Improving treatment outcomes and prognosticating gastric cancer risk in patients with *Helicobacter pylori* infection

By

Jonathon Paul Schubert

BSc, BEng(Hons I), MD, FRACP

A1161162

A thesis submitted for the degree of

Doctor of Philosophy

University of Adelaide

School of Medicine

Faculty of Health and Medical Sciences

November 2023

Table of contents

Contents

List of Figures

List of Tables

Publications arising from this thesis

Articles published

1. Schubert, J. P., Gehlert, J., Rayner, C. K., Roberts-Thomson, I. C., Costello, S., Mangoni, A. A., & Bryant, R. V. (2021). **Antibiotic resistance of Helicobacter pylori in Australia and New Zealand: A systematic review and meta-analysis**. *Journal of gastroenterology and hepatology*, *36*(6), 1450–1456.
2. Schubert, J. P., Warner, M. S., Rayner, C. K., Roberts-Thomson, I. C., Mangoni, A. A., Costello, S., & Bryant, R. V. (2022). **Increasing Helicobacter pylori clarithromycin resistance in Australia over 20 years**. *Internal medicine journal*, *52*(9), 1554–1560.
3. Schubert, J. P., Woodman, R. J., Mangoni, A. A., Rayner, C. K., Warner, M. S., Roberts-Thomson, I. C., Costello, S. P., & Bryant, R. V. (2022). **Geospatial analysis of Helicobacter pylori infection in South Australia: Should location influence eradication therapy?** *Journal of gastroenterology and hepatology*, *37*(7), 1263–1274.
4. Schubert, J. P., Ingram P.R., Warner M.S., Rayner C.K., Roberts-Thomson I.C., Costello S.P., Bryant R.V. (2023). **Refractory Helicobacter pylori infection in Australia: updated multicentre antimicrobial resistance** [published online ahead of print, 2023 Sep 13]. *Internal medicine journal*; 10.1111/imj.16226. doi:10.1111/imj.16226
5. Schubert JP, Rayner CK, Costello SP, Roberts-Thomson IC, Forster SC, Bryant RV. ***Helicobacter pylori*: Have potential benefits been overlooked?**. *JGH Open*. 2022;6(11):735-737. Published 2022 Nov 16. doi:10.1002/jgh3.12842

Articles in submission or under revision

6. Schubert, J. P., Tay C.Y., Lee, K.H, Leong, L., Rayner C.K., Warner M.S., Roberts-Thomson, I.C., Costello S.P., Bryant R.V. (2023). **Genomic analysis of Helicobacter pylori in Australia – antimicrobial resistance, phylogenetic patterns and virulence factors**. Submitted to *Helicobacter,* November 2023

Articles 1, 3 and 6 are published in American journals and the original American English spelling has been retained in these manuscripts in this thesis.

Abbreviations

BMI – Body mass index

cagPAI - cag Pathogenicity Island

GORD / GERD – Gastro oesophageal reflux disease

GWR – Geographically weighted regression

IARC - International Agency for Research on Cancer

IBD – Inflammatory bowel disease

IF – Impact factor

MALT - Mucosa-associated lymphoid tissue

MLST – Multi locus sequence typing

PBS – Pharmaceutical benefits scheme

P-CABs - Potassium-competitive acid blockers

PCR – Polymerise chain reaction

PPI - Proton pump inhibitor

QRDR - Quinolone resistance-determining regions

SA - South Australia

SNP - Single-nucleotide polymorphism

UBT – Urea breath test

vacA - Vacuolating cytotoxin gene A

WA - Western Australia

WGS – Whole genome sequencing

WHO – World Health Organisation

Abstract

Introduction

Helicobacter pylori (*H. pylori*) infection causes chronic gastritis and is strongly associated with the development of gastric cancer, peptic ulceration and mucosa-associated lymphoid tissue (MALT) lymphoma. Up to 90% of gastric cancer, which is the fourth leading cause of cancer mortality globally, is attributable to *H. pylori* infection. Treatment for people with *H. pylori* infection aims to eradicate the organism to reduce the risk of morbidity and mortality. Eradication of *H. pylori* has been shown to improve clinical outcomes; however, rates of eradication have been falling in the setting of rising antimicrobial resistance.

A contemporary and robust understanding of antimicrobial resistance patterns in a local and global setting is essential for prescribing *H. pylori* eradication therapy. In addition, an understanding of the risk factors associated with poor outcomes of *H. pylori* infection as well as treatment failure is required, to identify those most a risk of adverse outcomes and to prioritise testing and eradication therapy accordingly. This will lead to optimisation of eradication therapy and ultimately lower the morbidity and mortality associated with this common infectious bacteria.

Taking a broader view, *H. pylori* has co-evolved with humans over tens of thousands of years, and numerous studies have found an association with immune mediation. It plays important roles in immune signalling and its presence is inversely associated with gastro oesophageal reflux disease (GORD) and oesophageal adenocarcinoma. In the face of rising antimicrobial resistance, the difficulty of eradicating *H. pylori* is increasing. Treatment algorithms need to be individualised, including consideration of whether *H. pylori* eradication is needed and which combination of medications to select.

Aims

The aims of this thesis were to:

- Explore antimicrobial resistance patterns of *H. pylori* in Australia
- Characterise the prevalence, geographical origins and temporal trends of antimicrobial resistant *H. pylori* in Australia
- Assess risk factors for adverse clinical outcomes from *H. pylori* infection and guide best practice for eradication therapy

Methods

A systematic review and meta-analysis of resistance patterns in Australia and New Zealand was performed. Following this, a retrospective observational study was undertaken on resistance patterns locally over the last 20 years at a single tertiary hospital in Adelaide, South Australia. A multifactorial geospatial analysis was performed on the distribution of resistance in order to characterise the geographic factors associated with resistance. A multi-centre, prospective observational study was undertaken to provide updated national patterns of resistance as a guide to decision-making for antimicrobial eradication therapy. Prospective, whole genome sequencing of isolates was carried out to characterise genomic patterns of antimicrobial resistance and determine concordance with phenotypic resistant profile. Finally, genetic mutation analysis was used to determine the genetic region of origin of isolates and the virulence factors associated with adverse clinical outcomes, most notably gastric cancer.

Results

A paucity of recent *H. pylori* antimicrobial resistance data was identified in the systematic review and meta-analysis. Despite this, based on the best available information, rising rates of antimicrobial resistance were found, but there were few data over the last decade to guide clinical decision-making.

A retrospective analysis of local South Australian data found that rates of resistance had increased markedly over 20 years. In particular, rates of antimicrobial resistance to clarithromycin were at odds with current recommendations for its use in first-line antimicrobial therapy, bringing into question the need to revise current guidelines. Further analysis of geographical trends demonstrated clustering of antimicrobial resistance within migrant populations, emphasising the need for updated antimicrobial resistance data to guide appropriate eradication therapy in these groups. Moreover, the identification of populations that are at elevated risk of unsuccessful eradication therapy allows for a more individualised, rather than empiric, approach to eradication therapy.

A multi-centre study of contemporary rates of antimicrobial culture-based resistance from patients in Australia identified a high prevalence of antimicrobial resistance nationally, suggesting that current antimicrobial guidelines are unlikely to achieve adequate eradication rates and should be revisited. Concerningly, rates levofloxacin resistance were above 20%, and similar between two geographical regions in Australia, illustrating that levofloxacin may be an inappropriate choice for empirical use in patients with refractory *H. pylori* infection.

Prospective capture of whole genome sequencing from *H. pylori* isolates undergoing antimicrobial culture analysis demonstrated that genetic markers associated with antimicrobial resistance to clarithromycin and levofloxacin correlate strongly with phenotypic resistance. Phylogenetic origins determined by single-nucleotide polymorphisms (SNPs) revealed that the majority of strains originated from Europe and East Asia genetic ancestry, reflecting population migration trends in Australia over recent decades. *H. pylori* virulence factors associated with increased gastric cancer risk were present in the majority of isolates, especially those from East Asia. Correlations with pathological findings on histology from gastric biopsies obtained during endoscopy demonstrated that most patients with histological precursors for the development of gastric cancer contained mutations in *cagA*.

Conclusions

Rates of *H. pylori* antimicrobial resistance have been increasing in Australia, yet remain largely unrecognised. Currently recommended antimicrobial therapies for *H. pylori* are unlikely to achieve adequate eradication rates. Guidelines should therefore be revisited in order to reduce the morbidity and mortality associated with *H. pylori* infection. Clarithromycin and levofloxacin should no longer be used in first- and second-line empiric eradication respectively in Australia, in the populations studied. Antimicrobial resistance was found to be clustered in migrant populations, which represent a growing sector of the community at increased risk for treatment failure. Resistance was associated with migration from countries with a high prevalence of resistance, most notably East Asia and Europe.

Whole genome sequencing of isolates confirmed a strong correlation between phenotypic and genetically predicted antimicrobial resistance for levofloxacin and clarithromycin. As such, genetic testing for *H. pylori* resistance from faecal samples may be a simple and relatively cost-effective approach to guiding therapy is higher risk groups such as in migrant populations. Individualisation of patient risk, based on both host and genetically determined isolate factors, may enable risk factor stratification and guide optimal management of eradication. Looking forward, protocolised genetic testing and tracking of *H. pylori* resistance profiles is essential as a coordinated national effort to guide empirical therapy and best practice for eradicating *H. pylori* infection. Revised antimicrobial guidelines and recognition of risk factors associated with resistance are needed to improve the quality of care in people with *H. pylori* infection.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

The author acknowledges that copyright of published works contained within the thesis resides with the copyright holder(s) of those works. I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Dr Jonathon Paul Schubert

Acknowledgements

I would first like to thank A/Professor Rob Bryant for his guidance and mentorship, not only throughout my PhD, but during physician training and my entire training in Gastroenterology. I am eternally grateful to Rob for all of his time, energy, and effort invested in me, as well as for the numerous career-shaping opportunities he has afforded me. I am inspired by his ability to create opportunities, his outstanding execution and ongoing dedication to the IBD service at the Queen Elizabeth Hospital. I look forward to many future years of working together.

I would like to sincerely thank Professor Chris Rayner for his steadfast support, counsel, and leadership both during gastroenterology training and throughout my studies. He has had an extremely valuable influence on both my clinical and academic career and his wise advice and research mentorship has been of incredible value. I cherish our ongoing friendship and hope that this will continue for many years to come.

I would like to thank the dedicated and talented group of collaborators, with whom I have been lucky to work during this degree. To my collaborators at the Queen Elizabeth Hospital, including Dr Samuel Costello and Professor Ian Roberts-Thomson, both of whom have made a big impact on me both clinically and during my studies. I have benefited enormously from their expertise, willingness to help and approachability, and hope to carry this mantle forward in the future. I would also like to thank Professor Arduino Mangoni, who mentored and supervised me through my clinical pharmacology training during the first year of my studies, for his incredibly rational and planned approach, with unmatched research wisdom. Also to SA Pathology, in particular Dr Morgyn Warner who has pioneered and made possible the majority of the projects, and Dr Lex Leong for your expertise and help in our whole genome sequencing project. In addition, interstate collaborators at the Marshall Centre for Infectious Diseases Research and Training, Dr Alfred Tay and Dr Claire Lee for their whole genome sequencing expertise and Dr Paul Ingram for his antimicrobial resistance expertise through PathWest, Western Australia. In addition Professor Richard Woodman from Flinders University for his expert assistance and in-depth analysis in our geospatial analysis. Thank you for all your hard work, support and friendship over the course of this degree.

I would also like to acknowledge and thank the University of Adelade for their generous support through the provision of an Australian Government Research Training Program Scholarship and Adelaide Medical School Higher Degree Research Award.

I would like to thank the Schubert family, in particular my mother Sharyn and father Paul, for all of the unwavering love, support, (and babysitting) and assistance with home renovations over the course of this degree. Their interest in my research, even the seemingly mundane, is a great source of motivation and I am ever grateful for their insights and enthusiasm. I am very grateful for Paul's analytical mindset and rational approach, as it has greatly assisted me in developing sound research methodology.

Lastly, and most notably, I would like to thank my amazing wife Cindy. So much has happened over the span of this degree: home renovations, enormous business growth, a move to Brisbane and the birth of our beautiful daughter Isabella. Throughout all of this, your patient love and steadfastness has kept us afloat. Thank you for bearing with me, providing ongoing support, caring for and nurturing Isabella and for tolerating my lack of availability, all the while keeping the 'empire' afloat while the captain was absent from the helm. Uplifting our family to Brisbane during the final part of the degree was very disruptive, and I appreciate your unwavering support and encouragement to pursue my career goals.

The thesis was edited by Elite Editing and editing intervention was restricted to Standards D and E of the *Australian Standards for Editing Practice*.

CHAPTER 1: OVERVIEW

Helicobacter pylori (H. pylori) infection was estimated in 2015 to affect over half the world's population, or about 4.4 billion individuals.[1] Infection is most prevalent in developing countries, while in developed countries such as Australia and New Zealand, it is estimated that about 15-30% of the population are infected.[2-4] Infection with *H. pylori* causes chronic gastritis, and significantly increases the risk of peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer.[5] *H. pylori* is the leading infectious cause of cancer worldwide, responsible for 810,000 new cancer cases in 2018 and the third leading cause of cancer-related mortality.[6, 7] Chronic *H. pylori* infection has been estimated to be responsible for up to 90% of cases of gastric cancer globally and *H. pylori* eradication has been shown to reduce the incidence of gastric cancer at a population level.[8-11] However, eradication rates of *H. pylori* have fallen worldwide due to increasing rates of antibiotic resistance, particularly to clarithromycin and metronidazole.[12-15] These concerning trends have culminated in the World Health Organisation (WHO) in 2017 designating clarithromycin-resistant *H. pylori* a high priority for antibiotic research and development.[16] Characterising rates of resistance and temporal changes is essential to guide eradication therapy. Locally recommended first and second line eradication regimens have not changed for over a decade.[17] Comprehensive care of patients with *H. pylori* infection requires an understanding of local antimicrobial resistance patterns to guide eradication practices.[18] There is enormous geographical variation between resistance profiles globally, and treatment regimens need to be tailored to local resistance patterns.[19]

Long term outcomes of *H. pylori* infection also vary substantially between populations and geographically. There is significant geographical variation in both the prevalence of infection and gastric cancer risk. This is highlighted by regions in Africa where infection rates approach 100%, but rates of gastric cancer are very low.[20] The pathogenesis of gastric cancer is likely multifactorial and dependent on the genotype, alongside diet, lifestyle, host genetics and co-infection.[21, 22] The genotype of the *H. pylori* strain is also a determining factor in the risk of acquiring gastric cancer. Cag pathogenicity island (*cagPAI*) and the vacuolating cytotoxin gene A (*vacA*) gene are the best established pathogen virulence factors.[23-25] Cytotoxin-associated gene A (*cagA*) is the most investigated gene of *cagPAI*, and is an oncoprotein when injected into mammalian cells, undergoing phosphorylation by host cell kinases, and affecting cytoskeletal and tissue structure, as well as cell proliferation.[5, 26] Infection with *cagA*-positive *H. pylori* strains is associated with a high risk of peptic ulcers and gastric carcinoma.[25, 27] In East Asia, where rates of gastric cancer are high, virtually all *H.*

pylori isolates are *cagA*-positive, whereas in regions of Africa, where rates of gastric cancer are low, few isolates are *cagA*-positive.[28] Prognostication of adverse clinical outcomes from *H. pylori* infection depends on understanding co-factors including local isolates and their associated virulence factors.

A systematic review and meta-analysis of resistance profiles of *H. pylori* was performed to understand the current antimicrobial resistance landscape in Australasia. On review of the literature, a paucity of recent data could be identified to guide clinicians prescribing eradication therapy. However, from available data, concerning trends were revealed suggesting a previously unrecognised rise in the prevalence of antibiotic resistance to clarithromycin, our current first-line antimicrobial, over the last two decades.[17] This study is presented in Chapter 3: Manuscript: Schubert *et al.* "Antibiotic resistance of *Helicobacter pylori* in Australia and New Zealand: A systematic review and meta-analysis", published in *Journal of Gastroenterology and Hepatology,* 2021 [impact factor (IF) 2021 4.369]).

The systematic review and meta-analysis demonstrated a potential rise in antimicrobial resistance, which was not previously appreciated in Australia. To characterise this further, a retrospective observational study was performed on resistance patterns at a single tertiary centre over a 20 year period. This study demonstrated that rates of resistance to clarithromycin are rising, supporting calls for local prescribing guidelines to be revised. This study is presented in Chapter 4: Manuscript: Schubert *et al.* "Increasing *Helicobacter pylori* clarithromycin resistance in Australia over 20 years", published in *Internal Medicine Journal,* 2022 [IF 2022 2.048].

Rising rates of microbial resistance seen in earlier studies identified a need to improve the characterisation of resistance. Developing an understanding of the geospatial distribution of antimicrobial resistance is necessary to improve identification of patients at risk of treatment failure. A geospatial analysis was performed, using patient demographic data including post code, and linking this to known demographic data. This study demonstrated that *H. pylori* antimicrobial resistance was distributed heterogeneously in the greater Adelaide area. Furthermore, resistance was clustered in post code regions containing high numbers of migrants. This study is presented in Chapter 5: Manuscript: *Schubert et al.* "Geospatial analysis of *Helicobacter pylori* infection in South Australia: Should location influence eradication therapy?", published in *Journal of Gastroenterology and Hepatology*, 2022 [IF 2022 4.369].

The concerning rise in antimicrobial resistance calls for an updated evaluation of resistance patterns, not only in South Australia, but also nationally. A multi-centre study evaluating *H. pylori* sensitivity data from a broad geographical region, incorporating both South Australia and Western Australia, over the preceding four years (2018-2022) was undertaken. High rates of antibiotic and multi-drug resistance were demonstrated, generalisable between sites, implying that eradication guidelines should be revisited. This study is presented in Chapter 6: Manuscript: *Schubert et al*. "Contemporary antibiotic resistance patterns of Helicobacter pylori in Australia: a multi-centre analysis", published in *Internal Medicine Journal,* 2023 [IF 2023 2.1).

Adverse clinical outcomes from *H. pylori* infection vary substantially across the world. Characterisation of factors associated with morbidity and mortality was needed in order to prognosticate, identify populations at high risk for gastric cancer, and optimise eradication therapy. Genomic and phylogenetic data from whole genome sequencing can be used to identify the origins of isolates and reveal emerging trends in antimicrobial resistance. A multi-centre, multi-state, retrospective study was performed on isolates undergoing whole genome sequencing in South Australia and Western Australia. This demonstrated a heterogenous mix of *H. pylori* in "real world" Australian clinical practice, with the majority of isolates harbouring virulence factors associated with an increased risk for gastric cancer. This study is presented in Chapter 7: Manuscript: Schubert *et al*. "Genomic analysis of Helicobacter pylori in Australia – antimicrobial resistance, phylogenetic patterns and virulence factors", submitted to *Helicobacter*, 2023 [IF 2022 4.4].

The focus of management of *H. pylori* since its discovery has been on eradication, but the co-evolution of *H. pylori* with humans likely has evolutionary advantages, which have been largely overlooked in clinical practice. A literature review was performed to explore the multifaceted nature of the potential benefits of *H. pylori* as a commensal organism. This study is presented in Chapter 8: Manuscript: Schubert et al. "Helicobacter pylori: Have potential benefits been overlooked?", published in *Journal of Gastroenterology and Hepatology Open*, 2022 [IF 2022 1.658].

Finally, a discussion is presented summarising and integrating the findings of the thesis (Chapter 9). Strategies to optimise outcomes in patients with *H. pylori* infection in clinical practice are proposed, incorporating both an understanding of evolving antimicrobial resistance patterns, and factors associated with increased morbidity and mortality. Issues with the current body of evidence are discussed and future research directions outlined, to place the thesis within the contextual framework of current and future research, with the ultimate aim of improving the quality of care for patients with *H. pylori* infection.

REFERENCES:

1. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420-9.
2. Moujaber T, MacIntyre CR, Backhouse J, Gidding H, Quinn H, Gilbert GL. The seroepidemiology of Helicobacter pylori infection in Australia. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2008;12(5):500-4.
3. Peach HG, Pearce DC, Farish SJ. Helicobacter pylori infection in an Australian regional city: prevalence and risk factors. Med J Aust. 1997;167(6):310-3.
4. Robertson MS, Cade JF, Savoia HF, Clancy RL. Helicobacter pylori infection in the Australian community: current prevalence and lack of association with ABO blood groups. Internal medicine journal. 2003;33(4):163-7.
5. Wroblewski LE, Peek RM, Jr., Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clinical microbiology reviews. 2010;23(4):713-39.
6. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8(2):e180-e90.
7. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-53.
8. Gonzalez CA, Megraud F, Buissonniere A, Lujan Barroso L, Agudo A, Duell EJ, et al. Helicobacter pylori infection assessed by ELISA and by immunoblot and noncardia gastric cancer risk in a prospective study: the Eurgast-EPIC project. Ann Oncol. 2012;23(5):1320-4.
9. Lee YC, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, et al. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. Gastroenterology. 2016;150(5):1113-24.e5.
10. Rokkas T, Rokka A, Portincasa P. A systematic review and meta-analysis of the role of Helicobacter pylori eradication in preventing gastric cancer. Ann Gastroenterol. 2017;30(4):414-23.
11. Ford AC, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut. 2020.
12. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016;43(4):514-33.

13. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut. 2012;61(5):646-64.

14. Papastergiou V, Georgopoulos SD, Karatapanis S. Treatment of Helicobacter pylori infection: meeting the challenge of antimicrobial resistance. World J Gastroenterol. 2014;20(29):9898-911.

15. Nagaraja V, Eslick GD. Evidence-based assessment of proton-pump inhibitors in Helicobacter pylori eradication: a systematic review. World J Gastroenterol. 2014;20(40):14527-36.

16. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.

17. eTG, editor. Therapeutic Guidelines. eTG complete [digital]. Helicobacter pylori infection. 2016. Accessed August 2023.

URL: https://www.tg.org.au. Melbourne.: Therapeutic Guidelines.

18. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report. Gut. 2022.

19. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.

20. Holcombe C. Helicobacter pylori: the African enigma. Gut. 1992;33(4):429-31.

21. Whary MT, Sundina N, Bravo LE, Correa P, Quinones F, Caro F, et al. Intestinal helminthiasis in Colombian children promotes a Th2 response to Helicobacter pylori: possible implications for gastric carcinogenesis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2005;14(6):1464-9.

22. Correa P, Malcom G, Schmidt B, Fontham E, Ruiz B, Bravo JC, et al. Review article: Antioxidant micronutrients and gastric cancer. Aliment Pharmacol Ther. 1998;12 Suppl 1:73-82.

23. Peek RM, Jr., Crabtree JE. Helicobacter infection and gastric neoplasia. The Journal of pathology. 2006;208(2):233-48.

24. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer research. 1995;55(10):2111-5.

25. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40(3):297-301.

26. Hatakeyama M. Helicobacter pylori and gastric carcinogenesis. Journal of gastroenterology. 2009;44(4):239-48.

27. Kang J, Blaser MJ. Bacterial populations as perfect gases: genomic integrity and diversification tensions in Helicobacter pylori. Nature reviews Microbiology. 2006;4(11):826-36.

28. Ghoshal UC, Chaturvedi R, Correa P. The enigma of Helicobacter pylori infection and gastric cancer. Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology. 2010;29(3):95-100.

Chapter 2: INTRODUCTION

2.1 Background

The link between *H. pylori,* gastritis and peptic ulcer disease was first discovered in 1982 by Robin Warren and Barry Marshall who were awarded the 2005 Noble Prize in Physiology or Medicine for their discovery.[1] Various strains of *Helicobacter* colonise the stomach of most mammals and birds and have co-evolved with humans over more than 50,000 years.[2] Mechanisms that protect the organism within the acidic environment of the stomach include motility within the gastric mucus gel, adhesins that promote adherence to epithelial cells, ammonia produced by urea hydrolysis, and a degree of inhibition of immune responses. There is also substantial genetic diversity in *H. pylori* that involves a variety of bacterial proteins including the cag pathogenicity island (*cagA*) and vacuolating protein. *H. pylori* has been well recognised as a pathogen for several decades, with a causal role established for atrophic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer.[3]

2.2 Epidemiology

H. pylori colonises the gastric mucosa of more than half of the world's population.[4] However, only 10–20% of patients infected develop adverse outcomes .[5, 6] It is most prevalent in developing countries, while in developed countries such as Australia and New Zealand, it is estimated that about 15-30% of the population are infected.[7-9] Vertical transmission is thought to be the most common mode of transmission, with new infections thought to occur as a consequence of direct human-to-human transmission, via an oral-oral and/or faecal-oral route in early childhood, generally transmitted within close family members living in close proximity.[10-13] *H. pylori* has been detected in saliva, vomitus, gastric refluxate, and faeces, but there is no conclusive evidence for predominant transmission via any of these mediums.[14-19] As *H. pylori* is very sensitive to atmospheric oxygen pressure, highly dependent on nutrients, and requires maintenance of a temperature between 34-40°C, direct person-to-person transmission remains the most likely transmission route.[20]

2.3 Phylogenetic origins of present-day *H. pylori*

Given the co-evolution of *H. pylori* with humans, an understanding of the phylogenetic origins of *H. pylori* strains encountered in clinical practice in essential to improve quality of care. Rates of antimicrobial resistance are known to be higher overseas than in Australia and migration is a likely contributing factor to growing rates of resistance.[21] Understanding the origins of isolates encountered in Australian clinical practice will assist with characterising and monitoring patterns of antimicrobial resistance; the latter knowledge is needed to deliver effective eradication therapies. Furthermore, an understanding of the phylogenetic origins of *H. pylori* may assist in identifying strains harbouring virulence factors that put patients at increased risk of adverse clinical outcomes, including peptic ulceration and gastric cancer. Given the significant variability of clinical outcomes between different *H. pylori* genotypes, the risk of adverse clinical outcomes may be estimated and prognostication may be based on the specific region of origin of isolates.

