Transmission and evolution of bacteria during the course of enteritis outbreaks

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Abstract

Bacterial enteritis outbreaks are a worldwide problem. They are hard to investigate as the bacterial agents are often associated with multiple sources, closely-related bacteria often co-colonise these sources, highly discriminatory tests are often required to distinguish between these bacteria, and bacteria are continuously evolving, changing how they behave. In this thesis I investigated the transmission and evolution of bacteria over the course of enteritis outbreaks by integrating genomic, phenotypic and antibiotic susceptibility testing, and phylogenetic modelling in four studies.

The aim of the first study was to investigate the origin, evolution and transmission of *Salmonella enterica* serovar Typhimurium DT160 over a 14-year long outbreak in New Zealand. Genomic analysis of 109 DT160 isolates collected over this timeframe established that the DT160 strain was introduced into New Zealand approximately a year before the first human isolate was reported; there were frequent transmissions between the source groups investigated (human, wild bird, poultry and bovine); and there was no evidence of specific selective pressures imposed on DT160. This study demonstrated how genomic analyses can be used to investigate extended outbreaks of bacterial diseases.

The aim of the second study was to investigate whether two ancestral state reconstruction models (the discrete trait analysis and structured coalescent models) were applicable to salmonellosis outbreak investigations. Both models were used to estimate transmission and population parameters of simulated salmonellosis outbreaks. Comparisons between the models' estimates and the true transmission and population values for the simulations revealed that both models made assumptions that did not apply to outbreaks and prevented them from accurately predicting these parameters. This study highlighted the need for outbreak-specific phylogenetic transmission models.

The aim of the third study was to investigate the relationship between two strains of *Salmonella* that were the predominant causes of human salmonellosis in New Zealand in the 2000s (*S.* Typhimurium DT160 and *S.* Typhimurium DT56 variant), and identify potential reasons for one strain declining (DT160) as the other emerged (DT56 variant). This study demonstrated how genomic analyses can be used to compare *Salmonella* strains and identify genetic elements that may influence strain behaviour.

The aim of the fourth study was to investigate a patient that had presented excreting the same genotype of *Campylobacter*, *C. jejuni* ST45, on multiple occasions over a 10-year period. Genomic analyses, phenotypic testing and antimicrobial susceptibility testing of sixteen *Campylobacter* isolates collected from the

patient found that the patient was persistently colonised with *Campylobacter* over this period, and that the *Campylobacter* had adapted to long-term colonisation by altering its motily and developing resistance to the antibiotics the patient had been prescribed. This study demonstrated how genomic analyses can be used to investigate a patient's infection history.

These studies demonstrated the applicability and limitations of genomic analyses when investigating bacterial enteritis outbreaks, how genetics and the environment influence bacterial evolution, and highlighted areas in the fields of microbiology, phylogenetics, epidemiology and public health that require further research.

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Abbreviations

µl ..... Microlitre

°C ..... Degrees Celsius

×g ..... Times gravity

bp ..... Base pairs

CDC ..... Centre for Disease Control and Prevention

C. difficile ..... *Clostridium difficile*

CE ..... Common Era

C. jejuni ..... *Campylobacter jejuni*

CI ..... Confidence interval

COG ..... Cluster of Orthologous Group

CRC ..... Canterbury Regional Council

CVID ..... Common variable immune deficiency

Df ..... Degrees of freedom

DNA ..... Deoxyribonucleic acid

DT ..... Definitive type

DTA ..... Discrete trait analysis

EFSA ..... European Food Safety Authority

E. coli ..... *Escherichia coli*

ENA ..... European Nucleotide Archive

ESR ..... Institute of Environmental Science and Research

EUCAST ..... European Society of Clinical Microbiology and Infectious Diseases

GC ..... Guanine-cytosine

GMRF ..... Gaussian Markov Random Fields

GTR ..... Generalised time reversible

GWRC ..... Greater Wellington Regional Council

HBRC ..... Hawke's Bay Regional Council

HKY ..... Hasegawa, Kishino and Yano

HPD ..... Highest posterior density

HIV ..... Human immunodeficiency virus

IgA ..... Immunoglobulin A

IgG ..... Immunoglobulin G

INDELS ..... INsertions/DELetions

IVABS ..... Institute of Veterinary, Animal and Biological Sciences

kb ..... Kilo base pairs

Mb ..... Mega base pairs

MCDHB ..... MidCentral District Health Board

MCMC ..... Markov chain Monte Carlo

MDS ..... Multidimensional scaling

^{m}EpiLab ..... Massey University Molecular Epidemiology and Public Health Laboratory

ml ..... Millilitre

mm ..... Millimetre

mM ..... Millimolar

MLST ..... Multilocus sequence typing

MSE ..... Mean squared error

MSS ..... Mean sum of squares

MSSS ..... Manawatu sentinel surveillance site

NCBI ..... National Centre for Biotechnology Information

NZGL ..... New Zealand Genomics Limited

P. aeruginosa ..... *Pseudomonas aeruginosa*

PBS ..... Phosphate-buffered saline

PCR ..... Polymerase chain reaction

PFGE ..... Pulsed-field gel electrophoresis

PNMRF ..... Palmerston North Medical Research Fund

SC ..... Structured coalescent

S. enterica ..... *Salmonella enterica*

***S.* Enteritidis** ..... *Salmonella enterica* serovar Enteritidis

SIR ..... Susceptible-Infected-Recovered

SNP ..... Single nucleotide polymorphism

SS ..... Sum of squares

ST ..... Sequence type

STEC ..... Shiga toxin-producing *Escherichia coli*

***S.* Typhi** ..... *Salmonella enterica* serovar Typhi

***S.* Typhimurium** ..... *Salmonella enterica* serovar Typhimurium

UK ..... United Kingdom

US ..... United States

WHO ..... World Health Organisation

XML ..... Extensible Markup Language

Publications

Journals

Bloomfield, S.J., Benschop, J., Biggs, P.J., Marshall, J.C., Hayman, D.T.S., Carter, P.E., Midwinter, A.C., Mather, A.E. and French, N.P. (2017) Genomic analysis of *Salmonella enterica* serovar Typhimurium DT160 associated with a 14-year outbreak, New Zealand, 1998-2012. *Emerging Infectious Diseases* 23: 906-913

Conferences

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