear classification of bacteria into three categories. . . . .	84
3.9	Phenotyping on the Opera Phenix of <i>S. Typhimurium</i> after 2 h antimicrobial exposure. . . . .	85
3.10	Assessment of ciprofloxacin-treated <i>S. Typhimurium</i> after Opera Phenix imaging. . . . .	87
4.1	Geographic origin and phylogeny of four chosen <i>S. Typhimurium</i> isolates in a global context. . . . .	93
4.2	Example of a ciprofloxacin MIC ETEST. . . . .	96
4.3	Ciprofloxacin 24 h Time Kill Curves of four <i>S. Typhimurium</i> isolates. . . . .	97
4.4	TKC measurement of ciprofloxacin sustainability. . . . .	99
4.5	Bacteria grown in ciprofloxacin show desensitization over following 24 h. . . . .	100
4.6	Confocal imaging of <i>S. Typhimurium</i> D23580 over 24 h of ciprofloxacin exposure. . . . .	101
4.7	Confocal microscopy of ciprofloxacin sustainability after 24 h. . . . .	102
4.8	Single bacteria per well over 24 h of ciprofloxacin exposure. . . . .	104

## List of figures

---

4.9	<i>S. Typhimurium</i> bacterial length over 24 h in total single cell and 4x MIC treated population. . . . .	105
4.10	Scanning electron microscopy of <i>S. Typhimurium</i> D23580 at 2 and 8 h, 0x or 2x ciprofloxacin MIC exposure. . . . .	106
4.11	Characterization of <i>S. Typhimurium</i> SL1344 and D23580 spontaneous <i>gyrA</i> mutants. . . . .	108
4.12	SNP analysis of <i>S. Typhimurium</i> D23580 bacteria grown for 24 h in culture medium containing ciprofloxacin. . . . .	111
5.1	Heatmaps of differentially expressed genes upon 2 h of 2x ciprofloxacin MIC exposure. . . . .	118
5.2	Chromosome maps of differentially expressed genes of the four isolates upon 2 h of 2x ciprofloxacin MIC exposure. . . . .	119
5.3	Chromosome maps of differentially expressed genes of the four <i>S. Typhimurium</i> isolates after 2 h of 2x ciprofloxacin MIC exposure . . . . .	121
5.4	Heatmaps of differentially expressed genes upon 8 h of 2x ciprofloxacin MIC exposure. . . . .	123
5.5	Chromosome maps of differentially expressed genes of the four <i>S. Typhimurium</i> isolates after 8 h of 2x ciprofloxacin MIC exposure. . . . .	125
5.6	Time kill curves of D23580 $\Delta$ <i>sulA</i> and D23580 WT. . . . .	126
5.7	Heatmaps of differential expression of <i>S. Typhimurium</i> D23580 exposed to four different conditions. . . . .	127
5.8	Differential expression of <i>S. Typhimurium</i> D23580 chromosomal genes in response to four treatments. . . . .	128

---

5.9	Differential expression of D23580* <i>gyrA</i> relative to <i>S. Typhimurium</i> D23580 WT after 2 h. . . . .	130
5.10	Differential expression of <i>S. Typhimurium</i> D23580 WT and D23580* <i>gyrA</i> ciprofloxacin-treated relative to NT after 2 h growth. . . . .	132
5.11	Schematic of sucrose density gradient fractionation. . . . .	133
5.12	Light microscopy of <i>S. Typhimurium</i> D23580 separated by density fractionation. . . . .	134
5.13	Differential expression of <i>S. Typhimurium</i> D23580 bacteria separated by sucrose gradients following 2 h growth. . . . .	135
5.14	Chromosome maps of differentially expressed genes of ciprofloxacin-treated <i>S. Typhimurium</i> D23580 after sucrose density gradient separation. . . . .	136
5.15	Network analysis of genes highly downregulated in ciprofloxacin-treated <i>S. Typhimurium</i> D23580 within 60% sucrose gradient relative to 50% sucrose gradient. . . . .	137
6.1	Sequencing strategy for TraDIS. . . . .	143
6.2	Difference in coverage of matched reads of transposon library with known transposon tag depending on the mismatch threshold used. . . . .	146
6.3	Assessment of <i>S. Typhimurium</i> D23580 essential genes based on TraDIS. . . . .	148
6.4	Schematic of experiment measuring exposure of <i>S. Typhimurium</i> D23580 transposon mutant library to 2x MIC ciprofloxacin. . . . .	149
6.5	Venn diagrams of genes required for increase or decrease of ciprofloxacin susceptibility in <i>S. Typhimurium</i> D23580 at 2, 10.25, or 24 h post-exposure. . . . .	150