Using genetic analysis of multi-locus sequence typing (MLST) of seven "housekeeping" genes (atpA, efp, mutY, ppa, trpC, ureI, yphC) and one virulence-associated gene (vacA) of 370 strains isolated from 27 human populations, Falush et al. identified four main clusters of *H. pylori* populations with distinct geographical distribution identified as: 1) hpEurope, 2) hpAfrica1, 3) hpAfrica2, and 4) hpEastAsia.[22] Based on concatenated fragments of the 7 housekeeping genes, the evolutionary history of H. pylori and its correlation with the human "out-of-Africa" migration hypothesis has been established.[23-25] Additional analysis split hpEastAsia into three subpopulations: hspAmerind, hspEAsia and hspMaori. hpAfrica1 was subdivided into hspWAfrica and hspSAfrica. [22] More recent studies identified other main clusters: hpNEAfrica, hpAsia2, and hpSahul.[23, 24]

To summarise, *H. pylori* has been classified into 7 predominant populations, with some comprising subpopulations, that are associated with particular geographic areas: hpAfrica2, hpAfrica1, hpNEAfrica, hpEurope, hpAsia2, hpEastAsia and hpSahul.[22-24] The geographic distribution of hpEurope includes almost all *H. pylori* strains isolated from ethnic Europeans, including from countries colonised by Europeans. The majority of *H. pylori* isolates from Eastern Asia are hpEastAsia. hpEastAsia also includes the subpopulations hspMssaori (Polynesians, Melanesians and native Taiwanese), hspAmerind (native Americans) and hspEAsia (East Asia). hpAsia2 strains are isolated in South, Southeast and Central Asia. hpAfrica1 includes subpopulations hspWAfrica (West Africa, South Africa, and Afro-American) and hspSAfrica (South Africa). hpAfrica2 is very distinct and has only been isolated in South Africa. hpNEAfrica is predominantly made up of isolates from Northeast Africa (Table 1).[26]

Genetic ancestral population	Geographic/ethnic distribution
hpEurope	Europe, Middle East, India and Iran
hpAfrica1	Western Africa, South Africa
hpAfrica2	South Africa
hpNEAfrica	Ethiopia, Somalia, Sudan, northern Nigeria
hpEastAsia	hspMssaori (Melanesians, Polynesians, native Taiwanese) hspEAsia (East Asia), hspAmerinds (Native Americans)
hpAsia2	Northern India, Bangladesh, Thailand, Malaysia
hpSahul	Australia Aboriginals and Papua New Guineans

Table 2.1: *H. pylori* populations, subpopulations and geographic distribution[22-24, 27, 28]

Of specific note, hpSahul is named after the ancient Sahul continent, now mainland Australia, Tasmania and New Guinea, which were joined from 100,000 years ago until relatively recent times, estimated to be between 31,000–37,000 years ago.[24] hpSahul is only carried by Aboriginal Australians and highlanders in New Guinea and is thought to have been split from the East Asia *H. pylori* population when Aboriginal Australians first migrated to Australia 65,000 years ago.[29] In recent years, MLST has been superseded by core genome analysis, allowing for increase granularity of the evolution pathway but utilising the entire genome, rather than limiting phylogenetic origins to seven "housekeeping" genes.[30]

Populations with high rates of gastric cancer have a concordantly high prevalence of hpEastAsia (especially hspEAsia) *H. pylori* strains. Comparatively, the incidence of gastric cancer is very low in Africa and South Asia, where most strains are hpNEAfrica/ hpAfrica1/ hpAfrica2, or hpAsia2, respectively. It is thought that the so-called "African enigmas" and "Asian enigmas" might be at least partially explained by the different genotypes of *H. pylori* prevalent in specific geographical regions. More advanced genetic origins can be determined, at an individual gene level of granularity, using core genome sequencing, and genomic analysis has the potential to further probe the causes of variable clinical outcomes.[31]

2.4 Evolving diversity between *H. pylori* strains

H. pylori strains have significant diversity between populations, having co-evolved with humans over tens of thousands of years. This diversity includes genetic profiles, structure of genes and clinical outcomes, which vary substantially by geographical region.[25, 32, 33] There is considerable genetic recombination and exchange between strains, yielding significant adaptive benefits.[34, 35] *H. pylori* can incorporate genetic material from other organisms, with plasticity enabling the pathogen to adapt to its host.[36, 37] *H. pylori* antibiotic resistance also varies substantially geographically and has been increasing globally over time, with a corresponding fall in eradication rates.[38-40] As such, the World Health Organization has listed antibiotic resistant *H. pylori* as a high priority for antibiotic research and development.[41]

2.5 Clinical presentation

Infection with *H. pylori* causes chronic gastritis, and significantly increases the risk of peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer.[3] *H. pylori* is the leading infectious cause of cancer worldwide, responsible for 810,000 new cancer cases in 2018 and the third leading cause of cancer-related mortality.[42, 43] Chronic *H. pylori* infection has been estimated to be responsible for up to 90% of cases of gastric cancer globally and *H. pylori* eradication has been shown to reduce the incidence of gastric cancer at a population level.[44-47] In 1994, *H. pylori* was categorized as a class I (definite) carcinogen by the International Agency for Research on Cancer (IARC), a division of the World Health Organization (WHO).[48]

Almost all *H. pylori*-infected individuals have chronic active gastritis on biopsy, however most of these patients are asymptomatic, making *H. pylori* difficult to diagnose. Clinical presentations can range from asymptomatic, to severe complications such as peptic ulceration or gastric cancer. Several studies have reported that eradication therapy for *H. pylori* in healthy and asymptomatic patients reduces the risk of developing gastric cancer, however, this impact is most pronounced in patients early in the disease's clinical course.[46] In patients who have already developed pre-neoplastic lesions, such as intestinal metaplasia and dysplasia, the benefits of *H. pylori* eradication are limited.[45, 49]

2.6 Diagnosis

H. pylori infection can be diagnosed in several ways, with urea breath testing (UBT) regarded as a gold standard for non-invasive diagnosis. *H. pylori* produces large amounts of urease, and it has been estimated that up to 10% of the total protein content of *H. pylori* consists of this enzyme.[50] This forms the basis of the urea breath test; in the presence of urease-producing *H. pylori* in the stomach, ^{13}C-labelled urea is broken down and high levels of $^{13}CO_2$ are detected in the breath with a sensitivity of 96% and specificity of 93%.[51, 52] Faecal stool antigen is another non-invasive test used to detect the presence of *H. pylori*. *H. pylori* has also been detected in saliva, vomitus, and gastric refluxate, but these have not been used for detection to date in routine clinical practice.[14-19]

Invasive testing can include endoscopy to obtain gastric mucosa biopsies, which can be analysed using a rapid urease test (e.g. CLO test)[53], or by histopathology, as well as culture and antibiotic sensitivity testing. Diagnosis of antimicrobial sensitivity is usually via culture and E-test, but can include an agar dilution method. In addition, genetic mutations in isolates in geographic regions associated with antimicrobial resistance can be used to determine genomic resistance profiles.

2.7 Eradication therapy

H. pylori eradication has been shown to reduce the incidence of gastric cancer at a population level.[54] Current routine eradication therapy for *H. pylori* in Australia consists of a proton pump inhibitor (PPI), amoxicillin 1g twice daily and clarithromycin 500mg twice daily for 10-14 days.[55] This combination has been recommended since the late 1990s, prior to which metronidazole was used in place of clarithromycin as a first -line eradication therapy. For patients with an allergy to penicillins, metronidazole can be substituted for amoxicillin. For refractory infection, current regimens include levofloxacin triple therapy (comprising PPI, amoxicillin, and levofloxacin). Recommendations for levofloxacin-based regimens are based on a study from the mid-2010s showing high eradication rates of 90%, although it is worth noting that in the 20 isolates cultured, levofloxacin resistance was non-existent in this study.[56] Alternatives include bismuth quadruple therapy (bismuth, PPI, metronidazole, and tetracycline) and rifampicin based triple therapy (PPI, rifampicin, and amoxicillin).[55]

Antibiotics alone are not able to eradicate *H. pylori* and concurrent acid suppression is essential for successful therapy.[57] Many antimicrobials were not developed to penetrate the gastric mucosa and

do not act in the gastric lumen, especially in an acidic environment. Antibiotics including both metronidazole and clarithromycin are secreted in saliva and are particularly effective against *H. pylori*. Bismuth, used as part of quadruple therapy, acts topically on gastric mucosa. However as the drug does not penetrate the mucus layer, it is used in practice as part of quadruple therapy (with a PPI, metronidazole and tetracycline) to achieve eradication.[57]

To enable successful antimicrobial eradication, concurrent use of high dose acid suppression therapy is needed in order to maintain pH ≥6 in the stomach. The class of H_2 blockers (e.g. ranitidine, famotidine) are only competitive inhibitors of acid secretion and are not sufficient to raise the pH to adequate levels consistently and reliably. In contrast, PPIs usually provide sufficiently reliable and durable suppression of gastric acid that is needed for successful *H. pylori* eradication.[57] Despite this, some *H. pylori* may survive antimicrobial eradication therapy because of inadequate acid suppression even with PPIs.

2.8 Antimicrobial resistance

In Australia, there has been little attention paid to evolution of *H. pylori* antimicrobial resistance over recent years. Rates of primary *H. pylori* resistance to clarithromycin in Australia have historically been thought to be low (between 6-8%) based on data collected during the 1990s.[58, 59] Based on this assumption, clarithromycin along with amoxicillin and a proton pump inhibitor, are currently recommended for empiric eradication therapy.[55] Globally, it is known that *H. pylori* antimicrobial resistance is rising, and meta-analyses from the Asia-Pacific region have found a substantial increase in recent times, with clarithromycin-resistance increasing from 7% before 2000 to 21% between 2011-15.[21]

Factors driving these emerging trends overseas include widespread community antibiotic use, and population changes due to migration from countries with a high prevalence of *H. pylori* resistance.[55] The proportion of Australians born overseas has also been increasing over time (one third of Australians in the 2016 census), as has the number of immigrants from countries where clarithromycin resistance is above 30%, particularly Asia.[60, 61] This is likely to have resulted in increased resistance in Australia over time, so up-to-date monitoring is required to guide appropriate empiric eradication therapy.

2.9 Genetic determinants of antimicrobial resistance

Given the rising rates of antibiotic resistance globally, with a corresponding fall eradication rates[38-40], the World Health Organization has listed antibiotic resistant *H. pylori* as a high priority for antibiotic research and development.[41] Genomic profiles may be used to predict antimicrobial resistance; for example, mutations in the 23S ribosomal RNA gene demonstrate resistance to clarithromycin, while gyrA quinolone resistance-determining region (QRDR) mutations are associated with fluroquinolone resistance,[40, 62, 63] and tetracycline resistance is associated with 16S rRNA (AGA926-928TTC) mutations.[64] Mutations in the genes *frxA/rdx*A are associated with resistance to metronidazole[65], mutations in *rpo*B (500 to 545) to rifampin[66] and mutations in *pbp*1A to amoxicillin.[63, 67]

2.10 Evolutionary origins and potential benefits of colonisation with *H. pylori*

While the role of *H. pylori* in the pathogenesis of peptic ulcer and gastric cancer is widely acknowledged, less publicised is the multi-faceted physiological function that *H. pylori* plays as a commensal in the human gastric microbiome.[2] Given that *H. pylori* has survived and co-evolved in the human stomach over more than 50,000 years, it is likely that chronic infection confers survival advantages. In specific regions of Africa, the prevalence of *H. pylori* infection is nearly 100%, but it is much less prevalent in the developed world, so that overall just over half the world's population is colonised.

Recent evidence indicates a role of *H. pylori* as an immune modulator, with an inverse association observed between *H. pylori* colonisation and immune-mediated disorders including asthma and inflammatory bowel disease.[68, 69] In addition, there is burgeoning evidence to suggest that *H. pylori* modulates satiety hormones, including leptin and ghrelin, that may influence appetite and contribute to weight control. Yet another issue is the relationship of *H. pylori to* gastric acid secretion, gastro-oesophageal reflux disease and the rising incidence of adenocarcinoma of the lower oesophagus.[70, 71]

A detailed and informed understanding of these potential positive associations is needed to manage the burden of *H. pylori* infection, and to individualise patient management. Considering both rising rates of antimicrobial resistance, including development of multi-drug resistant *H. pylori* strains, and an organism that is present in more than half of the world's population, global eradication seems

unlikely. An improved understanding of the positive benefits of *H. pylori* is needed to inform future management, which likely will include "selective eradication", rather than "universal eradication".

2.11 Virulence factors and association with gastric cancer

Gastric cancer is the third leading global cause of cancer related mortality. Up to 90% of gastric cancer has been attributed to *H. pylori* infection, and *H. pylori* eradication has been shown to reduce the incidence of gastric cancer.[45] However, there is significant geographical variation in both the prevalence of *H. pylori* infection and gastric cancer risk. This is highlighted by regions in Africa where infection rates approach 100%, but rates of gastric cancer are very low.[72] The pathogenesis of gastric cancer is likely multifactorial and dependent on the genotype, in addition to diet, lifestyle, host genetics and co-infection.[73, 74]

The genotype of the infecting *H. pylori* strain is an important determinant in the risk of acquiring gastric cancer. Virulence factors including cag pathogenicity island (cag*PAI*) and the vacuolating cytotoxin gene A (*vacA*) gene are most established in gastric cancer pathogenesis.[75-77] Cytotoxin-associated gene A (*cagA*) is the most investigated gene of the oncoprotein *cagPAI,* with the strongest associations with gastric cancer of any known virulence factor.[3, 78] Infection with *cagA*-positive *H. pylori* strains is associated with a substantially increased risk of peptic ulceration, gastric atrophy and gastric carcinoma.[36, 77, 79] In Southeast Asia, where rates of gastric cancer are high, the vast majority of *H. pylori* isolates are *cagA*-positive. The frequencies of this gene in South Korea (97%), Japan (95%) and China (90%) are much higher compared to western countries, where rates of gastric cancer are low.[80, 81] The EPIYA motif is a membrane-targeting signal of Helicobacter pylori virulence factor cagA and is divided into four classes, A,B,C and D. The *cagA* genotype (ABD), which is highly prevalent in East Asia, has been associated with an increased risk of gastric cancer.[82]

vacA has a variable structure in the signal region with s1 or s2 alleles, and in the middle region with m1 and m2 alleles. The combination of these alleles determines the level of cytotoxin produced: *vacA* s1m1 strains produce high levels of cytotoxin *in vitro*, while s1m2 strains produce moderate levels, and s2m2 strains minimal levels. Comparatively, patients infected with vacA s1 or M1 strains may be at increased risk for developing peptic ulceration and/or gastric cancer, compared to those

with s2 or m2 strains.[83-85] Within east Asian countries, strains with the s1m1 allele are more prolific in regions of high gastric cancer risk, such as Japan and Korea. In studies outside of east Asian countries, the s1m1 genotype has also been associated with increased risk of peptic ulcer disease, chronic gastritis and gastric cancer.[86, 87] As such, it has been hypothesised that the s1m1 type is the most virulent type of *vacA*, conferring the highest risk of gastric cancer.

The pathogenesis of gastric cancer is likely multi-faceted, involving host factors (including smoking, diet, obesity, family history, age of acquisition and immunity to *H. pylori*) as well as pathogen factors (including the strain, ancestral origin, genotype and associated virulence factors). Pathogen risk factors are also likely to be multifactorial, with a range of mutations likely contributing to risk, many of which are not understood. A better understanding of this complex pathogenesis is needed to individualise treatment, prognosticate on the risk of adverse clinical outcomes and ultimately optimise management.

2.12 Research question

2.12.1 Rationale

Therapeutic advances in the medical management of *H. pylori* have largely involved antibiotics and drugs that suppress gastric acid secretion. While a 'treat to eradicate' strategy is currently recommended, there is growing evidence that treatment needs to be individualised on the basis of drug resistance and the degree of risk or benefit associated with persistent infection.

Quality care of patients infected with *H. pylori* necessitates management beyond the eradication of the organism. Risk stratification for adverse outcomes, including prognostication of peptic ulcer and gastric cancer risk versus potential positive effects of the organism needs to be considered. Risk stratification needs to include both patient factors and pathogen factors. Emerging technology including whole genome sequencing allows for analysis of gene level mutations to explore novel methods of prognosticating adverse clinical outcomes and has promise for better understanding of pathogen- related risk factors.

Current therapies for eradication of *H. pylori* are limited by poor efficacy, with empiric eradication therapies having decreasing rates of eradication over time. In the setting of rising resistance, and the emergence of multi-resistant strains, appropriate use of antimicrobials and an updated understanding of resistance trends are needed to guide eradication therapy. Australasian antimicrobial resistance studies have been few and far between in recent decades, and consideration of the optimum management of *H. pylori* has been widely overlooked in clinical practice.

2.12.2 Overarching aims of this thesis

The overarching aim of this thesis is to explore and characterise antimicrobial resistance in Australia, with the longer-term aim of improving quality of care and clinical outcomes for patients infected with *H. pylori*. This includes identification of factors that put patients at increased risk of failing eradication therapy, such as the development of multi-resistant strains. The secondary aim of this thesis is to characterise the genetic basis of antimicrobial resistance in Australia, as well as to characterise the phylogenetic origins of resistant isolates and their associations with known virulence factors and gastric cancer, and correlate these with clinical outcomes.

2.12.3 Research objectives

The objectives of this research were to:

1. Characterise patterns of *H. pylori* antimicrobial resistance in Australasia
2. Assess risk factors associated with antimicrobial resistance and failure of *H. pylori* eradication therapy
3. Evaluate the appropriateness of current antimicrobial regimens for *H. pylori* eradication therapy
4. Evaluate phylogenetic origins of *H. pylori* species encountered in clinical practice in Australia
5. Prognostic the risk of adverse clinical outcomes utilising whole genome sequencing of *H. pylori* isolates, exploring pathogenic virulence factors of *H. pylori*

2.12.4 Research process

Six studies were conducted to address the overarching aim. The research presented in this thesis was undertaken in Adelaide, South Australia and involved interstate collaboration with researchers in Perth, Western Australia and Melbourne, Victoria. The research may be broadly divided into 6 inter-related work-streams, the timelines for which overlapped during the course of the candidature.

- **Project 1**. The first work-stream set out to evaluate the current state of antimicrobial resistance of *H. pylori* in Australia and New Zealand (Adelaide, SA).

- **Project 2**. The second work-stream involved the retrospective evaluation of antimicrobial resistance encountered in local clinical practice in South Australia (Adelaide, SA).

- **Project 3**. The third work-stream involved a geospatial analysis to identify associations between geographic location and antimicrobial resistant strains (Adelaide, SA).

- **Project 4**. The fourth work-stream involved capturing current multicentre, multi-state antimicrobial resistance trends in South Australia and Western Australia (Adelaide, SA and Perth, WA).

- **Project 5.** The fifth work-stream involved exploring the role of co-evolution of *H. pylori* with humans as part of the upper GI microbiome. Characterisation of the advantages of

colonisation by *H. pylori* which have enabled it to survive in the human stomach was undertaken through a literature review (Adelaide, SA and Melbourne, VIC).

- **Project 6.** The sixth work-stream involved characterising *H. pylori* antimicrobial phenotypic, genomic resistance and virulence factors using whole genome sequencing. Analysis of these factors and their association with adverse clinical outcomes was performed, to improve risk stratification of patients and identify high risk populations for adverse clinical outcomes. (Adelaide, SA and Perth, WA)

REFERENCES

1. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet (London, England). 1983;1(8336):1273-5.
2. Atherton JC, Blaser MJ. Coadaptation of Helicobacter pylori and humans: ancient history, modern implications. The Journal of clinical investigation. 2009;119(9):2475-87.
3. Wroblewski LE, Peek RM, Jr., Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clinical microbiology reviews. 2010;23(4):713-39.
4. Suerbaum S, Michetti P. Helicobacter pylori infection. The New England journal of medicine. 2002;347(15):1175-86.
5. Pohl D, Keller PM, Bordier V, Wagner K. Review of current diagnostic methods and advances in Helicobacter pylori diagnostics in the era of next generation sequencing. World J Gastroenterol. 2019;25(32):4629-60.
6. Attila T, Zeybel M, Yigit YE, Baran B, Ahishali E, Alper E, et al. Upper socioeconomic status is associated with lower Helicobacter pylori infection rate among patients undergoing gastroscopy. Journal of infection in developing countries. 2020;14(3):298-303.
7. Moujaber T, MacIntyre CR, Backhouse J, Gidding H, Quinn H, Gilbert GL. The seroepidemiology of Helicobacter pylori infection in Australia. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2008;12(5):500-4.
8. Peach HG, Pearce DC, Farish SJ. Helicobacter pylori infection in an Australian regional city: prevalence and risk factors. Med J Aust. 1997;167(6):310-3.
9. Robertson MS, Cade JF, Savoia HF, Clancy RL. Helicobacter pylori infection in the Australian community: current prevalence and lack of association with ABO blood groups. Internal medicine journal. 2003;33(4):163-7.
10. Kivi M, Tindberg Y, Sörberg M, Casswall TH, Befrits R, Hellström PM, et al. Concordance of Helicobacter pylori strains within families. Journal of clinical microbiology. 2003;41(12):5604-8.
11. Konno M, Fujii N, Yokota S, Sato K, Takahashi M, Sato K, et al. Five-year follow-up study of mother-to-child transmission of Helicobacter pylori infection detected by a random amplified polymorphic DNA fingerprinting method. Journal of clinical microbiology. 2005;43(5):2246-50.
12. Raymond J, Thiberg JM, Chevalier C, Kalach N, Bergeret M, Labigne A, et al. Genetic and transmission analysis of Helicobacter pylori strains within a family. Emerging infectious diseases. 2004;10(10):1816-21.
13. Rowland M, Daly L, Vaughan M, Higgins A, Bourke B, Drumm B. Age-specific incidence of Helicobacter pylori. Gastroenterology. 2006;130(1):65-72; quiz 211.

14. Allaker RP, Young KA, Hardie JM, Domizio P, Meadows NJ. Prevalence of helicobacter pylori at oral and gastrointestinal sites in children: evidence for possible oral-to-oral transmission. Journal of medical microbiology. 2002;51(4):312-7.

15. Ferguson DA, Jr., Jiang C, Chi DS, Laffan JJ, Li C, Thomas E. Evaluation of two string tests for obtaining gastric juice for culture, nested-PCR detection, and combined single- and double-stranded conformational polymorphism discrimination of Helicobacter pylori. Digestive diseases and sciences. 1999;44(10):2056-62.

16. Ferguson DA, Jr., Li C, Patel NR, Mayberry WR, Chi DS, Thomas E. Isolation of Helicobacter pylori from saliva. Journal of clinical microbiology. 1993;31(10):2802-4.

17. Kabir S. Detection of Helicobacter pylori DNA in feces and saliva by polymerase chain reaction: a review. Helicobacter. 2004;9(2):115-23.

18. Leung WK, Siu KL, Kwok CK, Chan SY, Sung R, Sung JJ. Isolation of Helicobacter pylori from vomitus in children and its implication in gastro-oral transmission. The American journal of gastroenterology. 1999;94(10):2881-4.

19. Sinha SK, Martin B, Gold BD, Song Q, Sargent M, Bernstein CN. The incidence of Helicobacter pylori acquisition in children of a Canadian First Nations community and the potential for parent-to-child transmission. Helicobacter. 2004;9(1):59-68.

20. Kusters JG, Gerrits MM, Van Strijp JA, Vandenbroucke-Grauls CM. Coccoid forms of Helicobacter pylori are the morphologic manifestation of cell death. Infection and immunity. 1997;65(9):3672-9.

21. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, et al. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. The lancet Gastroenterology & hepatology. 2017;2(10):707-15.

22. Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, et al. Traces of human migrations in Helicobacter pylori populations. Science (New York, NY). 2003;299(5612):1582-5.

23. Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, et al. An African origin for the intimate association between humans and Helicobacter pylori. Nature. 2007;445(7130):915-8.

24. Moodley Y, Linz B, Yamaoka Y, Windsor HM, Breurec S, Wu JY, et al. The peopling of the Pacific from a bacterial perspective. Science (New York, NY). 2009;323(5913):527-30.

25. Maixner F, Krause-Kyora B, Turaev D, Herbig A, Hoopmann MR, Hallows JL, et al. The 5300-year-old Helicobacter pylori genome of the Iceman. Science (New York, NY). 2016;351(6269):162-5.

