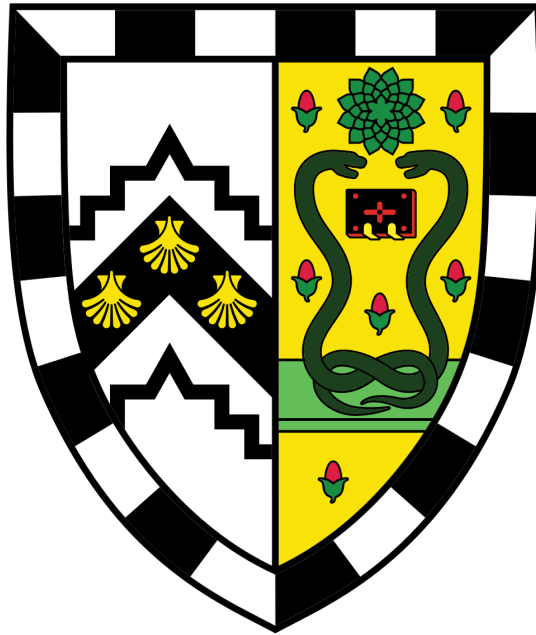


Characterising antibiotic susceptibility and resistance in human commensal gut bacteria



Lindsay Jacqueline Pike

Gonville and Caius College, University of Cambridge

Wellcome Sanger Institute

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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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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