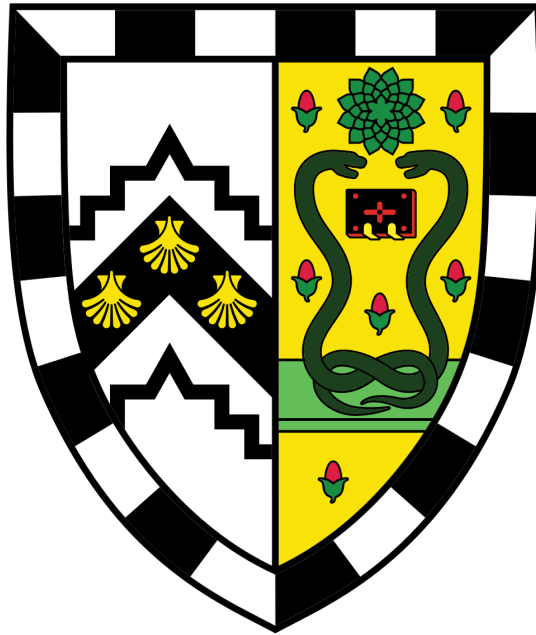


Characterising antibiotic susceptibility and resistance in human commensal gut bacteria



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Declaration

This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the word limit of 60,000 words (excluding bibliography, figures, and appendixes) as prescribed by the Degree Committee for the Faculty of Biology at the University of Cambridge.

Mr Mark Stares assisted with phenotyping gut bacteria against antibiotics and extracting DNA for whole genome sequencing. Mr Matthew Dorman assisted with cloning of candidate novel antibiotic resistance genes. Dr Simon Clare and members of his team looked after the mice used in this study and collected mouse faecal pellets. Dr Sam Forster, Dr B. Anne Neville, Dr Ana Zhu, Dr Elisa Viciani, Dr Hilary Browne and Mr Mark Stares assisted with mouse sample processing and isolating individual bacterial colonies. Dr Kevin Vervier helped analyse diversity indices in metagenomic samples.

Lindsay Jacqueline Pike

August 2019

'Life, uh... finds a way.'

Dr Ian Malcom

Jurassic Park (1993)

Dedication

For my Grampy, who always said I should be a weather girl on TV. Hopefully this isn't too much of a disappointment!

To my parents: it is only with your support and values you have instilled in me that I have achieved all that I have.

Unconventionally, as ever, I also dedicate this thesis to myself: it stands as a monument to my determination, tenacity and strength.

Abstract

The human commensal gut microbiota can act as a reservoir of antimicrobial resistance genes that can persist and spread to pathogens. However, the extent and diversity of antibiotic resistance encoded by human commensal bacteria remains to be determined. Due to immediate clinical relevance and our previous inability to culture these commensal bacteria, the majority of research into antibiotic resistance has focused on pathogenic organisms or well-characterized antibiotic resistance mechanisms. Here, I demonstrate the existence of unpredicted antibiotic resistance, not detected by several genome-based prediction methods, in diverse bacterial species from the human gastrointestinal tract.

178 antibiotic resistance genes and mutations were identified in a culture collection of 737 phylogenetically diverse gut bacteria from healthy humans. Recent developments in culturing anaerobic gut bacteria were used to determine antibiotic sensitivity phenotypes and observe the spectrum of clinically relevant antibiotics across the diversity of these isolates. These data were combined to assess the accuracy of genome-based predictions in human commensal gut bacteria, revealing multiple instances of unpredicted antibiotic resistance. This highlights the importance of combining computational genomic prediction with functional validation and increases our knowledge of antibiotic resistance in commensal human gut bacteria.

In addition, the impact of therapeutic amoxicillin treatment on antibiotic resistance in mice with human-derived gut microbiota was studied. These experiments model processes in humans and reveal community- and strain-level changes in antibiotic resistance following antibiotic therapy. These experiments further elucidate the role of the gut microbiota as a reservoir of antibiotic resistance and the influence of antibiotics on this reservoir.