26. Yamaoka Y. Helicobacter pylori typing as a tool for tracking human migration. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2009;15(9):829-34.

27. Devi SM, Ahmed I, Francalacci P, Hussain MA, Akhter Y, Alvi A, et al. Ancestral European roots of Helicobacter pylori in India. BMC genomics. 2007;8:184.

28. Tay CY, Mitchell H, Dong Q, Goh KL, Dawes IW, Lan R. Population structure of Helicobacter pylori among ethnic groups in Malaysia: recent acquisition of the bacterium by the Malay population. BMC microbiology. 2009;9:126.

29. Clarkson C, Jacobs Z, Marwick B, Fullagar R, Wallis L, Smith M, et al. Human occupation of northern Australia by 65,000 years ago. Nature. 2017;547(7663):306-10.

30. Muñoz-Ramírez ZY, Mendez-Tenorio A, Kato I, Bravo MM, Rizzato C, Thorell K, et al. Whole Genome Sequence and Phylogenetic Analysis Show Helicobacter pylori Strains from Latin America Have Followed a Unique Evolution Pathway. Frontiers in cellular and infection microbiology. 2017;7:50.

31. Lu W, Wise MJ, Tay CY, Windsor HM, Marshall BJ, Peacock C, et al. Comparative analysis of the full genome of Helicobacter pylori isolate Sahul64 identifies genes of high divergence. Journal of bacteriology. 2014;196(5):1073-83.

32. Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. The Journal of clinical investigation. 2004;113(3):321-33.

33. Kodaman N, Pazos A, Schneider BG, Piazuelo MB, Mera R, Sobota RS, et al. Human and Helicobacter pylori coevolution shapes the risk of gastric disease. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(4):1455-60.

34. Suerbaum S, Josenhans C. Helicobacter pylori evolution and phenotypic diversification in a changing host. Nature reviews Microbiology. 2007;5(6):441-52.

35. Falush D, Kraft C, Taylor NS, Correa P, Fox JG, Achtman M, et al. Recombination and mutation during long-term gastric colonization by Helicobacter pylori: estimates of clock rates, recombination size, and minimal age. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(26):15056-61.

36. Kang J, Blaser MJ. Bacterial populations as perfect gases: genomic integrity and diversification tensions in Helicobacter pylori. Nature reviews Microbiology. 2006;4(11):826-36.

37. Didelot X, Nell S, Yang I, Woltemate S, van der Merwe S, Suerbaum S. Genomic evolution and transmission of Helicobacter pylori in two South African families. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(34):13880-5.

38. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.

39. Graham DY, Shiotani A. New concepts of resistance in the treatment of Helicobacter pylori infections. Nature clinical practice Gastroenterology & hepatology. 2008;5(6):321-31.

40. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016;43(4):514-33.

41. Tacconelli E. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development. 2017.

42. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8(2):e180-e90.

43. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-53.

44. Gonzalez CA, Megraud F, Buissonniere A, Lujan Barroso L, Agudo A, Duell EJ, et al. Helicobacter pylori infection assessed by ELISA and by immunoblot and noncardia gastric cancer risk in a prospective study: the Eurgast-EPIC project. Ann Oncol. 2012;23(5):1320-4.

45. Lee YC, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, et al. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. Gastroenterology. 2016;150(5):1113-24.e5.

46. Rokkas T, Rokka A, Portincasa P. A systematic review and meta-analysis of the role of Helicobacter pylori eradication in preventing gastric cancer. Ann Gastroenterol. 2017;30(4):414-23.

47. Ford AC, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut. 2020.

48. Schistosomes, liver flukes and Helicobacter pylori. IARC monographs on the evaluation of carcinogenic risks to humans. 1994;61:1-241.

49. Ford AC, Forman D, Hunt RH, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. BMJ (Clinical research ed). 2014;348:g3174.

50. Bauerfeind P, Garner R, Dunn BE, Mobley HL. Synthesis and activity of Helicobacter pylori urease and catalase at low pH. Gut. 1997;40(1):25-30.

51. Berger A. Helicobacter pylori breath tests. BMJ (Clinical research ed). 2002;324(7348):1263.

52. Ferwana M, Abdulmajeed I, Alhajiahmed A, Madani W, Firwana B, Hasan R, et al. Accuracy of urea breath test in Helicobacter pylori infection: meta-analysis. World J Gastroenterol. 2015;21(4):1305-14.

53. Uotani T, Graham DY. Diagnosis of Helicobacter pylori using the rapid urease test. Annals of translational medicine. 2015;3(1):9.

54. Takenaka R, Okada H, Kato J, Makidono C, Hori S, Kawahara Y, et al. Helicobacter pylori eradication reduced the incidence of gastric cancer, especially of the intestinal type. Alimentary pharmacology & therapeutics. 2007;25(7):805-12.

55. eTG, editor. Therapeutic Guidelines. eTG complete [digital]. Helicobacter pylori infection. 2016. Accessed August 2023.

URL: https://www.tg.org.au. Melbourne.: Therapeutic Guidelines.

56. Katelaris PH, Katelaris AL. A prospective evaluation of levofloxacin-based triple therapy for refractory Helicobacter pylori infection in Australia. Internal medicine journal. 2017;47(7):761-6.

57. Tay A, Wise MJ, Marshall BJ. Helicobacteriology update %J Microbiology Australia. 2021;42(2):92-5.

58. Katelaris PH, Forbes GM, Talley NJ, Crotty B. A randomized comparison of quadruple and triple therapies for Helicobacter pylori eradication: The QUADRATE Study. Gastroenterology. 2002;123(6):1763-9.

59. Katelaris PH, Adamthwaite D, Midolo P, Yeomans ND, Davidson G, Lambert J. Randomized trial of omeprazole and metronidazole with amoxycillin or clarithromycin for Helicobacter pylori eradication, in a region of high primary metronidazole resistance: the HERO study. Alimentary pharmacology & therapeutics. 2000;14(6):751-8.

60. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.

61. Correa P, Piazuelo MB. Evolutionary History of the Helicobacter pylori Genome: Implications for Gastric Carcinogenesis. Gut and liver. 2012;6(1):21-8.

62. Cattoir V, Nectoux J, Lascols C, Deforges L, Delchier JC, Megraud F, et al. Update on fluoroquinolone resistance in Helicobacter pylori: new mutations leading to resistance and first description of a gyrA polymorphism associated with hypersusceptibility. International journal of antimicrobial agents. 2007;29(4):389-96.

63. Nishizawa T, Suzuki H. Mechanisms of Helicobacter pylori antibiotic resistance and molecular testing. Frontiers in molecular biosciences. 2014;1:19.

64. Gerrits MM, Berning M, Van Vliet AH, Kuipers EJ, Kusters JG. Effects of 16S rRNA gene mutations on tetracycline resistance in Helicobacter pylori. Antimicrobial agents and chemotherapy. 2003;47(9):2984-6.

65. Kwon DH, El-Zaatari FA, Kato M, Osato MS, Reddy R, Yamaoka Y, et al. Analysis of rdxA and involvement of additional genes encoding NAD(P)H flavin oxidoreductase (FrxA) and ferredoxin-like

protein (FdxB) in metronidazole resistance of Helicobacter pylori. Antimicrobial agents and chemotherapy. 2000;44(8):2133-42.

66. Hays C, Burucoa C, Lehours P, Tran CT, Leleu A, Raymond J. Molecular characterization of Helicobacter pylori resistance to rifamycins. Helicobacter. 2018;23(1).

67. Tran TT, Nguyen AT, Quach DT, Pham DT, Cao NM, Nguyen UT, et al. Emergence of amoxicillin resistance and identification of novel mutations of the pbp1A gene in Helicobacter pylori in Vietnam. BMC microbiology. 2022;22(1):41.

68. Wu XW, Ji HZ, Yang MF, Wu L, Wang FY. Helicobacter pylori infection and inflammatory bowel disease in Asians: a meta-analysis. World J Gastroenterol. 2015;21(15):4750-6.

69. Arnold IC, Hitzler I, Müller A. The immunomodulatory properties of Helicobacter pylori confer protection against allergic and chronic inflammatory disorders. Frontiers in cellular and infection microbiology. 2012;2:10.

70. Ho KY, Chan YH, Kang JY. Increasing trend of reflux esophagitis and decreasing trend of Helicobacter pylori infection in patients from a multiethnic Asian country. The American journal of gastroenterology. 2005;100(9):1923-8.

71. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer. 1998;83(10):2049-53.

72. Holcombe C. Helicobacter pylori: the African enigma. Gut. 1992;33(4):429-31.

73. Whary MT, Sundina N, Bravo LE, Correa P, Quinones F, Caro F, et al. Intestinal helminthiasis in Colombian children promotes a Th2 response to Helicobacter pylori: possible implications for gastric carcinogenesis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2005;14(6):1464-9.

74. Correa P, Malcom G, Schmidt B, Fontham E, Ruiz B, Bravo JC, et al. Review article: Antioxidant micronutrients and gastric cancer. Aliment Pharmacol Ther. 1998;12 Suppl 1:73-82.

75. Peek RM, Jr., Crabtree JE. Helicobacter infection and gastric neoplasia. The Journal of pathology. 2006;208(2):233-48.

76. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer research. 1995;55(10):2111-5.

77. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40(3):297-301.

78. Hatakeyama M. Helicobacter pylori and gastric carcinogenesis. Journal of gastroenterology. 2009;44(4):239-48.

79. Kamogawa-Schifter Y, Yamaoka Y, Uchida T, Beer A, Tribl B, Schöniger-Hekele M, et al. Prevalence of Helicobacter pylori and its CagA subtypes in gastric cancer and duodenal ulcer at an Austrian tertiary referral center over 25 years. PloS one. 2018;13(5):e0197695.

80. Kim SY, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, et al. Genotyping CagA, VacA subtype, IceA1, and BabA of Helicobacter pylori isolates from Korean patients, and their association with gastroduodenal diseases. Journal of Korean medical science. 2001;16(5):579-84.

81. Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, et al. Structure of cag pathogenicity island in Japanese Helicobacter pylori isolates. Gut. 1999;44(3):336-41.

82. Sahara S, Sugimoto M, Vilaichone RK, Mahachai V, Miyajima H, Furuta T, et al. Role of Helicobacter pylori cagA EPIYA motif and vacA genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC infectious diseases. 2012;12:223.

83. Atherton JC, Cao P, Peek RM, Jr., Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. The Journal of biological chemistry. 1995;270(30):17771-7.

84. Sugimoto M, Zali MR, Yamaoka Y. The association of vacA genotypes and Helicobacter pylori-related gastroduodenal diseases in the Middle East. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2009;28(10):1227-36.

85. Sugimoto M, Yamaoka Y. The association of vacA genotype and Helicobacter pylori-related disease in Latin American and African populations. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2009;15(9):835-42.

86. Gangwer KA, Shaffer CL, Suerbaum S, Lacy DB, Cover TL, Bordenstein SR. Molecular evolution of the Helicobacter pylori vacuolating toxin gene vacA. Journal of bacteriology. 2010;192(23):6126-35.

87. Román-Román A, Martínez-Carrillo DN, Atrisco-Morales J, Azúcar-Heziquio JC, Cuevas-Caballero AS, Castañón-Sánchez CA, et al. Helicobacter pylori vacA s1m1 genotype but not cagA or babA2 increase the risk of ulcer and gastric cancer in patients from Southern Mexico. Gut pathogens. 2017;9:18.

CHAPTER 3: CURRENT KNOWLEDGE OF H. PYLORI ANTIMICROBIAL RESISTANCE IN AUSTRALASIA

3.1 Background

Understanding the current landscape of *H. pylori* resistance is essential for the outcomes of eradication treatment to be improved. An understanding of both historic and present rates of antibiotic resistance is needed, including temporal and geographical trends. The Maastricht IV *H. pylori* Consensus Report recommends that clinicians understand the local prevalence of *H. pylori* resistance so that they can select the most appropriate first- and second-line eradication regimens.[13)]

Eradication rates of *H. pylori* have fallen worldwide due to increasing rates of antibiotic resistance, particularly to clarithromycin and metronidazole.[12-15] Evidence based prescribing of antimicrobial therapy to eradicate *H. pylori* requires contemporary local data to guide management. The aim of this project was to perform a systematic review and meta-analysis of all available literature on antimicrobial resistance in Australasia.

Presented in this chapter is the manuscript published in *Journal of Gastroenterology and Hepatology* 2021; 36 (6): 1450-56.

3.2 Specific Author Contributions

Title of Paper	Antibiotic Resistance of Helicobacter pylori in Australia and New Zealand: A Systematic Review and Meta-analysis
Publication Status	Published
Publication Details	Schubert JP, Gehlert J, Rayner CK, et al. Antibiotic resistance of Helicobacter pylori in Australia and New Zealand: A systematic review and meta-analysis. *J Gastroenterol Hepatol*. 2021;36(6):1450-1456. doi:10.1111/jgh.15352

Principal Author

Name of Principal Author (Candidate)	Jonathon P Schubert		
Contribution to the Paper	Conception, Acquiring data, Knowledge, Analysis, Drafting		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/9/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Jessica Gehlert		
Contribution to the Paper (4%)	Analysis, Drafting		
Signature		Date	30/10/2023

Name of Co-Author	Christopher K Rayner		
Contribution to the Paper (4%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Ian C Roberts-Thomson		
Contribution to the Paper (4%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Samuel P Costello		
Contribution to the Paper (4%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Arduino A Mangoni		
Contribution to the Paper (5%)	Conception, Knowledge, Drafting		
Signature		Date	27/9/2023

Name of Co-Author	Robert V Bryant		
Contribution to the Paper (4%)	Conception, Knowledge, Drafting		
Signature		Date	28/11/2023

[Manuscript 1] Antibiotic Resistance of Helicobacter pylori in Australia and New Zealand: A Systematic Review and Meta-analysis

Jonathon P Schubert[1,2], Jessica Gehlert[2] , Christopher K Rayner[1,3], Ian C Roberts-Thomson[1] , Sam Costello[1,4] , Arduino A Mangoni[2], Robert V Bryant[1,4].

[1]Faculty of Health Sciences, Adelaide Medical School, University of Adelaide, South Australia

[2]Department of Clinical Pharmacology, Flinders Medical Centre, Flinders University, South Australia

[3]Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, South Australia

[4]Department of Gastroenterology, The Queen Elizabeth Hospital, South Australia

Corresponding author

Dr Robert V Bryant

Department of Gastroenterology, The Queen Elizabeth Hospital, South Australia, 5011

Email: robert.bryant@sa.gov.au

Phone: +61882226000

Conflict of interests

JPS none to disclose. JG none to disclose. CKR has received research funding from AstraZeneca, Merck Sharp & Dohme, Eli Lilly and Company, Novartis, and Sanofi and has participated in advisory boards for Allergan (now AbbVie). IRT none to disclose. SC has received advisory, speaking fees, or research support from Ferring, Microbiotica, Janssen. Shareholder in BiomeBank. AAM none to disclose. RVB has received grant/research support/speaker fees (all paid to employer for research support): AbbVie, Ferring, Janssen, Shire, Takeda, Emerge Health. Shareholder in BiomeBank.

Acknowledgements

Jonathon Schubert receives a University of Adelaide Research Training Program Stipend.

ABSTRACT

Objective: While the global prevalence of antibiotic-resistant *Helicobacter pylori (H. pylori)* is increasing, there is much regional variation, and local data are required to guide eradication therapy. We performed a systematic review and meta-analysis to determine rates of *H. pylori* antibiotic resistance in Australia and New Zealand.

Study Design: Random effects meta-analysis of data from 15 published studies and three published abstracts reporting prevalence of primary or secondary *H. pylori* antibiotic resistance in Australasia.

Data Sources: PubMed, EMBASE, MEDLINE, PROSPERO and the Cochrane Library were searched until August, 2020.

Data Synthesis: Fifteen published studies and three published abstracts were identified; one study was excluded due to high risk of bias. Seventeen studies conducted between 1996-2013 were included in the final analysis, 12 reporting primary and five reporting secondary antibiotic resistance. Prevalence of primary resistance was: clarithromycin 7.4% (95%CI, 5.3-9.7%), metronidazole 50.0% (95%CI, 23.9-56.1%), fluoroquinolones 3.7% (95%CI, 0.004-14.8%), and both amoxicillin and tetracycline <0.5%. Subgroup analysis (last 20 years) showed doubling of clarithromycin resistance to 16.1% (95%CI 11.2-21.7%) with other resistance stable. Prevalence of secondary resistance was high for all antibiotics, particularly clarithromycin 78.7% (95%CI, 64.1-90.1%) and metronidazole 68.3% (95%CI, 59.9-76.1%).

Conclusions: The outcomes reveal an increase in primary *H. pylori* clarithromycin resistance since the year 2000, while metronidazole resistance has remained stable and primary resistance to amoxicillin, tetracycline and fluoroquinolones is low. Rates of secondary resistance to metronidazole and clarithromycin are high. The results highlight the need for contemporary local data on antibiotic resistance in Australia and New Zealand.

Key Words

Helicobacter pylori, resistance, antimicrobial, antibiotic, Australia, New Zealand

INTRODUCTION

Helicobacter pylori (H. pylori) infection was estimated to affect over half the world's population (~4.4 billion individuals) in 2015.[1] It is most prevalent in developing countries, while in developed countries such as Australia and New Zealand, it is estimated that about 15-30% of the population are infected.[2-4] Infection with *H. pylori* causes chronic gastritis, and significantly increases the risk of peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer.[5] *H. pylori* is the leading infectious cause of cancer worldwide, responsible for 810,000 new cancer cases in 2018 and the third leading cause of cancer-related mortality.[6, 7] Chronic *H. pylori* infection has been estimated to be responsible for up to 90% of cases of gastric cancer globally and *H. pylori* eradication has been shown to reduce the incidence of gastric cancer at a population level.[8-11] Eradication rates of *H. pylori* have fallen worldwide due to increasing rates of antibiotic resistance, particularly to clarithromycin and metronidazole.[12-15] These concerning trends have culminated in the World Health Organisation (WHO) designating clarithromycin-resistant *H. pylori* a high priority for antibiotic research and development in 2017.[16]

The Maastricht IV *H. pylori* Consensus Report recommends that clinicians understand the local prevalence of *H. pylori* resistance so that they can select the most appropriate first- and second-line eradication regimens.[13] Worldwide, clarithromycin-containing eradication regimens are no longer recommended for first-line empirical use due to inadequate eradication rates.[17, 18] In Australia and New Zealand, clarithromycin is still recommended in first line empiric therapies due to historically low rates of resistance, estimated from data collected in the 1990s at 6-8%.[19-21] However, Australian studies around the year 2000 identified increasing rates of clarithromycin resistance, which may influence first-line antibiotic prescribing patterns.[22, 23]

Given the vast disparity in rates of *H. pylori* antibiotic resistance across the globe, local resistance data are needed to inform the most appropriate empirical eradication regimens. In Australia and New Zealand, ongoing migration from developing countries along with high rates of antibiotic use, both in the community and in food production, are likely to have increased the prevalence of *H. pylori* resistance.[24] To date, two systematic reviews have assessed rates of primary antibiotic resistance in Australia and New Zealand; however their inclusion and exclusion criteria compromised complete data capture.[16, 17] Furthermore, meta-analyses have not been performed on local rates of secondary resistance. Therefore, the aim of this study was to evaluate the literature systematically and perform meta-analyses to ascertain the prevalence of primary and secondary *H. pylori* antibiotic resistance in Australia and New Zealand.

METHODS

Search Strategy and Study Selection

We performed a systematic review and meta-analysis of primary and secondary antibiotic resistance of *H. pylori* in Australia and New Zealand. Primary and secondary resistance was defined by whether patients had not, or had, received prior *H. pylori* eradication therapy, respectively. PubMed, EMBASE, Medline, PROSPERO and the Cochrane Library were searched for articles published from inception until August 14, 2020 using the following search terms: (Helicobacter pylori) AND (antibiotic OR antimicrobial OR antibacterial OR anti-bacterial OR drug OR clarithromycin OR metronidazole OR amoxicillin OR clarithromycin OR tetracycline) AND (resistance OR resistant) AND (Australia OR New Zealand OR Australasia). Individual study reference lists were searched for additional studies. The systematic review was registered with PROSPERO (Registry ID CRD42020172496), and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) guidelines for systematic reviews.[25]

Inclusion and Exclusion Criteria

To minimise selection bias, we established inclusion and exclusion criteria before the literature search. Inclusion criteria required a diagnosis of *H. pylori* based on culture and antibiotic susceptibility testing. Exclusion criteria were studies focusing on a special population (e.g. patients with diabetes mellitus), those with a mean or median age less than 18 years, studies with less than 35 isolates, and reviews or letters to the editor. Two reviewers (JS and JG) screened all titles and abstracts of retrieved articles independently and excluded irrelevant or duplicate articles. Both reviewers then independently assessed abstracts of the remaining articles for inclusion. Discrepancies were resolved by discussion.

Data Extraction

Full texts of relevant studies were retrieved and reviewed independently by two investigators (JS, JG) to determine final studies included, and any discrepancies were resolved by discussion. Articles were categorised into studies of primary resistance, secondary resistance, or both. Data from included studies were extracted independently by two reviewers (JS, JG) using a standardised data extraction sheet that included the following categories: mean age, gender, collection years, culture method,

antibiotic sensitivity method, prevalence of resistance to metronidazole, clarithromycin, amoxicillin, tetracycline and fluoroquinolones (encompassing studies of norfloxacin, moxifloxacin, doxycycline, levofloxacin and/or ciprofloxacin).

Quality of assessment

The quality of included studies was evaluated by two reviewers (JS, JG) independently. A modified Newcastle-Ottawa scale[26] was used to assess five categories for bias, including adequacy of selection (3 points), measurement of exposure (*H. pylori* infection – 1 point) and outcomes (*H. pylori* resistance – 1 point); each category was scored as either 1 (high risk or unclear) or 0 (low risk). Each study received a score between 0-5 points (0 = low risk of bias, 1-2 = moderate risk, 3-5 = high risk). Studies assessed as having a high risk of bias were excluded from the meta-analysis. Any differences between reviewers were resolved through discussion, and a third reviewer (AM) adjudicated in the event of disagreement.

Data analysis

We performed meta-analyses of the prevalence of both primary and secondary *H. pylori* resistance to antibiotics. As resistance rates in some studies were zero, we used the Freeman-Tukey double arcsine transformation to avoid exclusion of studies with estimated proportions of 0%.[27] A random-effects model was used to adjust for heterogeneity as there are likely to be significant variability in rates of antibiotic resistance within Australia and New Zealand, and the DerSimonian and Laird method was then used to calculate pooled estimates and 95% confidence intervals.[28]

The proportion of variability attributable to heterogeneity between studies, rather than sampling error, was expressed as the I^2 statistic (< 25% indicating low, 50% moderate, and 75% high levels of heterogeneity) and evaluated using Cochran's Q test.[29] Sub meta-analyses were performed on studies within Australia and New Zealand. Given that two studies[22, 23] demonstrated an increase in clarithromycin resistance in the late 1990s, an additional sub meta-analysis of data restricted to the last 20 years (2000 onwards) was performed. For studies that did not specify data collection periods, it was assumed that data were collected at least two years prior to publication. For studies that began before 2000 but extended after, only data from 2000 onwards were included in the subgroup analysis. All tests were two-sided, and the significance level was set at 0.05.

RESULTS

Our search retrieved 253 studies, with 184 unique results. We screened abstracts of 184 articles, 18 of which were eligible for analysis. The 18 articles were from two countries (Australia and New Zealand) and comprised 15 published studies and three published abstracts. They were published between 1996 and 2013 and comprised of a total of 2,570 H. pylori isolates. Thirteen studies reported primary antibiotic resistance, and five reported secondary resistance. Application of our modified Newcastle-Ottawa scale resulted in one study (Ahmed, 2004) being excluded from the meta-analysis due to a high risk of bias (Appendix A, Table 2). Data including study and patient characteristics, and rates of H. pylori antibiotic resistance, are shown in Table 1.

Meta-analysis

Primary Resistance

Mean overall prevalence of primary resistance to clarithromycin in Australasia was 7.4% (95% CI 5.3-9.7, Table 2), although between-study heterogeneity was high (I^2=60.0%, p=0.0075). Mean overall prevalence of resistance to metronidazole was 50.0% (95% CI 43.9-56.1), again with high between-study heterogeneity (I^2=95.2%, p<0.0001). Rates of primary antibiotic resistance to amoxicillin, tetracycline and fluoroquinolones were relatively low (Table 2).

In subgroup analyses of primary resistance, there was no significant difference between Australia and New Zealand, with 95% confidence intervals overlapping. When comparing subgroup resistance between studies conducted prior to 2000 and those after, the most striking result was a marked rise in clarithromycin resistance from 6.5% (95% CI 4.8-8.4%) pre-2000 to 16.1% (95% CI 11.2-21.7%) post 2000, with low heterogeneity between studies (I^2=0%, p=0.82). By contrast, there were no significant differences for resistance to metronidazole, amoxicillin, tetracycline or fluoroquinolones between pre- and post-2000 data.

