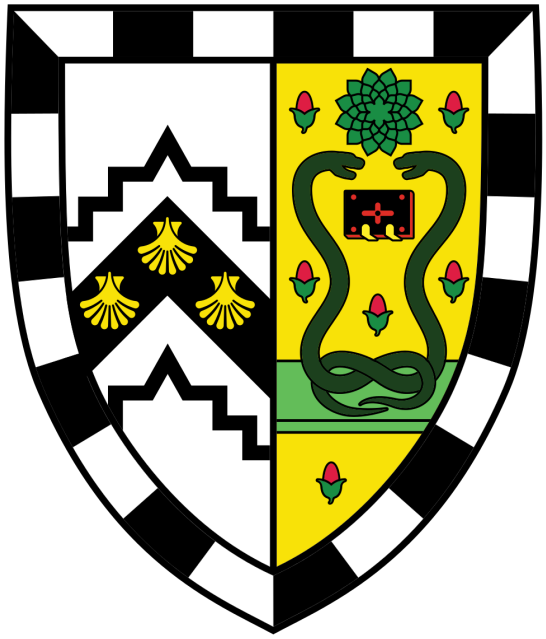
Characterising antibiotic susceptibility and resistance in human commensal gut bacteria



Lindsay Jacqueline Pike

Gonville and Caius College, University of Cambridge

Wellcome Sanger Institute

August 2019

This dissertation is submitted for the degree of Doctor of Philosophy

Supervised by Dr Trevor Lawley, Host-Microbiota Interactions Laboratory

Funded by the Medical Research Council and the Wellcome Sanger Institute

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.

Acknowledgements

Firstly, I must thank my supervisor, Dr Trevor Lawley, for his guidance throughout the four years of my PhD. He has very astute insight into project directions and the future of our field, which has been enormously helpful. In addition, he was very understanding when I experienced some personal issues over the last few years and supportive of my future aspirations, for which I am very grateful.

In addition, I would like to thank Professor Stephen Baker and Dr Estee Torok as members of my thesis committee. In addition, to Professor Nick Thompson, a member of the Committee of Graduate Studies at Sanger during my PhD. Their perspectives, advice and support – both academic and pastoral – were always gratefully received and helped to shape this thesis, as well as keeping me motivated at the times I found myself struggling.

I would also like to thank Professor Gordon Dougan and Professor Julian Parkhill – I have been extremely fortunate in receiving advice from these two eminent microbiologists due to the collaborative and supportive nature of the Parasites and Microbes programme at Sanger.

Dr Hilary Browne and Dr Sam Forster have demonstrated immense patience and diligence whilst reading and providing feedback on the various drafts of the chapters contained within this thesis. It is thanks to them and Trevor that my scientific writing has developed from when I arrived straight out of my undergraduate degree. It is also mainly thanks to Sam and Dr Nitin Kumar that my bioinformatics skills have advanced. I also owe Hilary, Nitin and Sam thanks for answering my endless questions – they are fountains of knowledge and expert scientists and I have learnt much from them. This is extended to all other current members of the Host-Microbiota Interactions Laboratory (Team 162): Mr Nick Dawson (the brave culture collection curator), Dr Junyan Liu (the plasmid expert), Yan Shao (the metagenomics magician), Mr Mark

Stares (the lab wizard), Dr Kevin Vervier (the statistics king), Dr Ana Zhu (the bioinformatics and now mouse experiment queen). In addition, to previous Team 162 members: Mr Matt Dunn (the fermenter guru), Dr B. Anne Neville (the most enthusiastic microbiologist I have ever met), Dr Giulia Falivelli (a role model for how to balance science and family), Dr Fernanda Schrieber (the cell culture pro), and Dr Elisa Viciani (an extremely diligent scientist). Thank you for your wisdom, patience, camaraderie and friendship.

Special thanks in particular must go to Sam, who assisted with the long days of mouse experiments, and anyone else who helped pick colonies in those times (at least Anne, Ana, Elisa, Mark). Also to Mark, for assisting with phenotyping gut bacteria against antibiotics and extracting DNA for whole genome sequencing – I would not have been able to perform as many tests or sequence as many bacteria without your help. Plus, to Mr Matthew Dorman, who lent time, knowledge and hands to plasmid cloning experiments. In addition, to Dr Simon Clare, Ms Cordelia Brandt, and other members of Simon's team for collecting mouse stool samples for this study. Finally, to Sam, Nitin and Kevin who helped me write scripts to analyse my data.

Thank you also to the Graduate Office at Sanger for their support: Dr Annabel Smith, Dr Christina Hedberg-Delouka and Dr Carl Anderson are always there to answer questions via email or when cornered unexpectedly in their office or the DiNA. Also, to Carl and other members of CoGS for giving me the opportunity to do the PhD at Sanger in the first place. It hasn't been easy, but I have always tried to remind myself how lucky I have been to study here and I am so glad to have done it.

On a more personal level, I would like to thank my fellow Sanger PhD buddies, Fiona Calvert and Tapoka Mkandawire. This triangle of mutual support has kept me going more times than

you will know and I can't wait to see where we go after our PhDs. I would like to extend this to my friends I have met through Gonville and Caius College, where I had the fortune of being part of a wonderful graduate community. The College porters as well deserve their own mention for making Caius such a special, friendly place to be. It truly has been my home for the last four years.

My friends from "home home" have also been instrumental in helping me stay grounded and in touch with reality (a PhD should not be your whole life!) – Katie, Natalie, Bonita, Christina, Robin, Adam, thank you for still being here despite my frequent moaning about PhD life when truthfully it's been pretty great overall. Also to my friends I met as an undergraduate – Suzy, Becky, Megan, Sophie, we've all been continuing further education at the same time and it's been enormously helpful to have that support network as well. I only hope I've been as supportive to you as you have been to me.

To a recent addition in my support network, Will ("Dr Dr" Hamilton) – thank you for reminding me how to enjoy science. With your positivity and encouragement I have enjoyed my final year and writing this thesis much more than I expected!

Finally, to all of my family – especially my mum Cathy, dad Julian and grandma Frances (my biggest fan) – thank you for your unconditional support not just over the last four years but the last 27. My success is all thanks to you.

Publications

Arising elsewhere:

Pike, L. J., Viciani, E. & Kumar, N. Genome watch: Microbial diversity knows no borders. *Nat Rev Microbiol* **16** (2), 66 (2018).

Pike, L. J. & Forster, S. C. Genome watch: A new piece in the microbiome puzzle. *Nat Rev Microbiol* **16** (4), 186 (2018).

Forster, S. C., Kumar, N., Anonye, B. A., Almeida, A., Viciani, E., Stares, M. D., Dunn, M., Mkandawire, T. T., Zhu, A., Shao, Y, **Pike, L. J.,** Louie, T., Browne, H. P., Mitchell, A. L., Neville, B. A., Finn, R. D. & Lawley, T. D. A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nat Biotechnol* **37**, 186-192 (2019).

Contents

List of Figures

List of Tables

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