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Publications

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Contents

Declaration.....	iii
Dedication.....	vii
Abstract.....	ix
Acknowledgements.....	xi
Publications.....	xv
Contents.....	xvii
List of Figures.....	xxi
List of Tables.....	xxvii
Glossary.....	xxix
Chapter 1: Antibiotics and commensal gut bacteria.....	1
1.1 Antibiotics.....	1
1.2 The history of antibiotics.....	2
1.3 Clinically relevant antibiotics.....	6
1.3.1 <i>Access antibiotics: first or second line of defence</i>	11
1.3.2 <i>Watch antibiotics: first or second line of defence with high resistance potential</i>	15
1.3.3 <i>Reserve antibiotics: last resort</i>	17
1.4 Bacterial genetics and antibiotic resistance.....	20
1.4.1 <i>Intrinsic resistance</i>	21
1.4.2 <i>DNA mutations</i>	21
1.4.3 <i>Antibiotic resistance genes and horizontal gene transfer</i>	22
1.5 Antibiotic resistance is a major global issue.....	24
1.6 Antibiotic misuse and overuse: a One Health problem.....	28

1.7 The gut microbiome as a reservoir of antibiotic resistance.....	33
1.8 Studying antibiotic resistance in bacteria.....	37
1.8.1 <i>Phenotyping</i>	37
1.8.2 <i>Genome sequencing</i>	39
1.9 Thesis aims.....	46
Chapter 2: Materials and methods.....	47
2.1 Anaerobic gut bacteria culture collection.....	47
2.2 Genome-based predictions of antibiotic resistance in the HBC.....	47
2.3 Genome-based predictions of antibiotic resistance in pathogenic genomes.....	49
2.4 Phenotypic antibiotic sensitivity in commensal gut bacteria.....	50
2.5 Defining a scale for categorising resistant/susceptible phenotypes.....	53
2.6 Further investigations of Unpredicted Resistance.....	54
2.7 Humanised microbiota mouse experiments.....	57
2.8 Colony count data.....	58
2.9 Isolation of individual isolates and analysis.....	59
2.10 Metascape and metagenomic analysis.....	61
Chapter 3: Characterisation of genomic antibiotic resistance in commensal gut bacteria.....	63
3.1 Introduction.....	63
3.2 Results.....	65
3.2.1 <i>Summary of resources used in this chapter</i>	65
3.2.2 <i>Computational predictions of antibiotic resistance in 737 whole genome sequences of anaerobic gut bacteria</i>	69
3.2.3 <i>Variation of predicted genomic resistance across the four key gut bacteria phyla</i>	72
3.2.4 <i>Variation of predicted genomic resistance across different human commensal bacterial families</i>	79

3.2.5	<i>Distribution of predicted genomic resistance between known and novel isolates</i>	81
3.2.6	<i>Comparison of predicted genomic resistance in commensal versus pathogenic isolates</i>	84
3.3	Discussion	90

Chapter 4: Determination of phenotypic antibiotic resistance in commensal gut bacteria and the accuracy of genotypes..... 99

4.1	Introduction	99
4.1.1	<i>Overview</i>	99
4.1.2	<i>Defining isolates as antibiotic-susceptible or -resistant</i>	100
4.2	Results	102
4.2.1	<i>Phenotypic screening of antibiotic resistance in a subset of 73 HBC isolates</i>	102
4.2.2	<i>Comparison of zone of inhibition sizes between isolates with and without genetic antibiotic resistance determinants</i>	107
4.2.3	<i>Defining a system for categorising gut bacteria as resistant or susceptible to antibiotics and considering the spectrum of antibiotics</i>	108
4.2.4	<i>Comparison of genomic predictions of antibiotic resistance with bacterial phenotypes and identification of unpredicted resistance</i>	111
4.2.5	<i>Comparison of antibiotic resistance databases and prediction methods</i>	118
4.2.6	<i>Identifying enrichment of unpredicted resistance to certain antibiotics in particular phyla</i>	120
4.2.7	<i>Further investigations of unpredicted resistance</i>	122
4.2.8	<i>Searching for novel antibiotic resistance determinants in human gut commensal microbiota</i>	125
4.3	Discussion	137