Secondary Resistance

All studies of secondary resistance were from Australia. Mean prevalence of secondary resistance to clarithromycin was 78.7% (95% CI 64.1-90.1%) with high between-study heterogeneity (I^2=94.2%, p<0.0001). Mean prevalence of secondary resistance to metronidazole was 68.3% (95% CI 59.9-76.1%), again with high between-study heterogeneity (I^2=79.4%, p=0.0022). Rates of secondary resistance to amoxicillin, tetracycline and fluoroquinolones were relatively low (Table 2).

DISCUSSION

This study represents the largest systematic review and meta-analysis of *H. pylori* resistance in Australia and New Zealand, and provides valuable regional data to inform as to appropriate empirical eradication regimens. While recent data are limited, the most striking observation is the increase in primary clarithromycin resistance over time, which has more than doubled to 16.1% since the year 2000 (95% CI 11.2-21.7%), while rates of metronidazole, amoxicillin, tetracycline and fluoroquinolone resistance have remained stable. The lack of recent local data on rates of primary *H. pylori* resistance, particularly in Australia, is concerning, and further studies to elucidate the prevalence of community resistance to *H. pylori* should be seen as a priority.

The data on clarithromycin resistance in Australia and New Zealand are in keeping with current global trends. The largest individual study of *H. pylori* resistance in these countries to date by number of isolates showed there was a four-fold increase in clarithromycin resistance between 1996-2000.[22] Recent meta-analyses from the Asia-Pacific region have found a substantial increase in clarithromycin-resistant *H. pylori* from 7% before 2000 to 21% during 2011-15.[17] Global meta-analyses have indicated that antibiotic resistance of *H. pylori* has reached alarming levels, with resistance to clarithromycin, metronidazole and levofloxacin above the 15% threshold identified by international guidelines[30, 31] as representing high resistance, in the majority of World Health Organisation regions.[16] Accordingly, most countries do not include clarithromycin in first-line empirical regimens for *H. pylori* infection because of inadequate eradication rates (<80%), unlike Australia and New Zealand.

A key issue identified in this systematic review is the lack of recent regional resistance data. The vast majority of the available data on primary antibiotic resistance (1,626 of 1,810 isolates, or 90%) were collected before 2000. The only published data available in Australia and New Zealand in the last 10 years have shown a prevalence of primary clarithromycin resistance of 16.4%.[32] This raises concerns as to an unrecognised increase in the prevalence of community antibiotic resistance of *H. pylori* over the past two decades. Clarithromycin resistance is a major risk factor for eradication failure[15] and where present, the standard triple therapy regimen containing clarithromycin, amoxicillin and a proton-pump inhibitor demonstrates eradication rates of only 25-61%.[33] Accordingly, the Maastricht IV *H. pylori* Consensus Report recommends that empiric clarithromycin-containing triple therapy, without prior susceptibility testing, should be abandoned when the clarithromycin resistance rate in the region is over 15-20%.[13] This systematic review suggests that resistance rates in Australia and New Zealand may be above this level currently, further highlighting the need for

more contemporary data to guide first-line empirical antibiotic therapy appropriately, and that alternative regimens such as bismuth containing quadruple therapy should be considered. [13]

The development of resistance by *H. pylori* to clarithromycin has been shown in prior studies to be caused by several point mutations in the *rrl* gene encoding two 23S rRNA nucleotides, 2142 and 2143.[18, 34] *H. pylori* resistance may develop due to exposure to clarithromycin for the treatment of other illnesses such as respiratory infections, or due to cross-resistance through exposure to other macrolide antibiotics.[35] A change in pattern of macrolide use in Australia occurred in the 1990s with the introduction of roxithromycin in the Australian Pharmaceutical Benefits Scheme in October 1992 and thereafter clarithromycin in August 1998, resulting in a halving of erythromycin use over a decade. The rise in *H. pylori* clarithromycin resistance rates correlates with the widespread use of roxithromycin upon its introduction, suggesting that roxithromycin exposure may be capable of inducing resistance of *H. pylori* to clarithromycin.[22] The influence of low grade macrolide exposure through intake of food products from antibiotic-treated animals also needs to be taken into consideration.[35] Alternative mechanisms, including the large increase in the number of migrants coming to Australia and New Zealand, often from developing countries with high prevalence of *H. pylori* antibiotic resistance, may also contribute to this trend.[24] The lack of emergence of resistance to other antibiotics is consistent with published global data. It is reassuring that rates of fluoroquinolone resistance were relatively low, in keeping with data suggesting that levofloxacin-based salvage regimens result in high eradication rates in patients who have failed previous *H. pylori* eradication therapy, irrespective of the number of prior treatment failures.[36]

The main strength of this study was the exhaustive search strategy which yielded a large sample size, with approximately four times the number of *H. pylori* isolates than the largest review of Australian and New Zealand data to date.[17] Previous studies have captured *H. pylori* resistance in only 657 isolates from Australia and 330 isolates from New Zealand, whereas the current study has reported on 2190 Australian and 380 new Zealand isolates.[17] Savoldi et al.[16] reported combined primary and secondary resistance, but the Australian data were limited to a single study of secondary antibiotic resistance[37] comprising 306 isolates, and a single study of primary resistance in New Zealand comprising 73 isolates.[32] While the high heterogeneity between studies in these meta-analyses may be due to the small numbers of included studies and isolates, they may also reflect changing rates of resistance over time, and that resistance may be distributed heterogeneously among local areas. A further strength of the current study was the robust methodology for assessment of bias, resulting in the exclusion of a study from the meta-analysis. Limitations of the current meta-analysis include a lack of control for prior antibiotic therapy in the analyses of primary antibiotic resistance;

none of the included studies addressed lifetime exposure to antibiotics, and while some of the studies excluded patients who were known to have had *H. pylori* therapy in recent months, others[22] acknowledged that it was not known how many patients had received previous eradication therapy.

Multiple authors[13, 36] have advocated personalized guidelines for *H. pylori* eradication based on geographic area, and more recent data are required to assess local resistance patterns. If the high rates of clarithromycin resistance seen since 2000 are representative of current levels, then it is likely that the optimal first line therapy in Australasia needs to be reviewed.

CONCLUSIONS

This systematic review and meta-analysis has revealed a previously unrecognized increase in the prevalence of primary *H. pylori* resistance to clarithromycin in the 21st century in Australia and New Zealand. The lack of recent regional data is concerning, and there is a need for more contemporary data to assess current *H. pylori* resistance rates and determine whether current clarithromycin-based first line empiric antibiotic therapy in Australia and New Zealand should be revised.

REFERENCES

1. Hooi, J.K.Y., et al., *Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis.* Gastroenterology, 2017. **153**(2): p. 420-429.
2. Moujaber, T., et al., *The seroepidemiology of Helicobacter pylori infection in Australia.* Int J Infect Dis, 2008. **12**(5): p. 500-4.
3. Peach, H.G., D.C. Pearce, and S.J. Farish, *Helicobacter pylori infection in an Australian regional city: prevalence and risk factors.* Med J Aust, 1997. **167**(6): p. 310-3.
4. Robertson, M.S., et al., *Helicobacter pylori infection in the Australian community: current prevalence and lack of association with ABO blood groups.* Intern Med J, 2003. **33**(4): p. 163-7.
5. Wroblewski, L.E., R.M. Peek, Jr., and K.T. Wilson, *Helicobacter pylori and gastric cancer: factors that modulate disease risk.* Clin Microbiol Rev, 2010. **23**(4): p. 713-39.
6. de Martel, C., et al., *Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis.* Lancet Glob Health, 2020. **8**(2): p. e180-e190.
7. Ferlay, J., et al., *Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods.* Int J Cancer, 2019. **144**(8): p. 1941-1953.
8. Gonzalez, C.A., et al., *Helicobacter pylori infection assessed by ELISA and by immunoblot and noncardia gastric cancer risk in a prospective study: the Eurgast-EPIC project.* Ann Oncol, 2012. **23**(5): p. 1320-4.
9. Lee, Y.C., et al., *Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis.* Gastroenterology, 2016. **150**(5): p. 1113-1124.e5.
10. Rokkas, T., A. Rokka, and P. Portincasa, *A systematic review and meta-analysis of the role of Helicobacter pylori eradication in preventing gastric cancer.* Ann Gastroenterol, 2017. **30**(4): p. 414-423.
11. Ford, A.C., Y. Yuan, and P. Moayyedi, *Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis.* Gut, 2020.
12. Thung, I., et al., *Review article: the global emergence of Helicobacter pylori antibiotic resistance.* Aliment Pharmacol Ther, 2016. **43**(4): p. 514-33.
13. Malfertheiner, P., et al., *Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report.* Gut, 2012. **61**(5): p. 646-64.
14. Papastergiou, V., S.D. Georgopoulos, and S. Karatapanis, *Treatment of Helicobacter pylori infection: meeting the challenge of antimicrobial resistance.* World J Gastroenterol, 2014. **20**(29): p. 9898-911.

15. Nagaraja, V. and G.D. Eslick, *Evidence-based assessment of proton-pump inhibitors in Helicobacter pylori eradication: a systematic review.* World J Gastroenterol, 2014. **20**(40): p. 14527-36.
16. Savoldi, A., et al., *Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions.* Gastroenterology, 2018. **155**(5): p. 1372-1382.e17.
17. Kuo, Y.T., et al., *Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis.* Lancet Gastroenterol Hepatol, 2017. **2**(10): p. 707-715.
18. Arslan, N., O. Yilmaz, and E. Demiray-Gurbuz, *Importance of antimicrobial susceptibility testing for the management of eradication in Helicobacter pylori infection.* World J Gastroenterol, 2017. **23**(16): p. 2854-2869.
19. Therapeutic Guidelines. eTG complete [digital]. Helicobacter pylori infection, 2016. Cited August 2020. Available from URL: https://www.tg.org.au
20. Katelaris, P.H., et al., *Randomized trial of omeprazole and metronidazole with amoxycillin or clarithromycin for Helicobacter pylori eradication, in a region of high primary metronidazole resistance: the HERO study*. Aliment Pharmacol Ther, 2000. **14**(6): p. 751-8.
21. Katelaris, P.H., et al., *A randomized comparison of quadruple and triple therapies for Helicobacter pylori eradication: The QUADRATE Study*. Gastroenterology, 2002. **123**(6): p. 1763-9.
22. Grove, D.I. and G. Koutsouridis, *Increasing resistance of Helicobacter pylori to clarithromycin: is the horse bolting?* Pathology, 2002. **34**(1): p. 71-3.
23. Mollison, L.C., et al., *Antibiotic resistance in Helicobacter pylori.* Med J Aust, 2000. **173**(10): p. 521-3.
24. Gibney, K.B., et al., *The profile of health problems in African immigrants attending an infectious disease unit in Melbourne, Australia.* Am J Trop Med Hyg, 2009. **80**(5): p. 805-11.
25. Shamseer, L., et al., *Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation.* Bmj, 2015. **350**: p. g7647.
26. Stang, A., *Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses.* Eur J Epidemiol, 2010. **25**(9): p. 603-5.
27. Freeman, M.F. and J.W. Tukey, *Transformations Related to the Angular and the Square Root.* The Annals of Mathematical Statistics, 1950. **21**(4): p. 607-611.
28. DerSimonian, R. and N. Laird, *Meta-analysis in clinical trials.* Control Clin Trials, 1986. **7**(3): p. 177-88.

29. Higgins, J.P., et al., *Measuring inconsistency in meta-analyses.* Bmj, 2003. **327**(7414): p. 557-60.
30. Malfertheiner, P., et al., *Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report.* Gut, 2017. **66**(1): p. 6-30.
31. Fallone, C.A., et al., *The Toronto Consensus for the Treatment of Helicobacter pylori Infection in Adults.* Gastroenterology, 2016. **151**(1): p. 51-69.e14.
32. Hsiang, J., et al., *Increasing primary antibiotic resistance and ethnic differences in eradication rates of Helicobacter pylori infection in New Zealand--a new look at an old enemy.* N Z Med J, 2013. **126**(1384): p. 64-76.
33. Fischbach, L.A., S. van Zanten, and J. Dickason, *Meta-analysis: the efficacy, adverse events, and adherence related to first-line anti-Helicobacter pylori quadruple therapies.* Aliment Pharmacol Ther, 2004. **20**(10): p. 1071-82.
34. Megraud, F., *Resistance of Helicobacter pylori to antibiotics.* Aliment Pharmacol Ther, 1997. **11 Suppl 1**: p. 43-53.
35. Hu, Y., et al., *Helicobacter pylori and Antibiotic Resistance, A Continuing and Intractable Problem.* Helicobacter, 2016. **21**(5): p. 349-63.
36. Katelaris, P.H. and A.L. Katelaris, *A prospective evaluation of levofloxacin-based triple therapy for refractory Helicobacter pylori infection in Australia.* Intern Med J, 2017. **47**(7): p. 761-766.
37. Tay, C.Y., et al., *Helicobacter pylori eradication in Western Australia using novel quadruple therapy combinations.* Aliment Pharmacol Ther, 2012. **36**(11-12): p. 1076-83.

TABLES AND FIGURES

[†]nofloxacin, [‡] moxifloxacin, [§] doxycycline, [¶] levofloxacin, [x] ciprofloxacin

Abbreviations: N/A: not available; G: gastritis; DUD duodenitis, PUD: peptic ulcer disease; DU: duodenal ulcer; GU: gastric ulcer; D: dyspepsia; NUD: non-ulcer dyspepsia; GERD: Gastroesophageal reflux disease; E-test: Epsilon test

Country/Authors	Data Collection (years)	Endoscopy Diagnosis	Mean / Median Age (years)	Male, n (%)	Born overseas (%)	Susceptibility test	Number of strains	Prevalence of Helicobacter Pylori Antibiotic Resistance, n (%)				
Primary Resistance - Australia								**Clarithromycin**	**Metronidazole**	**Amoxicillin**	**Tetracycline**	**Fluoroquinolones**
Katelaris et al. 2002	N/A	NUD	51	N/A	N/A	E-test	137	11/137 (8%)	73/137 (53%)	0/137 (0%)	1/137 (1%)	N/A
Grove et al. 2002	1996-2000	N/A	N/A	N/A	N/A	E-test	514	44/514 (8.56%)	273/514 (53%)	0/514 (0%)	1/514 (0.2%)	N/A
Mollison et al. 2000	1998-1999	N/A	56	226/434 (52%)	39%	Disc diffusion, E-test	86	9/86 (11%)	31/86 (63.9%)	0/86 (0%)	0/86 (0%)	N/A
Katelaris et al. 2000	N/A	DU	49.9	N/A	N/A	E-test	157	9/156 (6%)	88/157 (56%)	0/157 (0%)	N/A	1/86 (1.2%)[†]
Katelaris et al. 1998	N/A	NUD	N/A	N/A	N/A	E-test	232	N/A	136/232 (59%)	N/A	N/A	N/A
Xia et al. 1998	1997	NUD, PUD, GERD	54.5	46/66 (69·7%)	N/A	Disc diffusion	66	4/66 (6%)	44/66 (64%)	N/A	N/A	N/A
Forbes et al. 1998	N/A	PUD,G	51.2	39 (60)	N/A	Disc diffusion, E-test	65	0/45 (0%)	32/65 (49%)	N/A	N/A	N/A
Grove et al. 1998	N/A	N/A	N/A	N/A	N/A	E-test	135	7/135 (5.2%)	81/135 (60%)	0/135 (0%)	0/135 (0%)	N/A
Midolo et al. 1997	N/A	NUD, DU, GU, GERD	50	30/51 (58.8%)	N/A	Disc diffusion, E-test	51	4/51 (8%)	25/51 (49%)	N/A	N/A	N/A
Modolo et al. 1996	N/A	NUD, PU	52	24/37 (65%)	N/A	Agar dilution	37	N/A	12/37 (32%)	N/A	N/A	N/A
Primary Resistance - New Zealand								**Clarithromycin**	**Metronidazole**	**Amoxicillin**	**Tetracycline**	**Fluoroquinolones**
Hsiang et al. 2013	2012	N/A	59.8	35/73 (48%)	N/A	E-test	73	12/73 (16.4%)	36/73 (49.3%)	4/73 (5.5%)	0/73 (0%)	6/63 (9.5%)[‡]
Ahmed et al. 2004	N/A	DU, GU, DUG, G, D	N/A	N/A	N/A	E-test	50	0/50 (0%)	10/50 (20%)	0/50 (0%)	N/A	0/50 (0%)[§]
Fraser et al. 1999	1993-1998	N/A	N/A	172/265 (64.9%)	N/A	Disc diffusion	257	18/257 (7%)	84/257 (32.7%)	N/A	N/A	N/A
Secondary Resistance - Australia								**Clarithromycin**	**Metronidazole**	**Amoxicillin**	**Tetracycline**	**Fluoroquinolones**
Pateria et al. 2018	2012-2017	N/A	45 (median)	58/154 (38%)	(58%	N/A	154	135/154 (88%)	117/154 (76%)	1/154 (0.6%)	3/154 (2.0%)	19/154 (12%)[¶]
Kaushik et al. 2018	2015-2018	G, GERD, NUD, GU	53	14/37 (37.8%)	N/A	N/A	37	23/37 (62.1%)	N/A	N/A	N/A	N/A
Lee et al. 2017	N/A	N/A	49	32/98 (32.7%)	79%	N/A	98	64/98 (65%)	53/98 (54%)	20/98 (20%)	N/A	N/A
Tay et al. 2012	2007-2011	N/A	53	91/306 (29.3%)	N/A	E-test	306	288/306 (94.1%)	207/306 (67.6%)	0/306 (0%)	0/306 (0%)	17/306 (5.6%)[x]
Broody et al. 2006	N/A	NUD, GERD, DU	52	62/130 (47.7%)	N/A	E-test	115	86/115 (74.8%)	85/115 (73.9%)	0/115 (0%)	0/115 (0%)	N/A

Table 3.1: Patient characteristics and prevalence of antibiotic resistance of the included studies.

Antibiotic	Measure	Study Population	Studies (isolates)	Prevalence % (95% CI)	Heterogeneity I^2 (%), Cochran Q (P=)
Clarithromycin	**Primary Resistance**	Australasia	10 (1541)	7.35 (5.3-9.7)	60.0, 0.0075
		Australia	8 (1211)	6.63 (4.5-9.2)	56.3, 0.025
		New Zealand	2 (330)	11.1 (3.6-22.0)	81.3, 0.021
		Australasia 2000 onwards	2 (184)	16.1 (11.2-21.7)	0.0, 0.82
		Australasia pre-2000	9 (1357)	6.46 (4.75-8.42)	42.3, 0.086
	Secondary Resistance	Australia	5 (710)	78.7 (64.1-90.1)	94.2, <0.0001
Metronidazole	**Primary Resistance**	Australasia	12 (1810)	50.0 (43.9-56.1)	83.8, <0.0001
		Australia	10 (1480)	52.6 (47.7-57.5)	67.9, 0.0009
		New Zealand	2 (330)	40.2 (24.8-56.7)	84.8, 0.010
		Australasia 2000 onwards	2 (184)	50.5 (43.4-57.7)	0.0, 0.79
		Australasia pre-2000	11 (1626)	50.1 (43.5-56.8)	85.3, <0.0001
	Secondary Resistance	Australia	4 (673)	68.3 (59.9-76.1)	79.4, 0.0022
Amoxicillin	**Primary Resistance**	Australasia	6 (1102)	0.41 (0.012-1.4)	62.7, 0.020
		Australia	*N/A*	*N/A*	*N/A*
		New Zealand	*N/A*	*N/A*	*N/A*
		Australasia 2000 onwards	2 (184)	2.09 (0.27-11.3)	86.2, 0.0071
		Australasia pre-2000	5 (918)	0.13 (0.001-0.46)	0.0, 0.9851
	Secondary Resistance	Australia	4 (673)	2.50 (0.041-11.0)	95.2, <0.0001
Tetracycline	**Primary Resistance**	Australasia	5 (945)	0.35 (0.076-0.83)	0.0, 0.86
		Australia	4 (872)	0.35 (0.070-0.86)	0.0, 0.73
		New Zealand	*N/A*	*N/A*	*N/A*
		Australasia 2000 onwards	2 (184)	0.27 (0.041-1.52)	0.0, 0.88
		Australasia pre-2000	4 (761)	0.41 (0.082-0.99)	0.0, 0.77
	Secondary Resistance	Australia	3 (575)	0.53 (0.001-2.21)	68.4, 0.0425
Fluoroquinolones	**Primary Resistance**	Australasia	2 (230)	3.72 (0.0038-14.7)	88.1, 0.0037
		Australia	*N/A*	*N/A*	*N/A*
		New Zealand	*N/A*	*N/A*	*N/A*
		Australasia 2000 onwards	2 (184)	2.92 (0.58-16.6)	91.3, 0.0007
		Australasia pre-2000	*N/A*	*N/A*	*N/A*
	Secondary Resistance	Australia	2 (460)	8.36 (3.4-15.3)	80.5, 0.023

I^2 (25% low, 50% moderate, 75%+ high heterogeneity)

Cochran Q (P<0.05, result likely due to heterogeneity)

Table 3.2: Meta-analysis results including subgroup analysis by antibiotic.

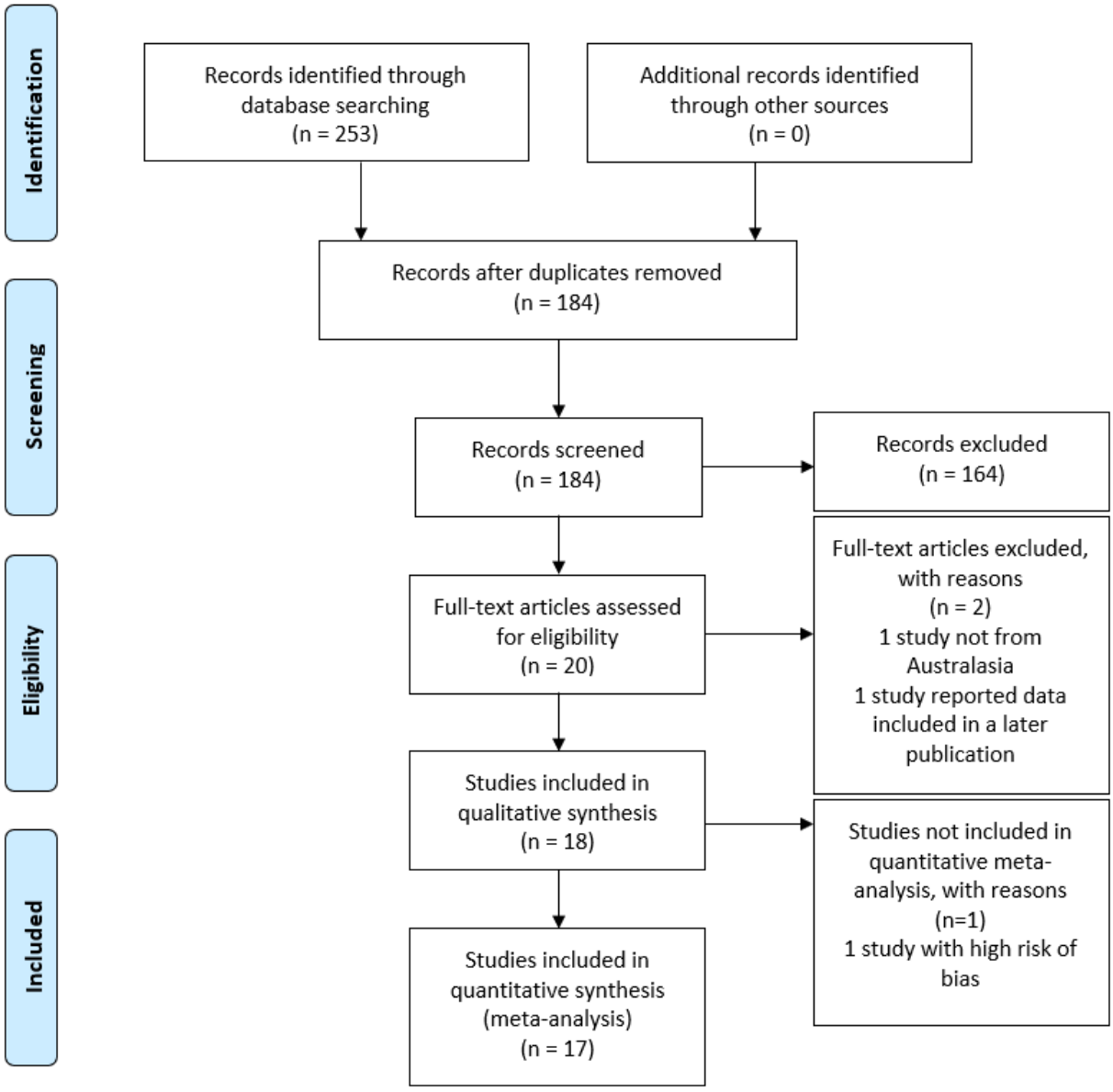


Figure 3.1: Review of literature for prevalence of primary and/or secondary antibiotic resistance of *H. pylori* in Australia and New Zealand

CHAPTER 4: CHARACTERISATION OF LOCAL RESISTANCE TRENDS IN A SINGLE TERTIARY CENTRE OVER 20 YEARS

4.1 Background

The systematic review and meta-analysis presented in Chapter 3 demonstrated a concerning rise in antimicrobial resistant *H. pylori* in Australia, not previously recognised in the literature. To investigate and characterise this potential trend further, a retrospective observational study was performed on local antimicrobial resistance patterns over the last 20 years in South Australia at a single tertiary centre. This allowed for the characterisation of temporal changes in resistance rates and clarification of whether the trend to resistance revealed in systematic review is supported by local data. This study demonstrated that rates of resistance to clarithromycin were rising over the last 20 years, informing necessary revisions to local prescribing guidelines.