## List of figures

---

6.6	Metabolic pathways implicated by genes required for <i>S. Typhimurium</i> D23580 increased ciprofloxacin susceptibility at 2, 10.25, and 24 h post-exposure. . . . .	153
6.7	Chromosome maps of <i>S. Typhimurium</i> D23580 genes implicated in increased and decreased susceptibility to 2x MIC ciprofloxacin using TraDIS. . . . .	154
6.8	Schematic of organoid invasion experiment using <i>S. Typhimurium</i> D23580 transposon insertion library. . . . .	155
7.1	Phylogenetic trees of global and 108 selected African <i>S. Typhimurium</i> isolates.	166
7.2	Comparison of SNPs in coding regions found in an DCS lineage of ST313 and 82 selected <i>S. Typhimurium</i> ST313 isolates. . . . .	169
7.3	Choice of 24 <i>S. Typhimurium</i> ST313 isolates and workflow for high-content imaging. . . . .	173
7.4	Number of analysed <i>S. Typhimurium</i> ST313 bacteria analysed per well across three replicates. . . . .	174
7.5	SYTOX Green mean fluorescence intensity for each of the 24 <i>S. Typhimurium</i> ST313 isolates. . . . .	176
7.6	Measurement of the length-to-width ratio across isolates and ciprofloxacin treatments of 24 imaged <i>S. Typhimurium</i> ST313 isolates. . . . .	177
7.7	Measurement of the DAPI radial relative deviation across isolates and ciprofloxacin treatments of 24 imaged <i>S. Typhimurium</i> ST313 isolates. . . . .	179
7.8	Measurement of the CSA threshold compactness 60% across isolates and ciprofloxacin treatments of 24 imaged <i>S. Typhimurium</i> ST313 isolates. . . . .	180
7.9	Principal component analysis of morphological parameters of 24 <i>S. Typhimurium</i> ST313 isolates following 0 or 1 $\mu$ g/ml ciprofloxacin treatment. . . . .	182

7.10 Principal component analysis of morphological parameters of 5 <i>S. Typhimurium</i> ST313 isolates with “intermediate” ciprofloxacin susceptibility following four different ciprofloxacin treatments. . . . .	183
7.11 Colony morphotypes of <i>S. Typhimurium</i> bacteria grown under biofilm-forming conditions. . . . .	184
7.12 Scanning electron microscopy and phylogeny of selected <i>S. Typhimurium</i> ST313 lineage II and lineage II.1 isolates grown under biofilm-forming conditions. . . . .	186



# List of tables

2.1	<i>S. Typhimurium</i> isolates and respective ciprofloxacin MIC linked concentrations used to generate growth curves. . . . .	56
2.2	Preparation of <i>S. Typhimurium</i> D23580 and VNS20081 cultures for imaging.	61
2.3	Treatment conditions for <i>S. Typhimurium</i> D23580 and D23580* <i>gyrA</i> . . . .	64
2.4	Primer sequences for gene knockouts. . . . .	68
2.5	Components of RDAR agar plates (1 L). . . . .	72
2.6	Isolates for RDAR growth and SEM. . . . .	73
3.1	$Z'$ -statistics for the 20 most important parameters to distinguish between ciprofloxacin-treated (2x MIC) and non-treated <i>S. Typhimurium</i> . . . . .	88
4.1	Ciprofloxacin susceptibility of four <i>S. Typhimurium</i> isolates. . . . .	94
4.2	<i>In silico</i> AMR analysis of four <i>S. Typhimurium</i> isolates. . . . .	95
6.1	Genes associated with increased ciprofloxacin susceptibility in common across time points. . . . .	151

---

7.1	Coding SNPs found in highest MIC isolates compared to lower MIC isolates.	170
7.2	Drug-associated genes containing SNPs amongst ST313 isolates. . . . .	170
A.1	All <i>S. Typhimurium</i> isolates used in this thesis . . . . .	272
A.2	<i>S. Typhimurium</i> ST313 isolates phenotyped on Opera Phenix . . . . .	272
B.1	Final plate coatings chosen for isolates used in Opera Phenix imaging. . . . .	277
B.2	Gram-negative rods analysis pipeline (Harmony v4.9). . . . .	277
B.3	Antimicrobials and determined MICs used for Opera Phenix imaging optimization. . . . .	277
B.4	$Z'$ statistics of <i>S. Typhimurium</i> D23580 treated for 2 h with 2x MIC ciprofloxacin versus no treatment. . . . .	287