Chapter 5: Modelling the development of antibiotic resistance <i>in vivo</i>	145
5.1 Introduction.....	145
5.2 Results.....	148
5.2.1 <i>Overview of mouse models</i>	148
5.2.2 <i>Impact of amoxicillin on the bacterial load in mice with humanised gut microbiota</i>	152
5.2.3 <i>Deep culturing and whole genome sequencing to improve taxonomic classification of metagenomic data</i>	155
5.2.4 <i>Impact of amoxicillin on the amoxicillin-resistant community</i>	164
5.2.5 <i>Characterisation of strain- and sequence-level changes in gut microbiota following exposure to amoxicillin</i>	172
5.3 Discussion.....	180
Chapter 6: Discussion	187
6.1 Key messages and future work.....	187
6.2 Concluding remarks.....	193
Bibliography	195
Appendix I: CARD determinant groupings	215
Appendix II: HBC antibiotic resistance determinant groupings	221
Appendix III: Gut microbiota community composition in mice with human-derived gut microbiota	232

List of Figures

Figure 1.1: Timeline of antibiotic discovery.....	3
Figure 1.2: Mechanism of action for antibiotics on the WHO List of Essential Medicines (2017)	6
Figure 1.3: Proportions of 538 species of bacterial pathogens belonging to particular phyla	26
Figure 1.4: Global aminopenicillin (including amoxicillin) resistance in <i>Escherichia coli</i>	27
Figure 1.5: Usage of antibiotics in the United Kingdom and Turkey.....	31
Figure 3.1: Phylogeny of the HBC commensal gut bacteria.....	66
Figure 3.2: The proportions of antibiotic categories in the Comprehensive Antibiotic Resistance Database.....	68
Figure 3.3: Proportions of 178 antibiotic resistance determinants identified in 737 isolates of human gut bacteria.....	70
Figure 3.4: Summary of genetic determinants of antibiotic resistance in the HBC.....	71
Figure 3.5: Observations of predicted antibiotic resistance in the HBC isolates against the core genome phylogeny.....	73
Figure 3.6: Proportions of isolates with at least one genetic antibiotic resistance determinant in each phyla, compared to the overall HBC.....	74

Figure 3.7: Interquartile range of number of antibiotic resistance determinants per isolate in each phyla, compared to the overall HBC.....	75
Figure 3.8: Interquartile range of number of antibiotic classes resistance is predicted to per isolate in each phylum, compared to the overall HBC.....	76
Figure 3.9: Proportions of isolates with at least one genetic antibiotic resistance determinant in taxonomic families.....	79
Figure 3.10: Interquartile range of number of antibiotic resistance determinants per isolate in commensal gut bacterial families.....	81
Figure 3.11: Proportions of resistant isolates in known versus novel isolates.....	82
Figure 3.12: Proportions of antibiotic categories that resistances are predicted against in HBC genomes.....	84
Figure 3.13: Interquartile range of number of antibiotic resistance determinants per isolate in pathogenic bacterial species.....	86
Figure 3.14: Proportions of antibiotic categories that resistances are predicted against in pathogenic bacterial genomes.....	87
Figure 3.15: Interquartile range of number of antibiotic resistance determinants per isolate in commensal versus pathogenic isolates.....	88
Figure 3.16: Proportions of antibiotic categories that resistances are predicted against in commensal HBC versus pathogenic bacterial genomes.....	89

Figure 4.1: A phylogeny of 73 isolates from the HBC selected for selective phenotypic screening of antibiotic sensitivity.....	103
Figure 4.2: Density curves of zone of inhibition size among 73 isolates of the four main phyla of human gut bacteria.....	106
Figure 4.3: Range of average zone of inhibition between isolates with and without the presence of genetic resistance determinants.....	108
Figure 4.4: Scale to define antibiotic resistance and susceptibility in human gut microbiota.....	109
Figure 4.5: Distribution of antibiotic sensitivity genotype/phenotype combinations in 73 phylogenetically diverse isolates of human gut bacteria reveals many “unpredicted” resistances.....	113
Figure 4.6: The proportion of genotype/phenotype combinations for each phylum.....	115
Figure 4.7: The proportion of genotype/phenotype combinations for each antibiotic.....	117
Figure 4.8: The proportion of genotype/phenotype combinations for each resistance database or method tested.....	119
Figure 4.9: The percentage of Unpredicted Resistance antibiotic genotype/phenotype combinations by which phyla those combinations were observed in.....	121
Figure 4.10: Ranking of isolates by ceftriaxone sensitivity.....	124
Figure 4.11: Comparison of ceftriaxone sensitivity in two sets of isolates from the HBC.....	126