Presented in this chapter is the manuscript published in *Internal Medicine Journal 2022*, *52*(9), 1554–1560.

4.2 Specific Author Contributions

Title of Paper	Increasing Helicobacter pylori clarithromycin resistance in Australia over 20 years
Publication Status	Published
Publication Details	Schubert JP, Warner MS, Rayner CK, et al. Increasing Helicobacter pylori clarithromycin resistance in Australia over 20 years. *Intern Med J*. 2022;52(9):1554-1560. doi:10.1111/imj.15640

Principal Author

Name of Principal Author (Candidate)	Jonathon P Schubert		
Contribution to the Paper	Conception, Acquiring data, Knowledge, Analysis, Drafting		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/9/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Morygn S Warner		
Contribution to the Paper (5%)	Acquiring data, Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Christopher K Rayner		
Contribution to the Paper (5%)	Conception, Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Ian C Roberts-Thomson		
Contribution to the Paper (5%)	Acquiring data, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Arduino A Mangoni		
Contribution to the Paper (5%)	Knowledge, Drafting		
Signature		Date	27/9/2023

Name of Co-Author	Samuel P Costello		
Contribution to the Paper (5%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Robert V Bryant		
Contribution to the Paper (5%)	Conception, Acquiring data, Drafting		
Signature		Date	28/11/2023

[Manuscript 2] Increasing Helicobacter pylori clarithromycin resistance in Australia over 20 years

Jonathon P Schubert[1,2], Morgyn S Warner[1,3], Christopher K Rayner[1,4], Ian C Roberts-Thomson[1], Arduino A Mangoni[2], Sam Costello[1,5], Robert V Bryant[1,5].

[1]Faculty of Health Sciences, Adelaide Medical School, University of Adelaide, Adelaide, Australia

[2]Department of Clinical Pharmacology, Flinders Medical Centre, Flinders University, Bedford Park, Australia

[3]Microbiology and Infectious Diseases Directorate, SA Pathology, Adelaide, Australia

[4]Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia

[5]Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, Australia

Corresponding author

Dr Jonathon P Schubert

Department of Gastroenterology, The Queen Elizabeth Hospital, South Australia, 5011

Email: Jonathon.schubert@sa.gov.au

Phone: (+61) (8) 8222 6000

Conflict of interests

JPS has none to disclose. MSW has none to disclose. CKR has received research funding from AstraZeneca, Merck Sharp & Dohme, Eli Lilly and Company, Novartis, and Sanofi and has participated in advisory boards for Allergan (now AbbVie). IRT has none to disclose. SPC has received advisory, speaking fees, or research support from Ferring, Microbiotica, Janssen. Shareholder in BiomeBank. AAM has none to disclose. RVB has received grant/research support/speaker fees (all paid to employer for research support): AbbVie, Ferring, Janssen, Shire, Takeda, Emerge Health. Shareholder in BiomeBank.

Acknowledgements

SA Pathology staff George Koutsouridis and Casey Moore for methodology and data collection. Suzanne Edwards for her assistance with statistical analysis. Medical student Benjamin Ngoi for his assistance with ethics application preparation. Jonathon Schubert receives a University of Adelaide Research Training Program Stipend.

ABSTRACT

Objectives: *Helicobacter pylori* infection is responsible for considerable morbidity and mortality worldwide, and eradication rates are falling in many countries, primarily due to clarithromycin and metronidazole resistance. However, there is a paucity of contemporary Australian data, which we sought to address by evaluating local rates of resistance of *H. pylori* to amoxicillin, clarithromycin, metronidazole, and tetracycline over the past 20 years.

Design: All gastric biopsy specimens collected at endoscopy to detect *H. pylori* infection at a single centre underwent routine culture and antibiotic susceptibility testing between 1998–2017. Specimens from 12,842 patients were cultured for *H. pylori,* of which 1,473 positive cultures were tested for antibiotic susceptibility.

Results: Antibiotic resistance to clarithromycin increased by 3.7% per year (IRR 1.037, p=0.014) over 20 years, with a corresponding 5.0% annual increase in minimum inhibitory concentration (MIC) (OR 1.050, p<0.001). Since 2010, average clarithromycin resistance has exceeded 20%, with >25% of isolates resistant in the last 2 years of data capture. By contrast, rates of resistance to metronidazole (35.3%), amoxicillin (0.14%) and tetracycline (0.34%) and their MICs have remained stable. Review of a representative sample of these patients (n=120, 8%) revealed that only 5% had documented prior *H. pylori* eradication therapy.

Conclusions: Over the last 20 years there has been a substantial rise in clarithromycin resistance, with stable metronidazole resistance and low rates of resistance to amoxicillin and tetracycline. Current first line *H. pylori* eradication therapy may fail to achieve adequate eradication rates, and optimal first line therapy in Australia should be revisited.

Keywords:
Helicobacter pylori, antibiotics, antimicrobial, resistance, antimicrobial resistance

INTRODUCTION

Helicobacter pylori infection is responsible for considerable worldwide morbidity and mortality, and is known to cause peptic ulceration, gastric cancer, and mucosa-associated lymphoid tissue lymphoma. *H. pylori* eradication has been shown to reduce the incidence of gastric cancer, the fifth most common neoplasm and the third most deadly cancer worldwide, at a population level.[1-4] However, rates of successful eradication have been declining in many countries due an increasing prevalence of antibiotic resistance, prompting the World Health Organisation in 2017 to list clarithromycin-resistant *H. pylori* as a high priority for antibiotic research and development.[5-7]

In developed countries such as Australia and New Zealand, both *H. pylori* infection and gastric cancer are less common, and it is estimated that about 15-30% of the population have *H. pylori* infection.[8, 9] 'Triple therapy', comprising a proton-pump inhibitor, clarithromycin, and amoxicillin, has been the most widely used first-line regimen, but this combination is achieving decreasing eradication rates globally and is no longer recommended for empiric use in most countries.[5, 6] In particular, clarithromycin resistance is a major risk factor for eradication failure, and has been shown to reduce eradication rates by as much as 70%.[10-12]

The prevalence of *H. pylori* antibiotic resistance varies between geographic regions, and changes over time within regions. Therefore, it is imperative to have up-to-date information that is locally relevant to optimise the selection of eradication regimens. Recent meta-analyses have raised concerns that clarithromycin resistance has increased in Australia and New Zealand since the year 2000, although there is a paucity of contemporary data.[13] The aim of the present study was to evaluate antibiotic resistance rates and minimum inhibitory concentrations (MICs) in *H. pylori* strains isolated from a single centre in Adelaide, Australia over the last 20 years.

METHODS

All gastric biopsy specimens collected to detect *H. pylori* infection at upper gastrointestinal endoscopy at The Queen Elizabeth Hospital (TQEH), a metropolitan teaching hospital in Adelaide, South Australia, underwent routine culture and antibiotic susceptibility testing between November 26, 1998 and September 16, 2017. Isolates from patients aged 18 or under were excluded from the study. Cultures were tested for susceptibility to amoxicillin, clarithromycin, tetracycline, and metronidazole. Gastroscopy and biopsy were performed as part of routine clinical practice and therefore informed consent to be included in this study was not obtained; however, the study received approval for publication by the Central Adelaide Human Research Ethics Committee (Reference number: 12902).

Demographic data for all patients, including age, gender and postcode, were obtained from patient records. Postcode data were used to estimate the proportion of patients born overseas based on Australian National Census Data (2006 and 2016 census for patients between 1998-2007 and 2008-2017, respectively).[10] To characterise the study population further, data including procedural indication, endoscopy findings and documentation of previous *H. pylori* therapy were systematically extracted from a representative sample of patients with positive *H. pylori* cultures. 120 patient records, representing approximately 8% of total isolates, were reviewed in four groups of 30 sequential patients derived from each five-year period evaluated (1998-2002, 2003-2007, 2008-2012 and 2013-2017).

***Helicobacter pylori* culture and antibiotic susceptibility test**

Gastric biopsies were cultured at 37°C on brain heart infusion plates (Difco Laboratories Pty Ltd) containing 7% horse blood under microaerobic conditions (5% O_2; 10% CO_2; 85% N_2) for 3-7days. If there was no visible microbial growth at 3 days, specimens were cultured for a further 3-4 days due to slower growth rates for some strains. Isolates were tested for susceptibility to amoxicillin, clarithromycin, metronidazole, and tetracycline using ETest strips (AB Biodisk, Sweden). Minimum inhibitory concentrations (MICs) to amoxicillin, clarithromycin, metronidazole, and tetracycline were determined for each isolate. Susceptibility breakpoints used were those recommended by European Committee on Antimicrobial Susceptibility Testing, including breakpoints for amoxicillin, tetracycline and clarithromycin of >0.125, >1, >0.5 mg/L respectively.[14] A metronidazole breakpoint of > 4 mg/L was used, as this breakpoint was validated locally at the laboratory processing the isolates.[15]

Statistical and subgroup analysis

Given the rise in antibiotic resistance in Australia and New Zealand since the late 1990s[13, 16, 17], sub-analyses were performed over four 5-year periods (1998-2002, 2003-2008, 2008-2013, 2013-2017) and two 10-year periods (1998-2007 and 2008-2017) to examine changes in resistance patterns over time. A p-value of <0.05 was considered statistically significant. Negative binomial regressions were performed on number of resistant cases for each antibiotic versus year (continuous) with offset being logarithmic transformation of total number of resistant and sensitive cases. SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

RESULTS

Patient demographics

12,842 adult patients underwent gastroscopy and biopsy to detect *H. pylori* infection, with a total of 1,473 (11.5%) positive *H. pylori* cultures. There were a greater number of subjects in the first 10 years of the study compared to the second 10 years. The mean age of included patients was 57.9 years, and this was lower (by mean 3.4 years, p=0.0002) in the latter 10 years of the study. 49.3% of participants were male, which did not change over time (Table 1). Estimates from postcode data, using the 2006 and 2016 national census data for each postcode, indicated that around 36.6% of participants would have been born overseas, which exceeds the national average of 30.7%.

From the representative sample of 120 patients (8% of the total) with positive *H. pylori* cultures, the most common indications for endoscopy were dyspepsia (53.3%), anaemia (17.5%) and upper gastrointestinal bleeding (10%). There was no difference between indications over 10-year time periods (Table 2). At endoscopy, the most common findings were gastritis (64.2%), reflux oesophagitis (15.8%), gastric ulceration (10%) and duodenitis (10%), and these did not vary between time periods. Prior *H. pylori* eradication attempts were documented in 6/120 patients (5%), and of the 6 corresponding *H. pylori* isolates, 1 had resistance only to metronidazole, 2 were resistant only to clarithromycin, 2 were resistant to both metronidazole and clarithromycin, and 1 was fully sensitive.

Prevalence of antibiotic resistance

Rates of antibiotic resistance to clarithromycin rose on average by 3.7% per year (IRR=1.037, 95% CI 1.007-1.068, p=0.014) over the 20 years studied with a corresponding 5.0% annual increase in minimum inhibitory concentration (MIC) (OR 1.050, p<0.001). Since 2010, the average clarithromycin resistance has exceeded 20% with more than 25% of isolates resistant in the last 2 years of data capture. Adjusting for the estimated 5% of patients who had received prior *H. pylori* eradication therapy, and that approximately 80% of patients who received prior eradication therapy are known to have clarithromycin resistance,[13] average estimated primary resistance to clarithromycin exceeded 20% by the end of data capture (Figure 2). By contrast, rates of metronidazole resistance and their MICs remained stable, while rates of resistance to amoxicillin and tetracycline were low (<0.5%) and did not change over time (Table 3).

Subgroup analysis

Subgroup analysis of 10-year periods showed that the prevalence of clarithromycin resistance was 16.0% between 1998-2007 and 21.2% between 2008-2017, however there was not a significant annual change within each 10-year period (Table 3). There was an increase in MIC values of 9.6% per year between 1998-2007 (OR=1.096, 95% CI 1.02-1.18, p=0.014), but no significant change in MIC values between 2008-2017. There was no difference in rates of resistance to any other antibiotic between these periods (Table 3).

DISCUSSION

This study represents the largest longitudinal evaluation of *H. pylori* antimicrobial resistance within Australasia and has revealed that clarithromycin resistance has increased by 3.7% per year over the last two decades. While it remains unclear whether the observed resistance patterns at a single centre are generalisable nationally, the study raises concerns regarding empiric clarithromycin eradication treatment in Australia and calls for the evidence to support this practice to be revisited at a national level. Given that only a minority (estimated at 5% from sampled data) of patients had received prior *H. pylori* therapy, the data are applicable to the primary resistance setting. Adjusting for those that had received prior eradication therapy also demonstrated a significant increase in clarithromycin resistance over time. This rise has culminated in rates of primary *H. pylori* resistance above internationally proposed thresholds where empiric treatment with clarithromycin may no longer be appropriate.[18] The study findings illustrate the importance of determining local resistance data to inform the most appropriate empirical eradication regimens for *H. pylori*

Rates of primary *H. pylori* resistance to clarithromycin in Australia have historically been thought to be around 6-8%, based on data collected during the 1990s.[19-21] Despite years of widespread community antibiotic use, and population changes due to migration from countries with a high prevalence of *H. pylori* resistance, there has been little attention paid to potentially rising *H. pylori* resistance in Australia, and there has been a paucity of local data to identify rates of clarithromycin resistant *H. pylori* since the turn of the century.[13] The results of the current study fill this gap and demonstrate a consistent local rise in clarithromycin resistance since 1998, which has continued until the end of data capture in 2017. Further Australian studies are warranted to provide insight into whether these trends are evident nationally.

Meta-analyses from the Asia-Pacific region have found a substantial increase in clarithromycin-resistant *H. pylori*, from 7% before 2000 to 21% during 2011-15, which is comparable to the rise seen in our study.[22] Of concern is that extrapolation of local data suggests that primary clarithromycin resistance in Australia may reach levels comparable to those presently seen in China (26%) and Vietnam (34%).[5]

The rise in clarithromycin resistant *H. pylori* in our study is likely due to several factors. The A2143G genetic mutation is associated with clarithromycin resistance and a reduced likelihood of eradication.[23] There is emerging evidence that low grade macrolide exposure through intake of food products from antibiotic-treated animals may contribute to *H. pylori* antibiotic resistance, particularly in migrants from regions where antimicrobial use in agriculture and farming is not

restricted.[24] The use of macrolides for the treatment of conditions such as pneumonia and sexually transmitted infections since the 1990s is also likely to have played a role. Furthermore, the percentage of Australians born overseas increased from 25.5% in 2001 to 33.3% in 2016, and this migration, commonly from countries such as China and India where resistance rates are known to be high[5, 10], may have contributed to increasing resistance rates.[23]

Our findings raise concern as to empirical prescribing recommendations for primary *H. pylori* eradication in Australia. The rising rates of clarithromycin resistance that we identified have exceeded the Maastricht V/Florence Consensus threshold, indicating that clarithromycin may be an inappropriate first-line antibiotic choice due to inadequate (<80%) primary eradication rates, although it is unclear whether these patterns of resistance are generalisable nationally. Whilst stable over the study period, metronidazole resistance remained high at >30% in all years sampled. Metronidazole is not currently part of empiric first-line *H. pylori* eradication therapy due to high rates of primary resistance, with previous studies showing resistance rates between 45%–50%, except in the case of penicillin allergy where it is recommended in place of amoxicillin.[25] Rates of resistance to amoxicillin and tetracycline remained low and did not change over the period studied, supporting their ongoing use in primary eradication regimens. Therefore, regimens that are currently considered salvage options for eradication may in fact be appropriate first line options for empirical therapy. The Maastricht V/Florence Consensus report recommends bismuth (proton pump inhibitor (PPI), bismuth, tetracycline and metronidazole), or non-bismuth (PPI, amoxicillin, clarithromycin and a nitroimidazole) quadruple therapies as first line treatment in areas of high (>15%) clarithromycin resistance.[18] In the setting of rising clarithromycin resistance, use of amoxicillin and tetracycline may be considered as first line options. Levofloxacin has been used in areas of Asia, however increasing resistance has been observed over time and this antibiotic is therefore unlikely to represent a long term option for eradication.[26] The development of new antibiotic agents may be required to meet this demand.

A major strength of this study is the large number of *H. pylori* isolates (three times that of the previous largest study of primary *H. pylori* antibiotic resistance in Australasia[16]), and the prolonged period of data collection, which to our knowledge is unmatched globally. Patient characterisation, albeit based on a representative sample of the study population, showed no difference in indication or endoscopic findings over time. Furthermore, the non-selective approach of routine culture of all specimens collected at endoscopy to detect *H. pylori* infection supports the notion that our data are representative of the primary resistance population. Postcode derived demographic information, while only providing an estimate, indicated migration trends comparable to those reported across

Australia. The proportion of patients in our study who were born overseas was marginally higher than for the general Australian population, likely reflecting our hospital's catchment area, but may be typical of hospitals in the Australian public health system. The number of patients for which biopsies were taken for *H. pylori* decreased in the second decade of the study, possibly due to increasing empiric management of *H. pylori* in the community setting, without referral for endoscopy.

A limitation was the inclusion of patients who had received prior *H. pylori* eradication therapy, but this applied only to a minority (~5%), so the data overwhelmingly represent primary *H. pylori* resistance. Furthermore, there was no change in the proportion of patients who had previously been treated over time, so this is unlikely to explain the increasing resistance rates, nor is there a change in the trend when adjusting for this (Figure 2). Many cases of H. *pylori* infection will be managed without endoscopy and therefore referral bias may be present in the cohort studied; further studies are indicated to determine generalisability of the results to the primary care setting. The low number of patients characterised in more detail, representing only 8% of the study population, is a further limitation. Whilst this characterisation adds clarity to the study population, these trends may not be generalisable to the remainder of the study cohort. Data capture was also limited to a single centre and further studies are recommended to assess whether the observations are generalisable to other Australasian centres.

CONCLUSIONS

Over the last 20 years there has been a marked rise in the rate of *H. pylori* resistance to clarithromycin in our Australian centre, while metronidazole resistance has been stable, and rates of amoxicillin and tetracycline resistance have remained low. This suggests that the current first line *H. pylori* eradication therapy may fail to achieve adequate eradication rate, and that further research to inform recommendations as to the optimal empiric eradication therapy in Australia should be a priority at a national level.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.

2. Lee YC, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, et al. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. Gastroenterology. 2016;150(5):1113-24.e5.

3. Rokkas T, Rokka A, Portincasa P. A systematic review and meta-analysis of the role of Helicobacter pylori eradication in preventing gastric cancer. Ann Gastroenterol. 2017;30(4):414-23.

4. Ford AC, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut. 2020.

5. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.

6. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016;43(4):514-33.

7. World Health Organisation, GLOBAL PRIORITY LIST OF ANTIBIOTIC-RESISTANT BACTERIA TO GUIDE RESEARCH, DISCOVERY, AND DEVELOPMENT OF NEW ANTIBIOTICS 2017 [Available from: https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/.

8. Moujaber T, MacIntyre CR, Backhouse J, Gidding H, Quinn H, Gilbert GL. The seroepidemiology of Helicobacter pylori infection in Australia. Int J Infect Dis. 2008;12(5):500-4.

9. Robertson MS, Cade JF, Savoia HF, Clancy RL. Helicobacter pylori infection in the Australian community: current prevalence and lack of association with ABO blood groups. Intern Med J. 2003;33(4):163-7.

10. Mégraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53(9):1374-84.

11. O'Connor A, Gisbert JP, McNamara D, O'Morain C. Treatment of Helicobacter pylori infection 2010. Helicobacter. 2010;15 Suppl 1:46-52.

12. Nagaraja V, Eslick GD. Evidence-based assessment of proton-pump inhibitors in Helicobacter pylori eradication: a systematic review. World J Gastroenterol. 2014;20(40):14527-36.

13. Schubert J, Gehlert, J, Rayner, C, Costello, S, Mangoni, AA, Bryant, RV. Antibiotic Resistance of Helicobacter pylori in Australia and New Zealand: A Systematic Review and Meta-analysis. Accepted for publication by the Journal of Gastroenterology and Hepatology. 2020.

14. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Accessed 31 August 2021. http://www.eucast.org/.

15. Grove DI, Koutsouridis G, Cummins AG. Comparison of culture, histopathology and urease testing for the diagnosis of Helicobacter pylori gastritis and susceptibility to amoxycillin, clarithromycin, metronidazole and tetracycline. Pathology. 1998;30(2):183-7.

16. Grove DI, Koutsouridis G. Increasing resistance of Helicobacter pylori to clarithromycin: is the horse bolting? Pathology. 2002;34(1):71-3.

17. Mollison LC, Stingemore N, Wake RA, Cullen DJ, McGechie DB. Antibiotic resistance in Helicobacter pylori. Med J Aust. 2000;173(10):521-3.

18. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66(1):6-30.

19. eTG complete. Helicobacter pylori infection 2016 [updated 2019].

20. Katelaris PH, Adamthwaite D, Midolo P, Yeomans ND, Davidson G, Lambert J. Randomized trial of omeprazole and metronidazole with amoxycillin or clarithromycin for Helicobacter pylori eradication, in a region of high primary metronidazole resistance: the HERO study. Aliment Pharmacol Ther. 2000;14(6):751-8.

21. Katelaris PH, Forbes GM, Talley NJ, Crotty B. A randomized comparison of quadruple and triple therapies for Helicobacter pylori eradication: The QUADRATE Study. Gastroenterology. 2002;123(6):1763-9.

22. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, et al. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. The lancet Gastroenterology & hepatology. 2017;2(10):707-15.

23. De Francesco V, Margiotta M, Zullo A, Hassan C, Troiani L, Burattini O, et al. Clarithromycin-resistant genotypes and eradication of Helicobacter pylori. Ann Intern Med. 2006;144(2):94-100.

24. Hu Y, Zhang M, Lu B, Dai J. Helicobacter pylori and Antibiotic Resistance, A Continuing and Intractable Problem. Helicobacter. 2016;21(5):349-63.

25. Katelaris PH, Nguyen TV, Robertson GJ, Bradbury R, Ngu MC. Prevalence and demographic determinants of metronidazole resistance by Helicobacter pylori in a large cosmopolitan cohort of Australian dyspeptic patients. Australian and New Zealand journal of medicine. 1998;28(5):633-8.

26. Liang CM, Tai WC, Hsu PI, Wu DC, Kuo CH, Tsay FW, et al. Trend of changes in antibiotic resistance in Helicobacter pylori from 2013 to 2019: a multicentre report from Taiwan. Therapeutic advances in gastroenterology. 2020;13:1756284820976990.

TABLES AND FIGURES

Time period	All years (1998-2017)	1998-2007	2008-2017	P (between decades)
Isolates (n)	1,473	907	566	N/A
Mean Age, years (95% CI)	57.9 (57.0-58.8)	59.2 (58.1-60.3)	55.8 (54.4-57.1)	0.0002*
Male (n, %)	726/1,473 (49.3)	451/907 (49.7)	275/566 (48.6)	0.67
Overseas Born[ŧ] (National average)	36.6% (30.7%)	35.3% (29.1%)	38.6% (33.3%)	N/A

* statistically significant

[ŧ] estimated from post-code derived data

Table 4.1: Demographic data for study participants undergoing gastroscopy

Indication	All years (n=120) n(%)	1998-2007 (n=60) n(%)	2008-2017 (n=60) n(%)	p (between decades)
Dyspepsia	64 (53.3)	31 (51.7)	33 (55.0)	0.71
Anaemia	21 (17.5)	8 (13.3)	13 (21.7)	0.23
Upper gastrointestinal bleeding	12 (10.0)	9 (15.0)	3 (5.0)	0.07
Altered bowel habit	8 (6.7)	6 (10.0)	2 (3.3)	0.14
Dysphagia	3 (2.5)	2 (3.3)	1 1.7)	0.65
Other	12 (10.0)	4 (6.7)	4 (6.7)	1

Table 4.2: Endoscopy indication for a representative sample of 120 patients undergoing gastroscopy

Time period		All years (1998-2017)			1998-2007			2008-2017		
Antibiotic Resistance		**Value**	**IRR/OR (95% CI)**	**p (annual change)**	**Value**	**IRR/OR (95% CI)**	**p (annual change)**	**Value**	**IRR/OR (95% CI)**	**p (annual change)**
Amoxicillin	Resistant strains (%)	0.14	0.891 (0.629-1.26)	0.52	0.22	1.18 (0.67-2.06)	0.58	0	1.177 (0.673-2.06)	0.58
	MIC (geometric mean, mg/ml)	0.02	0.9 (0.681-1.19)	0.46	0.02	1.21 (0.77-1.92)	0.41	0.022	1.211 (0.766-1.92)	0.41
Clarithromycin	Resistant strains (%)	18	1.037 (1.01-1.07)	0.014*	16	1.078 (0.99-1.18)	0.10	21.2	1.034 (0.964-1.11)	0.35
	MIC (geometric mean, mg/ml)	0.19	1.05 (1.021-1.079)	0.001*	0.07	1.096 (1.02-1.18)	0.014*	0.081	1.041 (0.963-1.13)	0.32
Metronidazole	Resistant strains (%)	35.2	1.012 (0.990-1.03)	0.29	32.3	0.981 (0.93-1.04)	0.49	39.9	0.99 (0.935-1.05)	0.73
	MIC (geometric mean, mg/ml)	1.55	1.02 (0.997-1.04)	0.09	1.30	0.956 (0.90-1.01)	0.14	2.054	0.997 (0.933-1.07)	0.94
Tetracycline	Resistant strains (%)	0.34	1.118 (0.935-1.34)	0.22	0.22	1.087 (0.62-1.91)	0.77	0.53	1.164 (0.777-1.75)	0.46
	MIC (geometric mean, mg/ml)	0.05	1.217 (0.950-1.56)	0.12	0.04	1.279 (0.57-2.85)	0.55	0.069	1.413 (0.846-2.36)	0.19

MIC (minimum inhibitory concentration)

* statistically significant

Table 4.3: Subgroup analysis of rates of resistance of H. pylori isolates over the entire study period and in 10-year periods

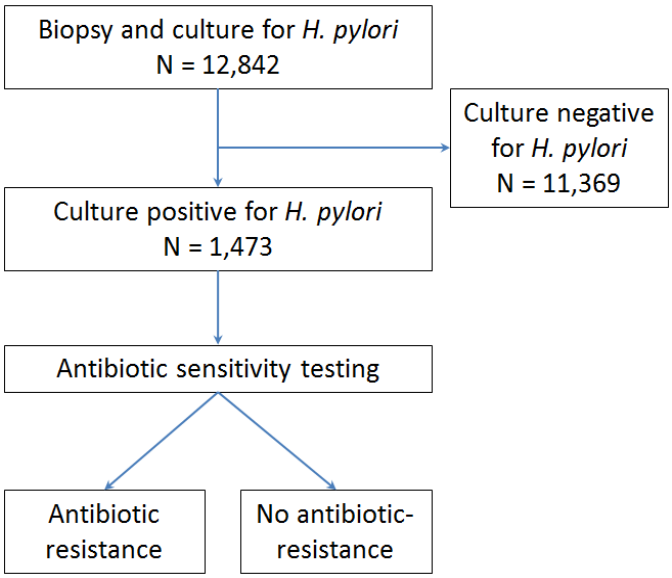
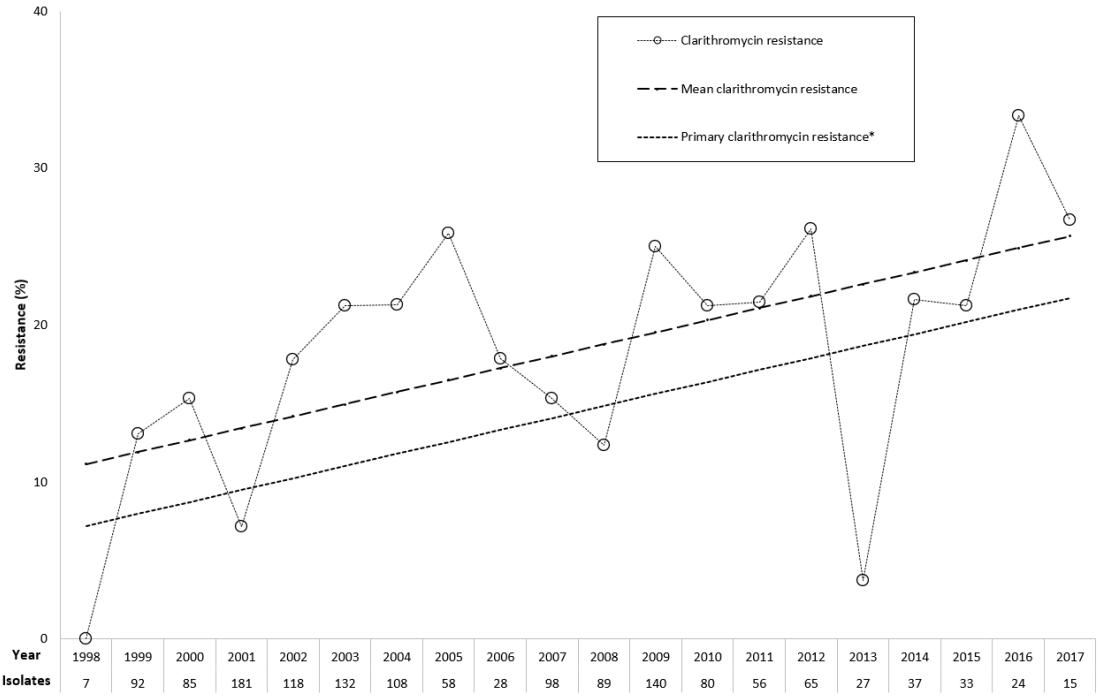


Figure 4.1: Flow chart of patients undergoing upper gastrointestinal endoscopy



*adjusted for the estimated 5% of patients who had received prior *H. pylori* antibiotic therapy

Figure 4.2: Annual rates of resistance of Helicobacter pylori to clarithromycin, 1998-2017.

CHAPTER 5: WHAT RISK FACTORS ARE ASSOCIATED WITH *H. PYLORI* INFECTION?

5.1 Background

Rising rates of microbial resistance seen in studies in Chapter 3 and confirmed in Chapter 4 identified a need to improve characterisation of antimicrobial resistance patterns. Development of an understanding of the geospatial distribution of antimicrobial resistance is necessary in order to improve identification of patients at risk of *H. pylori* treatment failure. A geospatial analysis was performed, using patient demographic data including post code, and linking these to known demographic data. This study demonstrated that *H. pylori* antimicrobial resistance was distributed heterogeneously in the greater Adelaide area, with resistance clustered in post code regions containing high numbers of migrants.

Presented in this chapter is a Geospatial analysis of *Helicobacter pylori* infection in South Australia, published in *Journal of gastroenterology and hepatology 2002*, *37*(7), 1263–1274.

5.2 Specific Author Contributions

Title of Paper	Geospatial analysis of *Helicobacter pylori* infection in South Australia: Should location influence eradication therapy?
Publication Status	Published
Publication Details	Schubert JP, Woodman RJ, Mangoni AA, et al. Geospatial analysis of Helicobacter pylori infection in South Australia: Should location influence eradication therapy?. *J Gastroenterol Hepatol*. 2022;37(7):1263-1274. doi:10.1111/jgh.15832

Principal Author

Name of Principal Author (Candidate)	Jonathon P Schubert		
Contribution to the Paper	Conception, Acquiring data, Knowledge, Analysis, Drafting		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/9/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

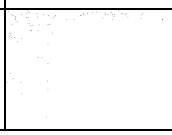
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Richard J Woodman		
Contribution to the Paper	Conception, Analysis, Drafting		
Signature		Date	27/9/2023

Name of Co-Author	Arduino A Mangoni		
Contribution to the Paper	Conception, Knowledge, Drafting		
Signature		Date	27/9/2023

Name of Co-Author	Christopher K Rayner		
Contribution to the Paper	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Morgyn S Warner		
Contribution to the Paper	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Ian C Roberts-Thomson		
Contribution to the Paper	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Samuel P Costello

Contribution to the Paper	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Robert V Bryant		
Contribution to the Paper	Knowledge, Drafting		
Signature		Date	28/11/2023

[Manuscript 3] Geospatial analysis of *Helicobacter pylori* infection in South Australia: Should location influence eradication therapy?

Jonathon P Schubert[1,2], Richard J Woodman [3], Arduino A Mangoni[4], Christopher K Rayner[1,5], Morgyn S Warner[1,6],Ian C Roberts-Thomson [1], Samuel P Costello[1,2], Robert V Bryant[1,2].

[1]Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia

[2]Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, Australia

[3]Flinders Centre for Epidemiology and Biostatistics, Flinders University, Bedford Park, Australia

[4]Department of Clinical Pharmacology, Flinders Medical Centre, Flinders University, Bedford Park, Australia

[5]Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia

[6]Microbiology and Infectious Diseases Directorate, SA Pathology, Adelaide, Australia

Corresponding author

Dr Jonathon P Schubert

Department of Gastroenterology, The Queen Elizabeth Hospital, South Australia, 5011

Email: Jonathon.schubert@sa.gov.au

Phone: (+61) (8) 8222 6000

Fax: (+61) (8) 8222 7188

Conflict of interests

No relevant conflicts of interest to disclose.

Acknowledgements

We wish to thank SA Pathology staff George Koutsouridis and Casey Moore for their assistance with data collection.

ABSTRACT

Background and Aim: Rates of antimicrobial-resistant *Helicobacter pylori* infection are rising globally, however geospatial location and its interaction with risk factors for infection have not been closely examined.

Methods: Gastric biopsy specimens were collected to detect *H. pylori* infection at multiple centres in Adelaide, South Australia between 1998–2017. The geospatial distribution of antibiotic resistant *H. pylori* in the Greater Adelaide region was plotted using choropleth maps. Moran's I was used to assess geospatial correlation, and multivariate linear regression (MLR) was used to examine associations between migration status, socioeconomic status, age, gender, and rates of *H. pylori* positivity and antibiotic resistance. Geographically weighted regression (GWR) was used to determine the extent to which the associations varied according to geospatial location.

Results: Of 20,108 biopsies across 136 postcodes within the Greater Adelaide region, 1,901(9.45%) were *H. pylori* positive. Of these, 797(41.9%) displayed clarithromycin, tetracycline, metronidazole, or amoxicillin resistance. In MLR, migration status was associated with the rate of *H. pylori* positivity (β=3.85% per 10% increase in a postcode's migrant population; $p<0.001$). *H. pylori* positivity and resistance to any antibiotic were geospatially clustered (Moran's I=0.571 and 0.280, respectively; $p<0.001$ for both). In GWR, there was significant geospatial variation in the strength of the migrant association for both *H. pylori* positivity and antibiotic resistance.

Conclusion: Our study demonstrates the heterogeneous geospatial distribution of *H. pylori* positivity and antibiotic resistance, as well as its interaction with migrant status. Geographic location and migrant status are important factors to consider for *H. pylori* eradication therapy.

Keywords:

Helicobacter pylori, antibiotics, antimicrobial, resistance, antimicrobial resistance, geospatial

INTRODUCTION

Geospatial technology has helped to target disease prevention programs through characterisation of disease distribution and aetiology.[1] *H. pylori* infection has been estimated to be responsible for up to 90% of cases of gastric cancer globally and *H. pylori* eradication has been shown to reduce the incidence of gastric cancer at a population level.[2-5] The prevalence of antimicrobial resistant *H. pylori* has been increasing globally and represents a significant public health threat.[6] Eradication rates have been declining in many countries due to antimicrobial resistance, culminating in the World Health Organisation listing clarithromycin-resistant *H. pylori* as a high priority for antibiotic research and development.[7]

H. pylori antibiotic resistance varies between geographic regions, has increased over time in most areas of the world, and is inversely associated with eradication rates.[8-11] In Australia, between 15-30% of the population have *H. pylori* infection.[12, 13] Varying ranges of antibiotic resistance and increasing rates of clarithromycin resistance have also been reported in Australia.[14, 15] Known risk factors for the development of resistant strains include prior antibiotic exposure, national antibiotic consumption and smoking.[16-18] Other proposed mechanisms of increasing resistance in Australia include migration from countries with a high prevalence of *H. pylori* infection and resistance, as well as low grade antibiotic exposure through intake of food products from antibiotic-treated animals.[19] The use of macrolides for the treatment of conditions such as pneumonia and sexually transmitted infections since the 1990s is also likely to have played a role.[20] Fluroquinolone resistance has been largely attributed to increased community exposure, with rising rates described overseas.[16, 21] Metronidazole resistance is also rising due to its widespread use and is more common in developing countries, where it is used for parasitic diseases and gynaecological infections, and women have been shown to have higher rates of resistance than men.[22, 23]

Geospatial analyses are underutilised for studying antimicrobial resistance and have not previously been applied to *H. pylori*. Globally, geospatial analyses have been useful for understanding the distribution of infection in antimicrobial resistant *Staphylococcus aureus*[24], *Streptococcus pneumoniae*[25] and *Escherichia coli*.[26] Given the large variation in *H. pylori* resistance rates, a better understanding of the geospatial distribution of both infection and resistance, and its influence on risk factors for resistance, is needed to guide eradication therapy. Most of the data on resistance

rates have been derived from large geographical regions, often comprising entire countries, whereas more granularity is needed to determine the extent of local variation and thereby inform local therapy. No established regional or national *H. pylori* resistance monitoring systems exists within Australia, supporting a need for better characterization of the distribution of disease and antibiotic resistance.

The present study combined clinical and geospatial data to assess both the rates and spatial distribution of *H. pylori* infection and antibiotic resistance using the largest Australia database of isolate susceptibility data collected between 1998-2018. It was hypothesised that an understanding of the geospatial distribution of infection, resistance and risk factor associations, may assist clinicians with risk stratification for *H. pylori* infection, particularly in those at increased risk of harbouring resistant strains, and assist in guiding eradication therapy.

METHODS

Gastric biopsy specimens collected from five centres in South Australia underwent histopathology testing for *H. pylori* infection. The Greater Adelaide region of South Australia comprises a multicultural population of approximately 1.4 million people, with a varied range of ethnicities and significant number of migrants from areas including Europe and Asia. All specimens taken between November 1998 and December 2018 were analysed. Isolates positive for *H. pylori* were cultured and tested for susceptibility to amoxicillin, clarithromycin, tetracycline, and metronidazole. All samples were collected as part of routine clinical practice, and therefore informed consent to be included in this study was not obtained. Analysis of the data was conducted following approval by the Central Adelaide Human Research Ethics Committee (Reference number: 12902).

Data collection

Demographic data including age, gender, year of presentation and postcode were obtained from patient records. The patient postcode was linked to information on the percentage of each postcode's population that was born within Australia and overseas, and the Socioeconomic Index For Areas (SEIFA) index, using the 2016 national census data.[23]

Geospatial data

The spatial area of interest was defined by the Greater Adelaide Planning Region of South Australia which contains 185 different postcodes (Figure 1). A Shapefile containing the polygons of all Australian postcodes was obtained from the Australian Bureau of Statistics,[27] and the Greater Adelaide Planning Region shapefile polygon was obtained from the South Australian Government Data Dictionary.[28] Patients from postcodes outside of the Greater Adelaide Planning Region were excluded from the analysis.

H. pylori culture and antibiotic susceptibility test

Gastric biopsies were cultured at 37°C on brain heart infusion plates (Difco Laboratories Pty Ltd) containing 7% horse blood under microaerobic conditions (5% O_2; 10% CO_2; 85% N_2) for 3-7 days. If there was no visible microbial growth at 3 days, specimens were cultured for a further 3-4 days due to slower growth rates for some strains. Positive *H. pylori* isolates were tested for susceptibility to amoxicillin, clarithromycin, metronidazole, and tetracycline using ETest strips (AB

Biodisk, Sweden), from which minimum inhibitory concentrations were determined. European Committee on Antimicrobial Susceptibility Testing guidelines breakpoints were used for clarithromycin, amoxicillin, tetracycline and metronidazole of >0.5, >0.125, >1, and >8 mg/L, respectively .[29]

Statistical Analysis

Spatial distribution and rates of *H. pylori* infection were assessed, as well as antibiotic resistance of *H. pylori* to clarithromycin, metronidazole, amoxicillin, and tetracycline. Postcode level data for migration status (percentage of the population born overseas) and the SEIFA were retrieved for each post code from the 2016 Australian national census data.[30]

Multivariate linear regression was performed with postcode data to examine the predictors of *H. pylori* positivity and rates of antibiotic resistance. Models included the mean age, percentage of males, migration status (percentage born overseas in each postcode), the SEIFA, and the period (1998-2007 versus 2008-2018) of the testing. We plotted the overall rates of *H. pylori* positivity and antibiotic resistance over time, and examined whether there was evidence of a linear trend using Poisson or negative binomial regression as appropriate. The degree of spatial clustering of *H. pylori* isolates and antibiotic resistance was assessed using Moran's I,[31] and *H. pylori* infection rates and antibiotic resistance rates for each postcode were plotted with choropleth maps. Hotspots were defined as postcodes with rates above the 90th percentile and p<0.001 for local Moran's I. Cold-spots were defined as postcodes with rates below the 10th percentile and p<0.001 for local Moran's I.

In addition to the multivariate linear regression, we also performed geographically weighted regression (GWR) to determine the extent to which geospatial location contributed to variability in *H. pylori* infection and antibiotic resistance, and to determine the spatial variation in the strength of the regression coefficients. The optimal spatial weights for the calculation of Moran's I and the GWR were determined using the k-nearest neighbours' method with varying bandwidths.[32] The proportion of explained variance for infection and resistance rates was described using the R-squared statistic from the GWR. A 2-sided type 1 error rate of α=0.05 was used for all hypothesis tests. Descriptive statistics and linear regression were performed using Stata version 16.0 (StataCorp, USA, Texas). Geospatial analysis was performed using Python (version 3.8.5) and the

Python geospatial packages geopandas (v 0.8.1), pysal (v 2.1.0), libpysal (v 4.4.0), esda (v 2.1.1), contextily (v 1.1.0) and mgwr (v 2.1.2).

RESULTS

Patient demographics

The study population consisted of 20,108 subjects across 136 of the 185 Greater Adelaide Planning region postcodes (Table 1). Amongst all subjects, 9,050 (45.0%) were male and the mean (SD) age was 57.5 (17.8) years. A total of n=1,901 (9.5%) biopsies tested were *H. pylori* positive. Of these, 797 (41.9%) displayed resistance to clarithromycin (19%), tetracycline (1.8%), metronidazole (31%), or amoxicillin (3.3%), with 222 of these isolates (28%) demonstrating resistance to two or more antibiotics. Across the 136 post codes, the minimum number of subjects included per postcode was 10, and the median (IQR) was 75 (28-192.5). There were several differences in the demographics between subjects with biopsies that were *H. pylori* positive, compared to the those that were *H. pylori* negative, including age, gender, number of subjects tested within each postcode, migration status, SEIFA, and period of testing (Table 5.1).

Predictors of *H. pylori* positivity and antibiotic resistance

An absolute increase in the rate of *H. pylori* positivity was associated with higher migrant density (β=3.85% per 10% increase in the postcode's migrant population; $p<0.001$) and with decade of analysis, but not with the SEIFA index, mean age for the postcode, or proportion of males in the postcode. There were also positive associations between migrant status and resistance to any antibiotic, and resistance to metronidazole, amoxicillin and clarithromycin (Table 5.2). There was an overall 10.9% linear decline in the rate of *H. pylori* positivity per year (IRR=0.89, 95% CI=0.87, 0.92; $p<0.001$) (Appendix: Figure 5.A2). However, amongst subjects who tested positive for *H. pylori*, there was an overall 2.2% increase per year in the rate of antibiotic resistance, with all antibiotics tested increasing over the study period. Specifically, the rate of amoxicillin resistance increased by 9.0% per year, clarithromycin by 5.7% per year, metronidazole by 1.6% per year and tetracycline by 13.7% per year (Figure 5.3).

Geospatial distributions

The distribution of resistance to *H. pylori* and rates of overall antibiotic resistance within the Greater Adelaide planning region postcodes showed strong evidence of clustering (Figure 5.A3). The pattern of resistance rates with antibiotics showed a similar trend (Figure 5.2). Moran's I showed a positive spatial auto-correlation, indicating significant geospatial clustering for rates of *H. pylori* (I=0.573, p<0.001), overall antibiotic resistance (I=0.280, p<0.001) and resistance to clarithromycin (I=0.157, p<0.001) and metronidazole (I=0.182, p<0.001), but not amoxicillin (I=0.022, p=0.382) or tetracycline (I=-0.028, p=0.298). The pattern of both rates of *H. pylori* positivity and antibiotic resistance across the postcodes each closely matched the respective migrant status (Figure 5.4).

Geographically weighted regression (GWR)

In GWR, which included the independent variables mean postcode age, gender, SEIFA and migrant status, the global regression R-squared statistic was 0.225, whilst the ensemble R-squared for the local model was 0.371. The difference in these R-squared values demonstrated that 14.6% of the variation in *H. pylori* positivity was due to a spatial component i.e. there was substantial variation in the individual postcode regression coefficients (Appendix; Figure 5.A4). Specifically, the regression coefficients for migrant status ranged from 0.0265 to 0.0415, indicating that a 10% increase in migrant density was associated with an absolute increase in *H. pylori* positivity of between 2.65%-4.15% depending on the postcode.

Local regression coefficients from the GWR for antibiotic resistance and migrant status ranged from 1.22% to 1.79% (Appendix; Figure 5.A5) indicating an absolute 1.22%-1.79% increase in antibiotic resistance rates for persons positive to *H. pylori* per 10% increase in the migrant density. Similar to *H. pylori* positivity, all 136 local coefficients for antibiotic resistance were significant for migrant status, whilst none were significant for gender, age, or SEIFA. Based on the R^2 statistics, each local model explained between 14.8% and 26.9% of the variation in antibiotic resistance rates for that postcode.

DISCUSSION

This is the first population-based study that provides evidence for a heterogeneous distribution of *H. pylori* resistance within a city. The data also reveal an association between the migrant density of a suburb and the rate of resistant *H. pylori* isolates. These findings illustrate that a patient's geospatial location may be a useful surrogate marker for risk of *H. pylori* resistance, which may help to guide clinicians in appropriate empirical *H. pylori* eradication therapy.

This study illustrates the role of risk factor stratification for *H. pylori* resistance to include geospatial location alongside previously established risk factors. Prior studies have demonstrated that migrants have higher rates of *H. pylori* infection as a consequence of emigrating from regions of higher antibiotic resistance than the general Australian population.[33, 34] Clustering of resistant *H. pylori* strains is likely to relate to both migration status and local transmission of *H. pylori* among close contacts within the suburb. Recognition of a patient's individual migrant status as well as a geospatial regions associated with higher migrant density, should alert a clinician to a higher risk of harbouring a resistant *H. pylori* strain. Prompt consideration should then be given to additional culture and sensitivity analyses, as well as consideration of alternative (even second-line) empirical eradication therapy.

Rates of primary *H. pylori* resistance to clarithromycin in Australia have historically been thought to be low (between 6-8%) based on data collected during the 1990s.[35-37] However, recent studies have demonstrated increasing resistance in Australia and New Zealand, with particular evidence of increasing clarithromycin resistance since the year 2000.[14, 15] Yet, there has been little recognition of rising rates of *H. pylori* resistance, and risk factors for resistance, in current Australian guidelines. Factors driving these emerging trends include widespread community antibiotic use, and population changes due to migration from countries with a high prevalence of *H. pylori* resistance.[37] The proportion of Australians born overseas has been increasing over time (1/3 of Australians in the 2016 census), and the number of immigrants has been rising from countries, particularly in Asia, where clarithromycin resistance rates are known to be above 30%.[23, 38] This study is the first to demonstrate that patients originating from postcodes which are associated with higher migrant density are more likely to harbour antibiotic-resistant strains.

Risk factor stratification should be advocated in the individualised management of *H. pylori*, which should be modified not only according to risk of antibiotic resistance, but also to risk of developing complications of *H. pylori* infection, including known gastric metaplasia or a family history of gastric cancer. Migrant populations are frequently burdened by both the risk of resistance and complications of *H. pylori* infection.

Individualising eradication therapy, particularly in nations where health care resources are in short supply and rates of treatment failure are increasing, has potential for economic cost savings. Effective empiric eradication therapy will reduce the probability of treatment failure and need for repeated courses of antibiotic therapy. While there is a proven benefit for *H. pylori* eradication at a population level for reducing the incidence of gastric cancer, the benefits may be greater in specific subpopulations, such as those with atrophic or non-atrophic gastritis.[4, 39] For low-risk patients without a family history or other risk factors for gastric cancer, the benefits of eradication are not established. Further identification and characterisation of specific strains, and environmental and bacterial genetic factors, would help to identify determinants of poor clinical outcomes. Both genetic factors and virulence factors with a high gastric cancer risk may be associated with the host and the *H. pylori* strain, and these would help to stratify subgroups of patients into those more or less likely to benefit from eradication therapy.

The reason for variable long term clinical outcomes in patients infected with *H. pylori*, such as development of gastric cancer or duodenal ulceration, remains unclear. However genetics modulating the immune response towards the infection, bacterial genetics and age at acquisition of *H. pylori* infection, in addition to environmental factors such as smoking and high intake of salt have been shown to play a role.[40] Testing for *H. pylori* as a gastric cancer pre-screening strategy has been described in Japan, and the importance of *H. pylori* eradication as a cancer prevention strategy is emphasized in Japanese guidelines.[41] Increased virulence and malignant potential of *H. pylori* strains have been linked to mutations in vacuolating cytotoxin (*vacA*) and the *Cag* pathogenicity island (*cagPAI*).[42, 43] Geospatial analyses have previously been used to link virulence factors of *H. pylori*, such as *cagA*, such that a relationship has been observed between areas with high rates of *cagA* and those with a strong prevalence of diffuse gastric cancer.[44] Additionally, prior studies have demonstrated that sociodemographic variables may result in high risk patients being overlooked for *H. pylori* testing.[45] An understanding of patients at risk of *H. pylori* infection and resistance may help to reduce this bias.