Figure 4.12: Ceftriaxone MIC in isolates with and without the Group 2384 candidate beta-lactamase gene.....	130
Figure 4.13: Group 2384 gene sequences from twelve HBC isolates.....	132
Figure 4.14: GeneArt construct containing Group 2384 candidate beta-lactamase gene.....	133
Figure 4.15: Gene phylogenies of candidate shared beta-lactamases in three HBC <i>Bacteroides faecis</i> isolates.....	136
Figure 5.1: Diagram of experiments assessing the impact of amoxicillin on mice with human-derived gut microbiota.....	151
Figure 5.2: Bacterial load over the course of humanised microbiota mouse amoxicillin model experiments.....	153
Figure 5.3: Proportion of classified metagenomic sequences reads from metascrapes of cultured faecal bacteria from mice with humanised gut microbiota.....	156
Figure 5.4: Diversity of the contigs assembled de novo from unclassified metagenomic sequence reads.....	158
Figure 5.5: A rarefaction curve of the number of OTUs observed against the number of colonies picked.....	159
Figure 5.6: A phylogeny of the consensus sequences of 367 OTUs identified from 8838 full length 16S sequences generated in this study.....	160
Figure 5.7: Diversity of OTUs isolated by culture from mice with human-derived microbiota.....	161

Figure 5.8: The phylogenetic relationship between the HBC and 198 new genomes generated in this study.....	162
Figure 5.9: Comparisons of proportion of classified metagenomic sequences reads from metascrapes of cultured faecal bacteria from mice with humanised gut microbiota using different databases of reference bacterial genomes.....	163
Figure 5.10: Changes in alpha diversity in metascrapes of cultured faecal bacteria from mice with humanised gut microbiota treated with amoxicillin.....	165
Figure 5.11: Changes in beta diversity in metascrapes of cultured faecal bacteria from mice with humanised gut microbiota treated with amoxicillin.....	167
Figure 5.12: Relative abundance of species in metascope samples cultured from mice following amoxicillin therapy.....	169
Figure 5.13: A phylogeny of the <i>cfxA</i> gene from 46 genomes from isolates cultured from mice with human-derived microbiota.....	175
Figure 5.14: Relative abundance of <i>Odoribacter splanchnicus</i> in metascope samples cultured from mice following amoxicillin therapy.....	176
Figure 5.15: The key findings from the humanised microbiota mouse experiments.....	181
Appendix III Figure A3.1: The relative abundance of species cultured from stool of Donor 2- and Donor 7-derived mice under anaerobic vegetative conditions.....	231
Appendix III Figure A3.2: The relative abundance of amoxicillin-resistant species cultured from stool of Donor 2- and Donor 7-derived mice under anaerobic vegetative conditions.....	232

Appendix III Figure A3.3: Antibiotic resistance genes (ARGs) identified in Donor 2- and Donor
7-derived mice 233

List of Tables

Table 1.1: Sources of antibiotics.....	5
Table 1.2: The antibiotics included in the 20 th Edition of the WHO List of Essential Medicines (2017).....	7
Table 1.3: Summary of databases and tools for predicting antibiotic resistance genotypes from sequence data.....	41
Table 2.1: Modified YCFA media.....	51
Table 2.2: Single-concentration antibiotic disks used for phenotypic susceptibility testing...	52
Table 3.1: Taxonomic information for the HBC.....	67
Table 3.2: The proportion of isolates with resistance to particular antibiotic categories is compared between phyla and the overall HBC.....	78
Table 4.1: Single-concentration antibiotic disks used for phenotypic sensitivity testing.....	104
Table 4.2: Zone of inhibition limits used to categorise isolates of human gut bacteria as antibiotic-resistant or -susceptible.....	110
Table 4.3: Genotype/phenotype combinations of antibiotic sensitivity.....	111
Table 4.4: Numbers of resistant-unique and shared core genes with similarity to beta-lactamase markers in the human gut bacteria isolate <i>Bacteroides faecis</i> 18048_2#66.....	127

Table 4.5: A summary of candidate beta-lactamases that may explain an unpredicted ceftriaxone resistance phenotype observed in the human gut bacteria isolate <i>Bacteroides faecis</i> 18048_2#66	128
Table 5.1: Summary of experiments assessing the impact of amoxicillin on mice with human-derived gut microbiota and samples or data generated	152
Table 5.2: Candidate OTUs of potential increase in amoxicillin resistance following <i>in vivo</i> exposure to amoxicillin	173
Table 5.3: Mutations in open reading frames and non-coding regions of gut bacteria isolated after amoxicillin therapy in mice with human-derived gut microbiota	178
Appendix I Table A1.1: Genetic determinants of antibiotic resistance as described in CARD were grouped by the antibiotic to which they are described as conferring resistance	215
Appendix II Table A2.1: The genetic determinants of antibiotic resistance predicted in the HBC genomes, as described in CARD, were grouped by the antibiotic to which they are described as conferring resistance	221
Appendix II Table A2.2: A complete list of HBC isolates and the antibiotic resistance genes and mutations identified in their genomes	230

Glossary

AMR: antimicrobial resistance

AMX: amoxicillin

ANI: average nucleotide identity

ARIBA: Antibiotic Resistance Identification By Assembly

ARG: antibiotic resistance gene

AST: antibiotic susceptibility testing

BNF: British National Formulary

Bp: base pairs

BSAC: British Society of Antimicrobial Chemotherapy

CARD: Comprehensive Antibiotic Resistance Database

CARD-RGI: Comprehensive Antibiotic Resistance Database – Resistance Gene Identifier

CFU: colony forming units

CIA: critically important antimicrobials

CLSI: Clinical Laboratory Standards Institute

Confirmed Resistance: genetic resistance and phenotypic resistance to a particular antibiotic both observed in an isolate

Confirmed Susceptibility: no genetic or phenotypic resistance to a particular antibiotic observed in an isolate

CRE: carbapenem-resistant Enterobacteriaceae

D7AMX1: the first Donor 7 mouse experiment performed in this study

D-Ala-D-Ala: D-Alanyl-D-Alanine

DDD: defined daily doses

DRI: drug-resistant infection

ESBL: extended-spectrum beta-lactamase

ESKAPE: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.

EUCAST: European Committee on Antibiotic Susceptibility Testing

FMT: faecal microbiota transplant

GF: germ-free

GI: gastrointestinal

HAI: hospital acquired infection

HBC: Human Gastrointestinal Bacteria Culture Collection

HGT: horizontal gene transfer

iTOL: Interactive Tree of Life

KAPE: *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.

LJP01: the second Donor 7 mouse experiment performed in this study

LJP02: the Donor 2 mouse experiment performed in this study

MAG: metagenome assembled genome

MDR: multi-drug resistant

MGE: mobile genetic element

MIC: minimum inhibitory concentration

MLPS: Macrolide, Lincosamide, Pleuromutilin and Streptogramin antibiotics

MRSA: methicillin-resistant *Staphylococcus aureus*

NGS: next generation sequencing

ORF: open reading frame

OTU: operational taxonomic unit

PATRIC: Pathosystems Resource Integration Center

PBPs: penicillin-binding proteins

R&D: research and design

SEM: standard error of the mean

SNP: single nucleotide polymorphism

STC: sodium taurocholate

TB: tuberculosis

Unpredicted Susceptibility: also referred to as “False Positive”; genetic resistance predicted to a particular antibiotic in an isolate, but the isolate was phenotypically susceptible

Unpredicted Resistance: also referred to as “False Negative”; no genetic resistance to a particular antibiotic predicted in an isolate, but the isolate was phenotypically resistant

UTI: urinary tract infection

VRE: vancomycin resistant enterococci

WGS: whole genome sequencing

WGS-AST: whole genome sequencing based antibiotic susceptibility testing

WHO: World Health Organisation

WSI: Wellcome Sanger Institute