Major strengths of this study include the multicentre approach, the large number of *H. pylori* isolates, and the prolonged period of data capture, allowing comparisons both over time and across metropolitan areas. A limitation of this study was the lack of information on the migration status of individual patients as well as detailed clinical information including historic antibiotic exposure. However, if the migrant-to-resistance association is valid, our data are likely, if anything, to have underestimated the true strength of this relationship. In addition, each patient's geospatial location was based on postcode, which can cover a wide geographical area and may therefore be either a higher or lower indicator of the true rate of infection rate in the patient's location. Again, assuming that a real spatial component exists in the data, the estimated degree of spatial correlation is likely to be an under- rather than an over-estimate. Data capture was limited to Greater Adelaide, and further studies are recommended to confirm whether the associations we observed are generalisable to other locations. Geospatial analyses across a broader context will confirm whether these relationships are observed nationally and internationally, and may assist in risk-stratifying patients for antibiotic resistant isolates.

CONCLUSIONS

This study demonstrates a heterogeneous distribution of *H. pylori* resistance, which is increased particularly in isolates originating from suburbs with a high percentage of migrants. This provides evidence that the current 'one-size-fits-all' approaches to antibiotic eradication therapy may not be suited to the general population, and that further risk-stratification of patients for resistant strains should be considered in routine clinical practice. Further studies of eradication therapy success rates are warranted in regions with high numbers of migrants.

Acknowledgements

We wish to thank SA Pathology staff George Koutsouridis and Casey Moore for their assistance with data collection.

REFERENCES

1. Cromley EK. GIS and disease. Annual review of public health. 2003;24:7-24.

2. González CA, Megraud F, Buissonniere A, Lujan Barroso L, Agudo A, Duell EJ, et al. Helicobacter pylori infection assessed by ELISA and by immunoblot and noncardia gastric cancer risk in a prospective study: the Eurgast-EPIC project. Annals of oncology : official journal of the European Society for Medical Oncology. 2012;23(5):1320-4.

3. Lee YC, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, et al. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. Gastroenterology. 2016;150(5):1113-24.e5.

4. Rokkas T, Rokka A, Portincasa P. A systematic review and meta-analysis of the role of Helicobacter pylori eradication in preventing gastric cancer. Annals of gastroenterology. 2017;30(4):414-23.

5. Ford AC, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut. 2020;69(12):2113-21.

6. Goh KL, Chan WK, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori infection and public health implications. Helicobacter. 2011;16 Suppl 1(0 1):1-9.

7. World Health Organisation. Global priority list of Antibiotic-resistance bacteria to guide rsearch, discovery, and development of new antibiotics. Accessed August 2021. URL: https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/. 2017.

8. Meyer JM, Silliman NP, Wang W, Siepman NY, Sugg JE, Morris D, et al. Risk factors for Helicobacter pylori resistance in the United States: the surveillance of H. pylori antimicrobial resistance partnership (SHARP) study, 1993-1999. Annals of internal medicine. 2002;136(1):13-24.

9. Graham DY. Helicobacter pylori update: gastric cancer, reliable therapy, and possible benefits. Gastroenterology. 2015;148(4):719-31.e3.

10. Graham DY, Shiotani A. New concepts of resistance in the treatment of Helicobacter pylori infections. Nature clinical practice Gastroenterology & hepatology. 2008;5(6):321-31.

11. Gisbert JP, Pajares JM. Helicobacter pylori "rescue" therapy after failure of two eradication treatments. Helicobacter. 2005;10(5):363-72.

12. Moujaber T, MacIntyre CR, Backhouse J, Gidding H, Quinn H, Gilbert GL. The seroepidemiology of Helicobacter pylori infection in Australia. Int J Infect Dis. 2008;12(5):500-4.

13. Robertson MS, Cade JF, Savoia HF, Clancy RL. Helicobacter pylori infection in the Australian community: current prevalence and lack of association with ABO blood groups. Intern Med J. 2003;33(4):163-7.

14. Schubert JP, Gehlert J, Rayner CK, Roberts-Thomson IC, Costello S, Mangoni AA, et al. Antibiotic resistance of Helicobacter pylori in Australia and New Zealand: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2020.

15. Schubert JP, Warner S, Rayner CK, Roberts-Thomson IC, Costello S, Mangoni AA, et al. Increasing Helicobacter pylori clarithromycin resistance in Australia over 20 years. World Journal of Gastroenterology (Accepted for publication). 2021.

16. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62(1):34-42.

17. Perez Aldana L, Kato M, Nakagawa S, Kawarasaki M, Nagasako T, Mizushima T, et al. The relationship between consumption of antimicrobial agents and the prevalence of primary Helicobacter pylori resistance. Helicobacter. 2002;7(5):306-9.

18. Mhaskar RS, Ricardo I, Azliyati A, Laxminarayan R, Amol B, Santosh W, et al. Assessment of risk factors of helicobacter pylori infection and peptic ulcer disease. Journal of global infectious diseases. 2013;5(2):60-7.

19. Hu Y, Zhang M, Lu B, Dai J. Helicobacter pylori and Antibiotic Resistance, A Continuing and Intractable Problem. Helicobacter. 2016;21(5):349-63.

20. McMullan BJ, Mostaghim M. Prescribing azithromycin. Australian prescriber. 2015;38(3):87-9.

21. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, et al. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. The lancet Gastroenterology & hepatology. 2017;2(10):707-15.

22. Glupczynski Y, Mégraud F, Lopez-Brea M, Andersen LP. European multicentre survey of in vitro antimicrobial resistance in Helicobacter pylori. European journal of clinical microbiology &

infectious diseases : official publication of the European Society of Clinical Microbiology. 2001;20(11):820-3.

23. Mégraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53(9):1374-84.

24. Tirabassi MV, Wadie G, Moriarty KP, Garb J, Konefal SH, Courtney RA, et al. Geographic information system localization of community-acquired MRSA soft tissue abscesses. Journal of pediatric surgery. 2005;40(6):962-5; discussion 5-6.

25. Mouro A, Kiffer C, Koga PC, Monteiro AM, Camargo EC, Pignatari AC. Spatial exploration of Streptococcus pneumoniae clonal clustering in São Paulo, Brazil. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases. 2011;15(5):462-6.

26. Kiffer CR, Camargo EC, Shimakura SE, Ribeiro PJ, Jr., Bailey TC, Pignatari AC, et al. A spatial approach for the epidemiology of antibiotic use and resistance in community-based studies: the emergence of urban clusters of Escherichia coli quinolone resistance in Sao Paulo, Brasil. International journal of health geographics. 2011;10:17.

27. Australian Bureau of Statistics. Australian Statistical Geography Standard (ASGS): Volume 3. Accessed August 2021. URL: https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/1270.0.55.003July%202016?OpenDocument. 2016.

28. Australian Bureau of Statistics. Greater Adelaide Planning Region. 2015. Accessed August 2021. URL: https://plan.sa.gov.au/__data/assets/pdf_file/0005/282974/G16_2015.pdf.

29. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Accessed 31 August 2021. http://www.eucast.org/.

30. Australian Bureau of Statistics. Census of Population and Housing: Socio-Economic Indexes for Areas (SEIFA), Australia, 2016. Accessed August 2021. URL: https://www.abs.gov.au/ausstats/abs@.nsf/mf/2033.0.55.001.

31. Moran PA. Notes on continuous stochastic phenomena. Biometrika. 1950;37(1-2):17-23.

32. Altman NS. An Introduction to Kernel and Nearest-Neighbor Nonparametric Regression. The American Statistician. 1992;46(3):175-85.

33. Pandeya N, Whiteman DC. Prevalence and determinants of Helicobacter pylori sero-positivity in the Australian adult community. Journal of gastroenterology and hepatology. 2011;26(8):1283-9.

34. Mitchell H, Katelaris P. Epidemiology, clinical impacts and current clinical management of Helicobacter pylori infection. The Medical journal of Australia. 2016;204(10):376-80.

35. Katelaris PH, Adamthwaite D, Midolo P, Yeomans ND, Davidson G, Lambert J. Randomized trial of omeprazole and metronidazole with amoxycillin or clarithromycin for Helicobacter pylori eradication, in a region of high primary metronidazole resistance: the HERO study. Aliment Pharmacol Ther. 2000;14(6):751-8.

36. Katelaris PH, Forbes GM, Talley NJ, Crotty B. A randomized comparison of quadruple and triple therapies for Helicobacter pylori eradication: The QUADRATE Study. Gastroenterology. 2002;123(6):1763-9.

37. https://www.tg.org.au TGecdHpiAAU.

38. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.

39. Ford AC, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis. 2020;69(12):2113-21.

40. Bornschein J, Malfertheiner P. Gastric carcinogenesis. Langenbeck's archives of surgery. 2011;396(6):729-42.

41. Kato M, Ota H, Okuda M, Kikuchi S, Satoh K, Shimoyama T, et al. Guidelines for the management of Helicobacter pylori infection in Japan: 2016 Revised Edition. Helicobacter. 2019;24(4):e12597.

42. Polk DB, Peek RM, Jr. Helicobacter pylori: gastric cancer and beyond. Nature reviews Cancer. 2010;10(6):403-14.

43. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer research. 1995;55(10):2111-5.

44. Dominguez RL, Cherry CB, Estevez-Ordonez D, Mera R, Escamilla V, Pawlita M, et al. Geospatial analyses identify regional hot spots of diffuse gastric cancer in rural Central America. BMC cancer. 2019;19(1):545.

45. Ma I, Guo M, Pillai DR, Church DL, Naugler C. Is the Utilization of Helicobacter pylori Stool Antigen Tests Appropriate in an Urban Canadian Population? American journal of clinical pathology. 2020;153(5):686-94.

TABLES AND FIGURES

	All subjects (n=20,108)	***H. pylori* negative (n=18,207)**	***H. pylori* positive (n=1,901)**	**p-value[1]**
Age, mean (SD)	57.5 (17.8)	57.5 (17.9)	56.7 (16.7)	0.046
Gender				
Male, n (%)	9,050 (45.01)	8,145 (44.7)	905 (47.6)	0.017
Female, n (%)	11,058 (55.0)	10,062 (55.3)	996 (52.4)	
N per postcode, median (IQR)	75 (28-192.5)	75 (28-192)	91 (43-211)	<0.001
Overseas born, median (IQR)	28.95 (23.6-34.4)	30.8 (25.9-36.3)	34.2 (28.9-39.3)	<0.001
SEIFA, median (IQR)	972 (929-1,016)	975 (929-1,019)	944 (895-1,000)	<0.001
Period, n (%)				
1998-2007	7,069 (35.2)	5,929 (32.6)	1,140 (60.0)	<0.001
2008-2018	13,039 (64.8)	12,278 (67.4)	761 (40.0)	
Any antibiotic resistance, n (%)	797 (3.96)	0 (0.00)	797 (41.9)	<0.001
Specific resistance				
Amoxicillin, n (%)	63 (0.31)	0 (0.00)	63 (3.31)	<0.001
Clarithromycin, n (%)	364 (1.81)	0 (0.00)	364 (19.2)	<0.001
Metronidazole, n (%)	592 (2.94)	0 (0.00)	592 (31.1)	<0.001
Tetracycline, n (%)	34 (0.17)	0 (0.00)	34 (1.79)	<0.001

[1]P-value from chi-squared, Mann-Whitney or t-test as appropriate.

Table 5.1: Demographic characteristics and antibiotic resistance of subjects by *H. pylori* status.

	β (95% CI)	p-value[1]
H. pylori		
Age (10 years)	-0.58 (-2.99, 1.33)	0.604
Gender (male vs female)	-5.70 (-13.00, 1.59)	0.125
Migrant status (per 10 % of the postcode population born overseas)	3.85 (2.27, 5.44)	<0.001
SEIFA (per 100-unit increase)	1.50 (-0.35, 3.35)	0.111
Period (2008-2018 vs 1998-2007)	-10.09 (-12.64, -7.53)	<0.001
Any antibiotic resistance		
Age (10 years)	-0.48 (-1.74, 0.77)	0.448
Gender (male vs female)	3.50 (-1.31, 8.31)	0.153
Migrant status (per 10 % of the postcode population born overseas)	1.77 (0.72, 2.81)	0.001
SEIFA (per 100-unit increase)	0.57 (-0.64, 1.79)	0.353
Period (2008-2018 vs 1998-2007)	-4.06 (-5.74, -2.38)	<0.001
Clarithromycin		
Age (10 years)	-0.27 (-1.01, 0.48)	0.479
Gender (male vs female)	1.25 (-1.60, 4.10)	0.390
Migrant status (per 10 % of the postcode population born overseas)	1.13 (0.51, 1.75)	<0.001
SEIFA (per 100-unit increase)	0.06 (-0.67, 4.10)	0.879
Period (2008-2018 vs 1998-2007)	-1.46 (-2.46, -0.46)	0.004
Amoxicillin		
Age (10 years)	-0.01 (-0.12, 0.10)	0.146
Gender (male vs female)	-0.08 (-0.51, 0.33)	0.721
Migrant status (per 10 % of the postcode population born overseas)	0.14 (0.04, 0.23)	0.004
SEIFA (per 100-unit increase)	-0.05 (-0.16, 0.06)	0.383
Period (2008-2018 vs 1998-2007)	0.19 (0.04, 0.34)	0.015
Metronidazole		
Age (10 years)	-0.13 (-1.11, 0.85)	0.796
Gender (male vs female)	2.78 (-0.97, 6.52)	0.145
Migrant status (per 10 % of the postcode population born overseas)	1.05 (0.24, 1.86)	0.012
SEIFA (per 100-unit increase)	0.52 (-0.43, 1.47)	0.280
Period (2008-2018 vs 1998-2007)	-2.92 (-4.23, -1.61)	<0.001
Tetracycline		
Age (10 years)	-0.01 (-0.20, 0.19)	0.949
Gender (male vs female)	0.04 (-0.70, 0.78)	0.914
Migrant status (per 10 % of the postcode population born overseas)	0.15 (-0.01, 0.31)	0.068
SEIFA (per 100-unit increase)	0.14 (-0.04, 0.33)	0.131
Period (2008-2018 vs 1998-2007)	-0.05 (-0.31, 0.21)	0.708

[1]From multivariate linear regression Wald statistic.

Table 5.2: Multivariate linear regression results for the percentage of subjects with H. pylori positivity, any antibiotic resistance and specific antibiotic resistance.

N=271 postcode-level observations (n=135 in 1998-2007 and n=136 in 2008-2018).

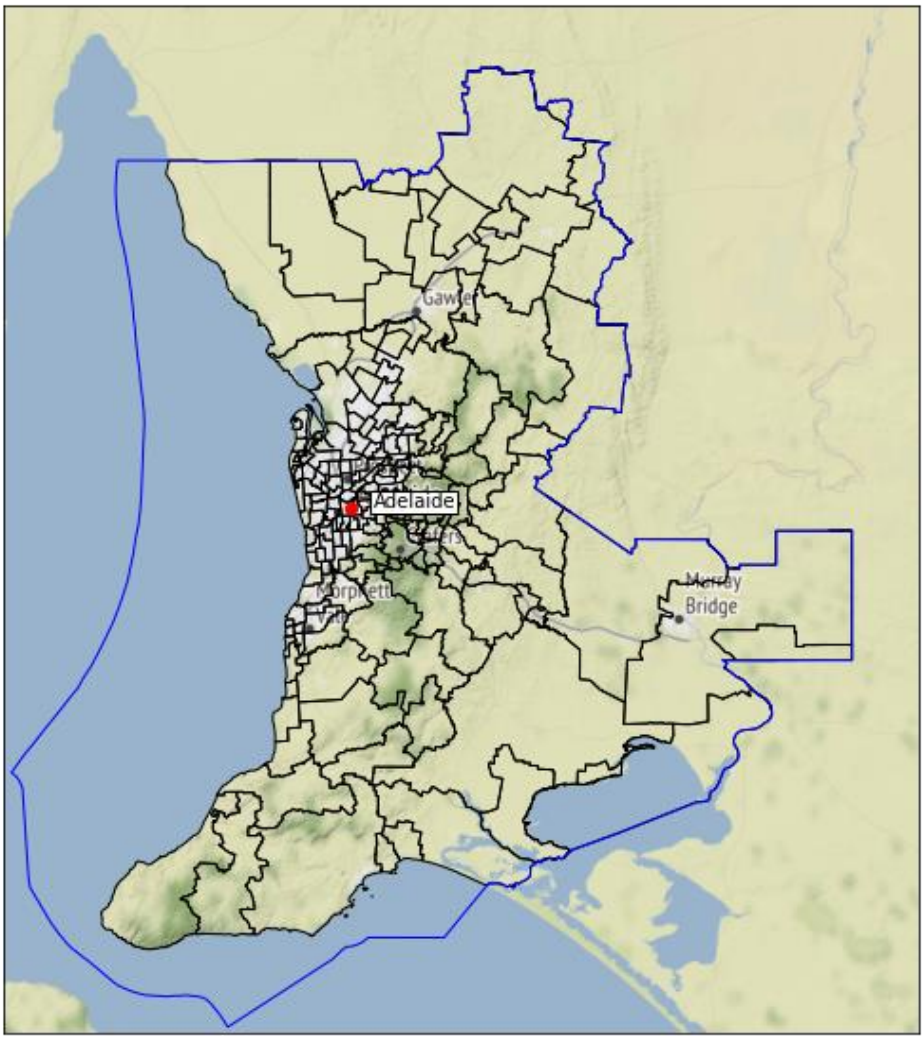


Figure 5.1: The boundary and 185 postcodes of the Greater Adelaide Planning region

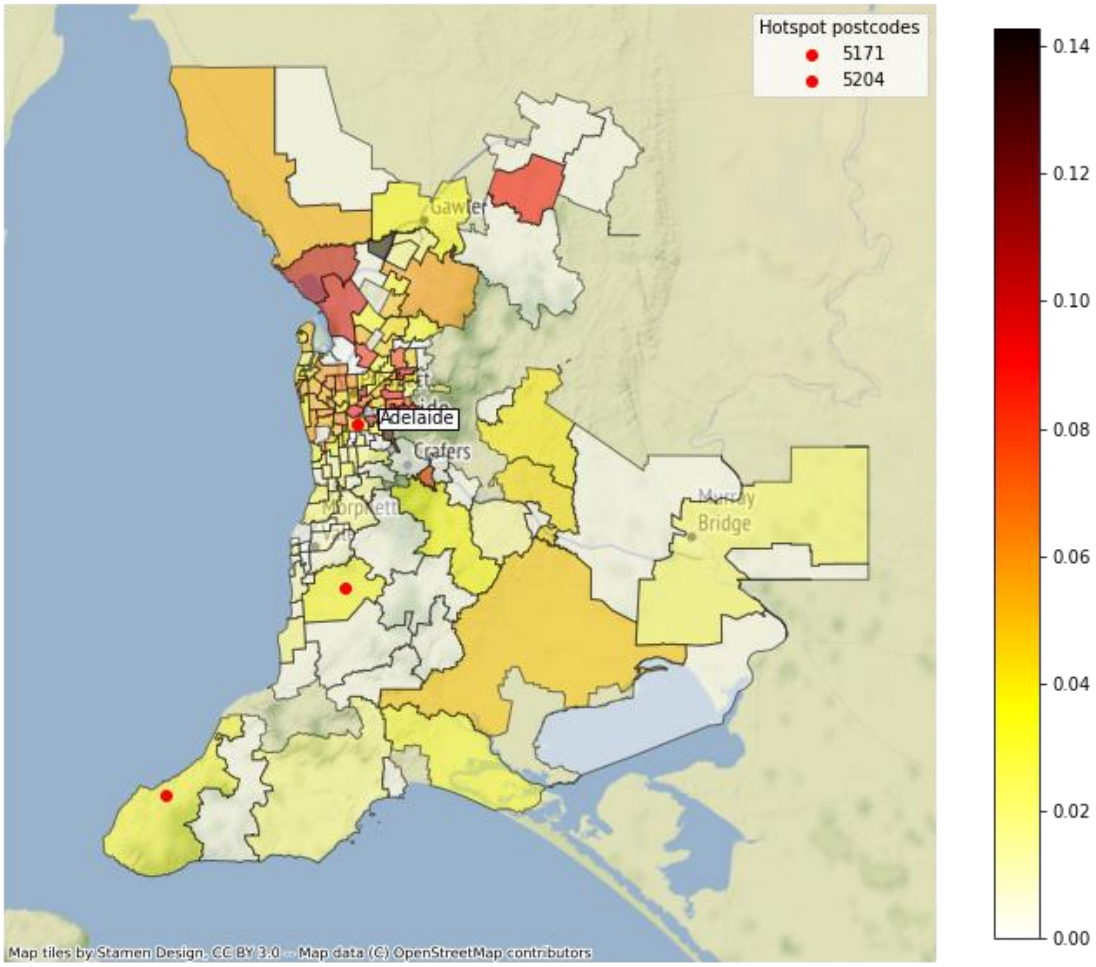


Figure 5.2: Choropleth map of antibiotic resistance rates amongst those subjects positive for H. pylori by Greater Adelaide Planning region postcode 1998-2018.

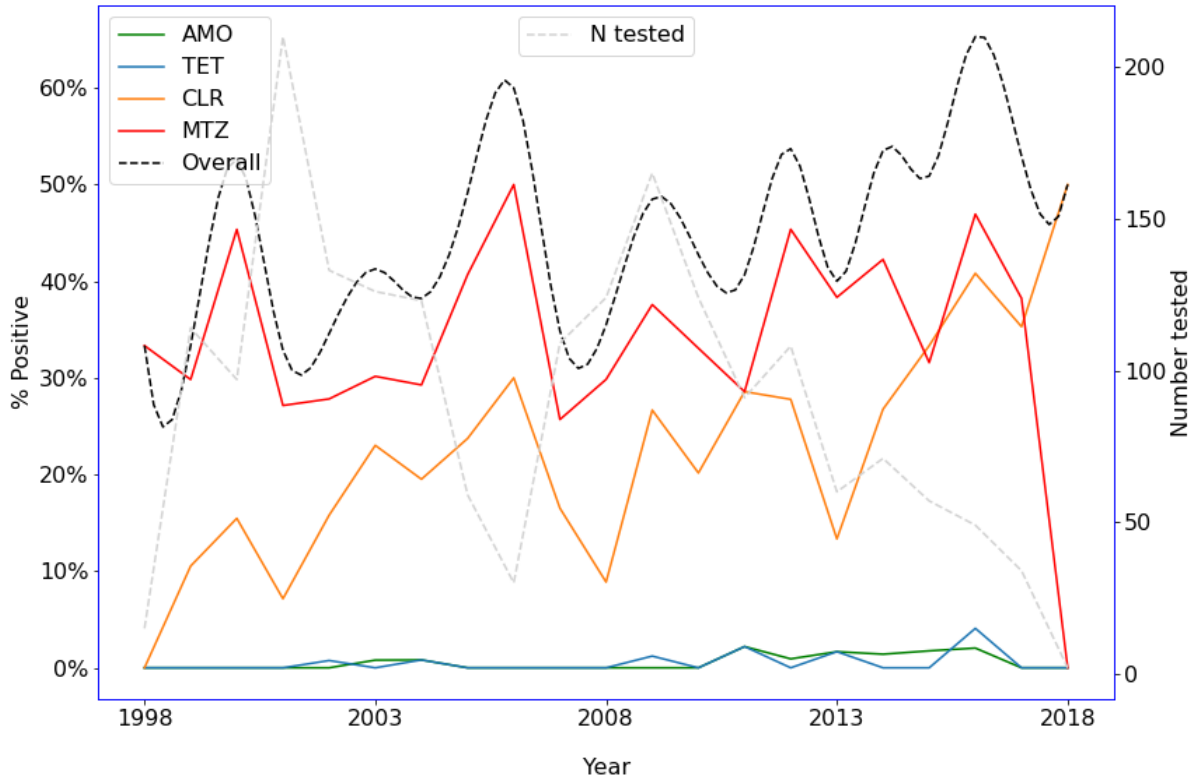


Figure 5.3: Change in rates of antibiotic resistance amongst those positive for *H. pylori* by year

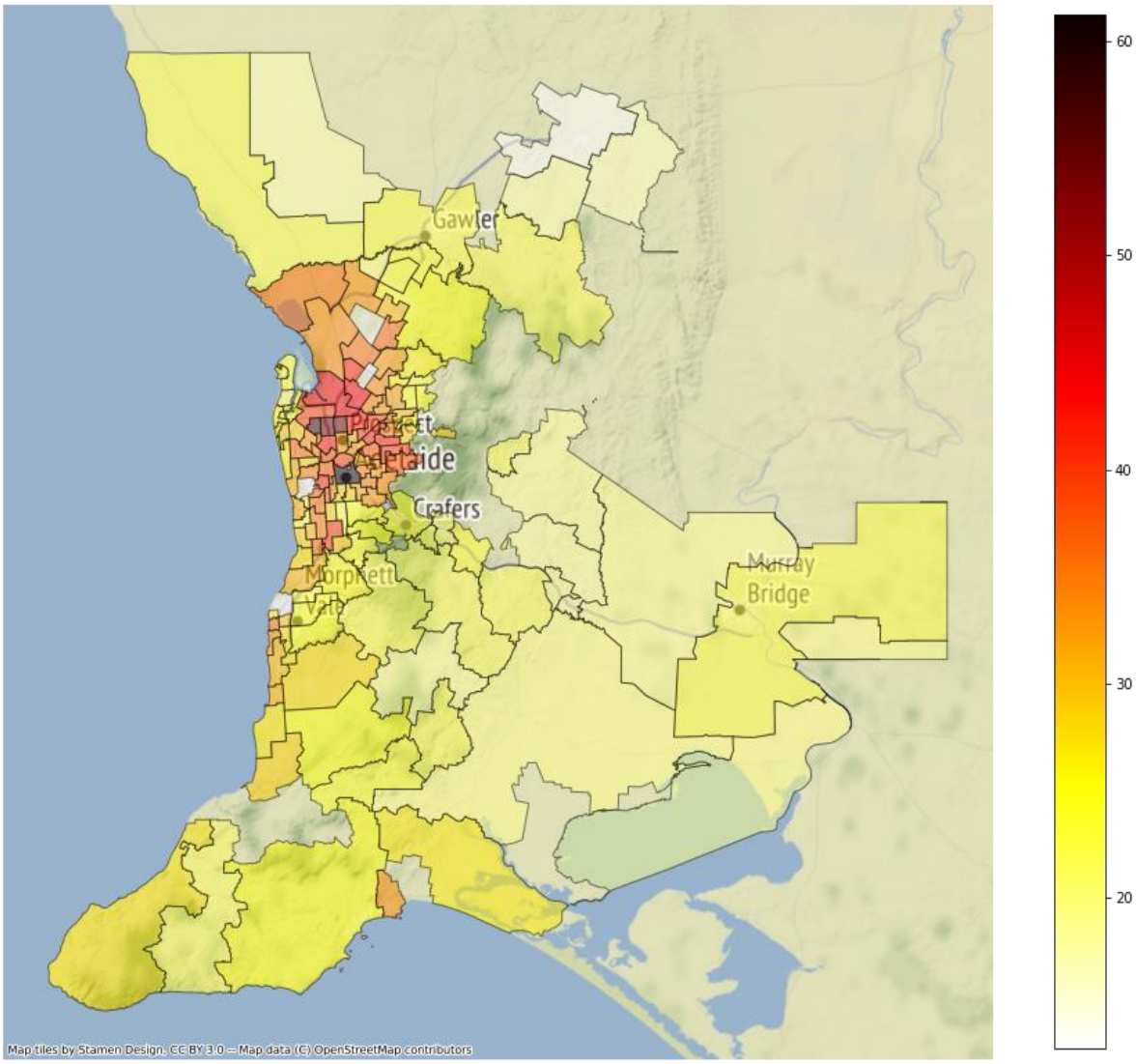


Figure 5.4: Choropleth map of migrant rates by postcode in the Greater Adelaide Planning region for 1998-2018.

APPENDIX

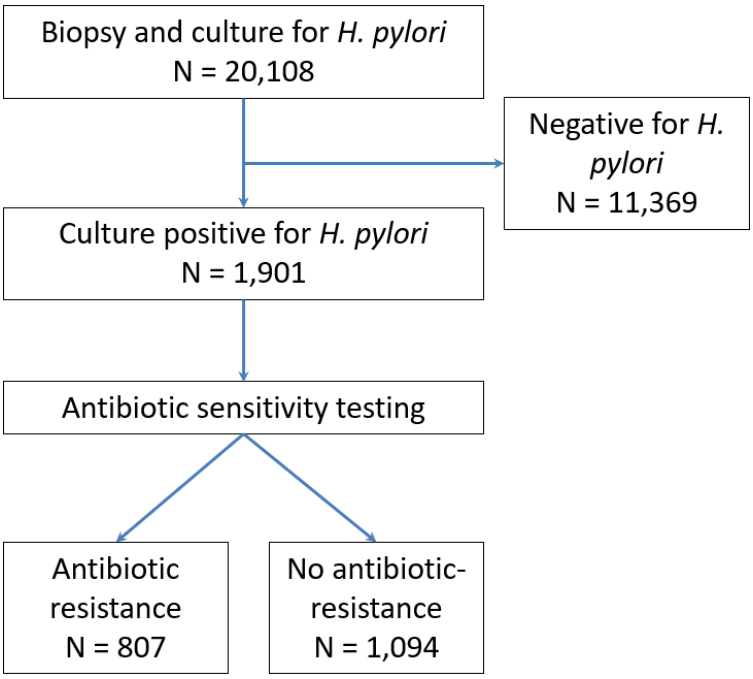


Figure 5.A1: Flow chart of patients undergoing upper gastrointestinal endoscopy

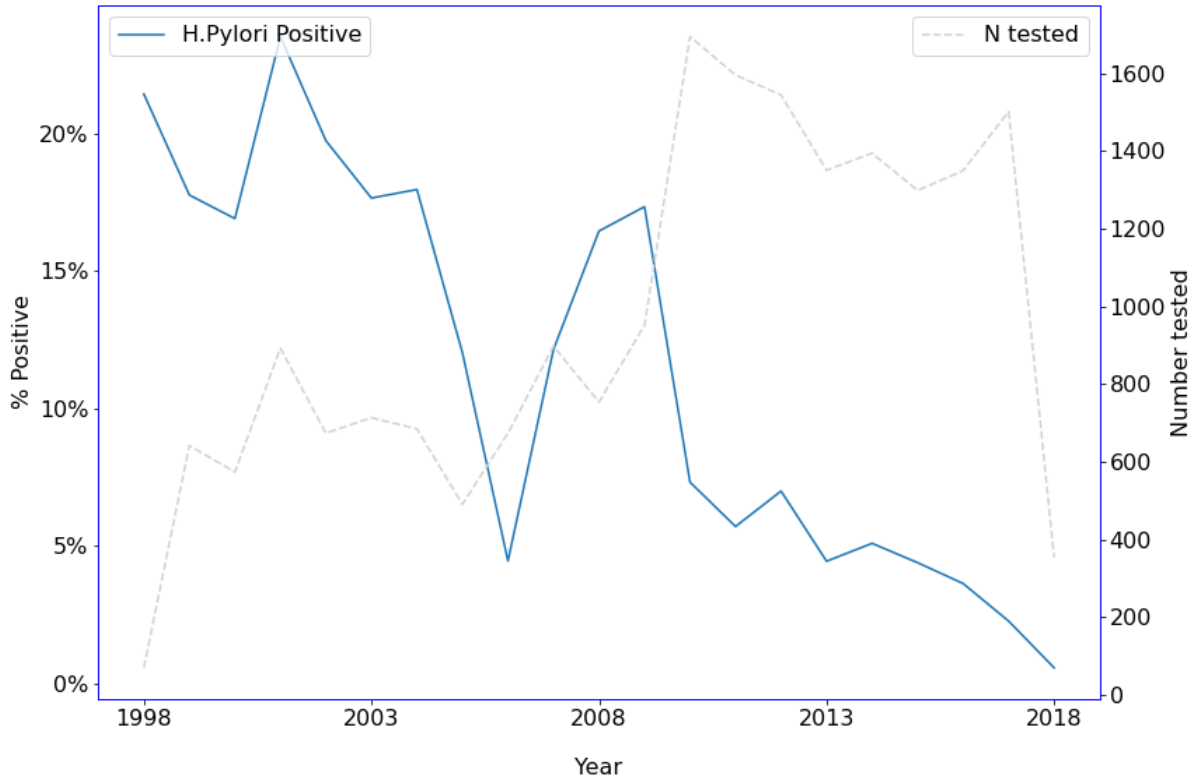


Figure 5.A2: Change in rates of *H. pylori* positivity by year

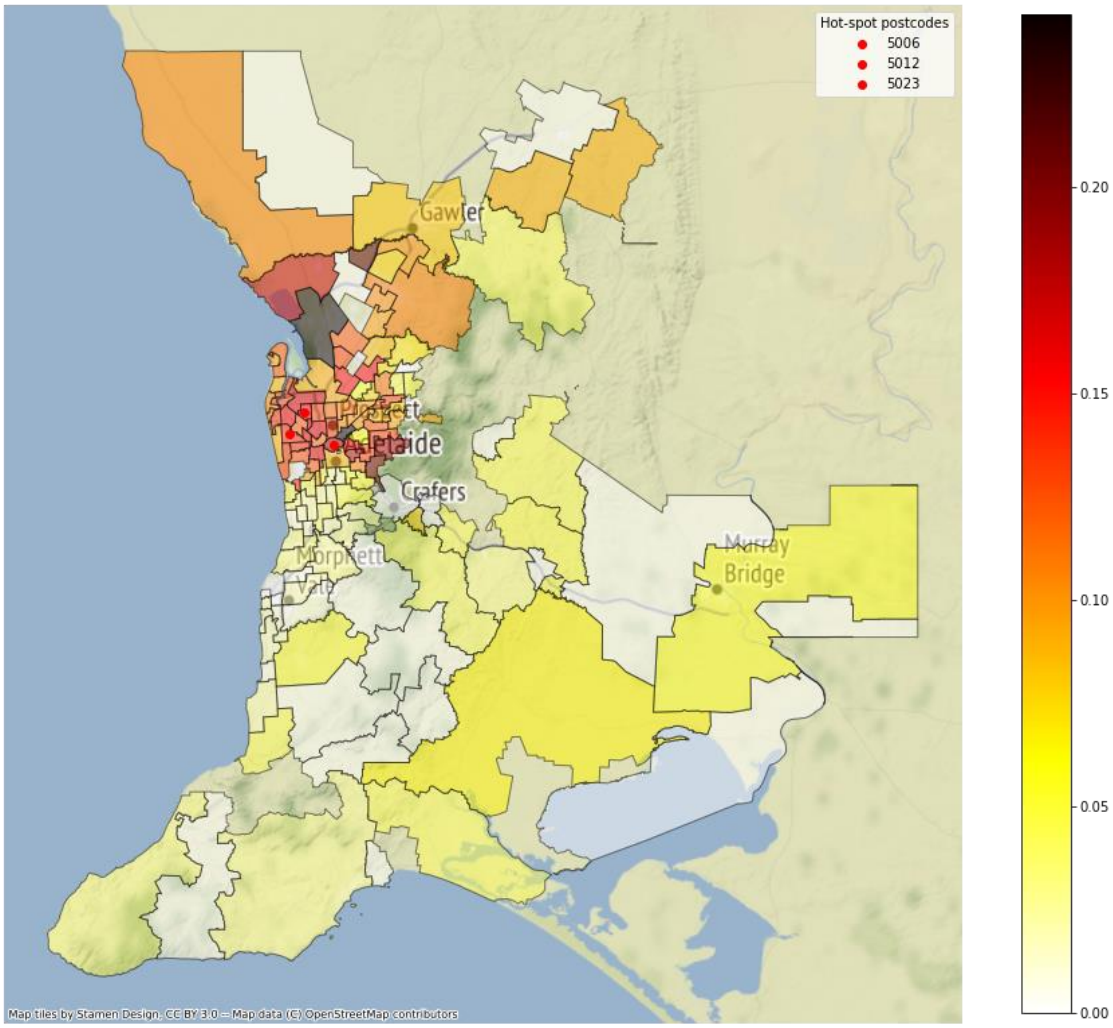


Figure 5.A3: Choropleth map of *H. pylori* positivity rates Greater Adelaide Planning region postcode 1998-2018.

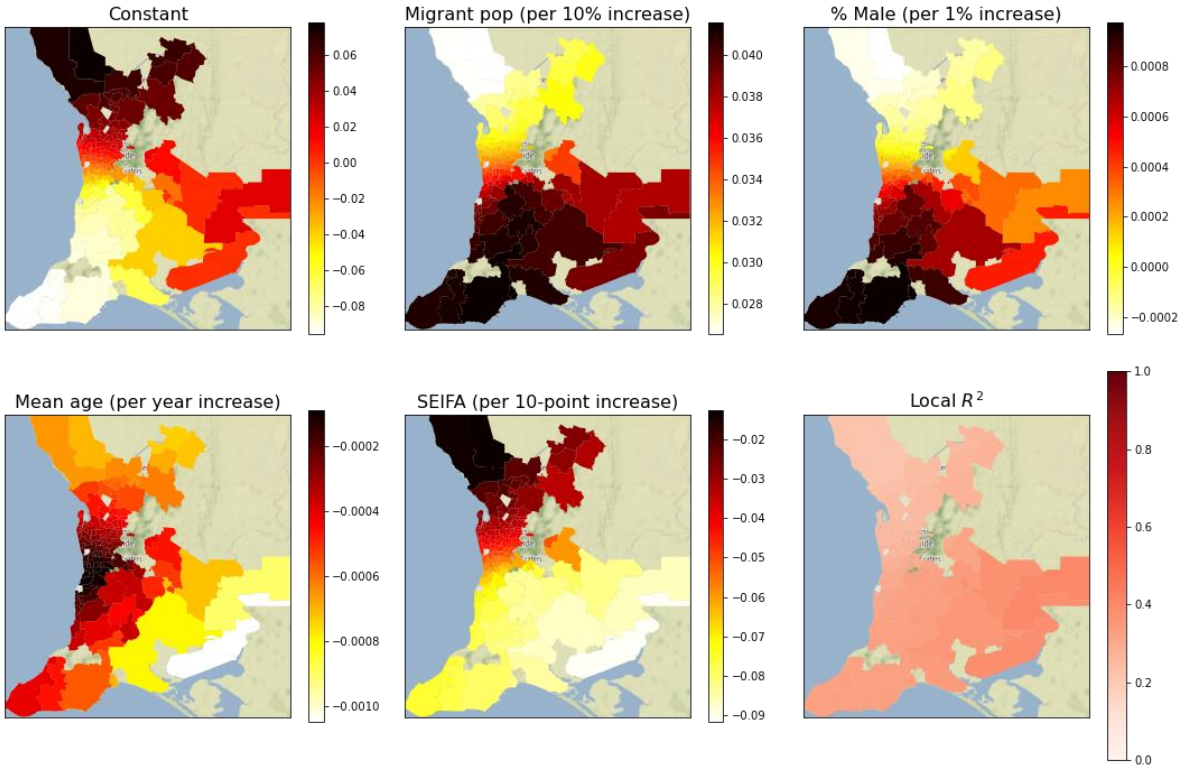


Figure 5.A4: Choropleth map of the postcode-level coefficients and R-squared from the Geographically Weighted Regression of *H. pylori* positivity rates.

Legend: Each plot represents the regression coefficient between H. Pylori and the demographic variable within each postcode. All 136 postcodes showed a significant migrant density-to-H. Pylori association ($p<0.05$), with the strength of the association varying from a 2.65% to 4.15% increase in the absolute rate of H. Pylori per 10% increase in the migrant density. The colour bar indicates the value of the local coefficient or R^2 statistic.

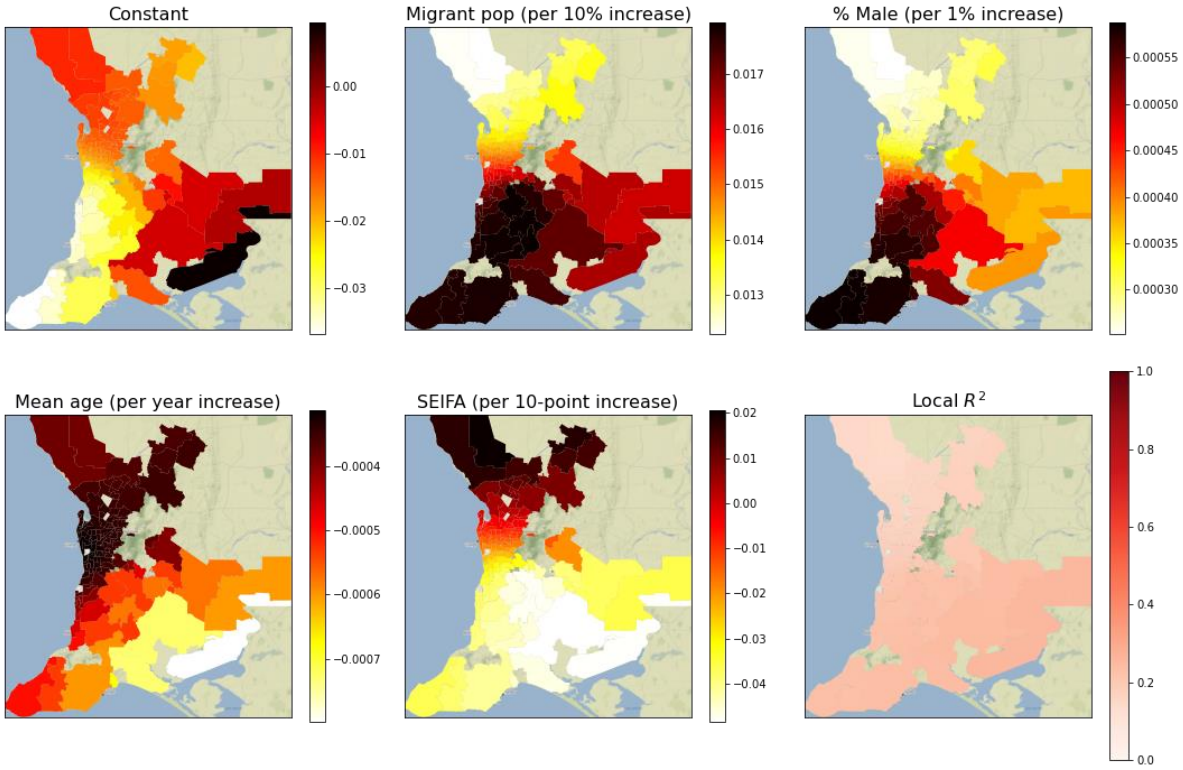


Figure 5.A5: Choropleth map of the postcode-level coefficients and R-squared from the Geographically Weighted Regression of rates of antibiotic resistance.

Legend: Each plot represents the regression coefficient between antibiotic resistance and the demographic variable within each postcode. All 136 postcodes showed a significant migrant density-to-*H. pylori* association ($p<0.05$), with the strength of the association varying from a 1.22% to 1.79% increase in the absolute rate of antibiotic resistance per 10% increase in the migrant density. The colour bar indicates the value of the local coefficient or R^2 statistic.

CHAPTER 6: WHAT IS THE OPTIMAL REGIMEN FOR PATIENTS WHO HAVE FAILED PRIOR *H. PYLORI* ERADICATION THERAPY?

6.1 Background

The concerning rise in *H. pylori* antimicrobial resistance prompted direction for an updated evaluation of resistance patterns, not only in South Australia, but also nationally. A multi-centre study comprising *H. pylori* sensitivity data from a broad geographical region, incorporating South Australia and Western Australia over the preceding 4 years (2018-2022), was undertaken. This demonstrated high rates of antibiotic and multi-drug resistance, generalisable between sites, highlighting the importance of updated guidelines for empirical eradication therapy.

Presented in this chapter is the manuscript published in *Internal Medicine Journal 2023, 13 September 2023*, https://doi.org/10.1111/imj.16226.

6.2 Specific Author Contributions

Title of Paper	Refractory Helicobacter pylori infection in Australia: updated multi-center antimicrobial resistance
Publication Status	Published
Publication Details	Schubert JP, Ingram PR, Warner MS, et al. Refractory Helicobacter pylori infection in Australia: updated multicentre antimicrobial resistance [published online ahead of print, 2023 Sep 13]. *Intern Med J*. 2023;10.1111/imj.16226. doi:10.1111/imj.16226

Principal Author

Name of Principal Author (Candidate)	Jonathon P Schubert		
Contribution to the Paper	Conception, Acquiring data, Knowledge, Analysis, Drafting		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/9/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

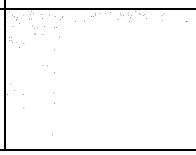
ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Paul R Ingram		
Contribution to the Paper (10%)	Acquiring data, Knowledge, Analysis, Drafting		
Signature		Date	28/9/2023

Name of Co-Author	Morgyn S Warner		
Contribution to the Paper (8%)	Acquiring data, Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Christopher K Rayner		
Contribution to the Paper (3%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Ian C Roberts-Thomson		
Contribution to the Paper (3%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Samuel P Costello		
Contribution to the Paper (3%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Robert V Bryant		
Contribution to the Paper (3%)	Knowledge, Drafting		
Signature		Date	28/11/2023

[Manuscript 4] Contemporary antibiotic resistance patterns of Helicobacter pylori in Australia: a multi-centre analysis

Jonathon P Schubert[1,2], Paul R Ingram[3,4], Morgyn S Warner[1,5], Christopher K Rayner[1,6], Ian C Roberts-Thomson[1], Samuel P Costello[1,2], Robert V Bryant[1,2]

Author Affiliations

[1]Faculty of Health Sciences, Adelaide Medical School, University of Adelaide, Adelaide, Australia

[2]Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, Australia

[3]Department of Microbiology, PathWest Laboratory Medicine, Fiona Stanley Hospital, Perth, Australia

[4]School of Pathology and Laboratory Medicine, University of Western Australia, Perth, Australia

[5]Microbiology and Infectious Diseases Directorate, SA Pathology, Adelaide, Australia

[6]Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia

Corresponding author

Dr Jonathon P Schubert

Faculty of Health Sciences, Adelaide Medical School, University of Adelaide, Adelaide, Australia

Email: jonathon.schubert@adelaide.edu.au

Conflict of interests

JPS, PRI, MSW, IRT none to disclose. CKR has received research funding from AstraZeneca, Merck Sharp & Dohme, Eli Lilly and Company, Novartis, and Sanofi and has participated in advisory boards for Allergan (now AbbVie). SPC has received advisory, speaking fees, or research support from Ferring, Microbiotica, Janssen. Shareholder in BiomeBank. RVB has received grant/research support/speaker fees (all paid to employer for research support): AbbVie, Ferring, Janssen, Shire, Takeda, Emerge Health. Shareholder in BiomeBank.

ABSTRACT

Background and Aim: *Helicobacter pylori* infection is responsible for considerable morbidity and mortality worldwide and eradication rates are falling globally due to increasing antimicrobial resistance. However, there is a paucity of local data to guide the choice of eradication therapy in Australia. This study aimed to evaluate current Australian rates of *H. pylori* antibiotic resistance in patients who had failed prior eradication therapy.

Methods: Retrospective analysis of routine culture and antibiotic susceptibility data from two pathology laboratories servicing multiple tertiary referral hospitals in Western Australia and South Australia, between 2018-2022. Rates of antimicrobial resistance and prevalence of multi-resistant isolates in both South Australia and Western Australia and comparison of temporal trends and differences between the two states.

Results: A total of 796 *H. pylori* isolates revealed a clarithromycin resistance rate of 82%, metronidazole 68%, amoxicillin 4.4% and tetracycline 0.5%. Resistance to levofloxacin was observed in 22% and rifampicin 14%. Rates of resistance to clarithromycin were lower in South Australia compared to Western Australia (incidence rate ratio 0.69, p=0.0001). Multi-resistant isolates were discovered in 63% of patients, with lower rates in South Australia compared to Western Australia (incidence rate ratio 0.74, p=0.002).

Conclusion: This first multi-center, multi-state study of *H. pylori* resistance in Australian patients exposed to prior therapy demonstrated high rates of antimicrobial resistance, including to levofloxacin (>20%). This raises concern about recommending levofloxacin in empirical second line therapies. Increased monitoring and awareness of current *H. pylori* resistance rates in Australia are needed to guide local eradication practices.

Keywords:
Helicobacter pylori, antibiotic, antimicrobial, antimicrobial resistance, levofloxacin.

INTRODUCTION

Helicobacter pylori infection is responsible for considerable worldwide morbidity and mortality, through its causative role in peptic ulceration, gastric cancer, and mucosa-associated lymphoid tissue lymphoma. *H. pylori* eradication has been shown to reduce the incidence of gastric cancer, the fifth most common neoplasm and the third highest cause of cancer mortality worldwide, at a population level.[1, 2] However, rates of successful eradication have been declining in many countries due to increasing antibiotic resistance.[3, 4]

The prevalence of antibiotic resistance amongst *H. pylori* varies between geographic regions, and changes over time within regions. Therefore, current local information is pertinent for optimal selection of empirical eradication regimens. In Australia and New Zealand, there is a paucity of data regarding antimicrobial resistance over the last 20 years.[5] Furthermore, recent publications have raised concern about increasing rates of antimicrobial resistance in Australia.[6-8]

The aim of the present study was to evaluate antibiotic resistance rates of *H. pylori* across multiple centres in Australia over the last four years, with a focus on patients who have had prior unsuccessful eradication therapy.

METHODS

Gastric biopsy specimens from patients who had failed prior eradication therapy underwent culture and antibiotic susceptibility testing across multiple centres between January 1, 2018 and Jan 19, 2022 in PathWest Laboratory Medicine, Western Australia (WA), and between January 1, 2018 and October 24, 2022 in SA Pathology, South Australia (SA). Cultures were tested for susceptibility to amoxicillin, clarithromycin, tetracycline, metronidazole, levofloxacin and rifampicin. Gastroscopy and biopsy were performed as part of routine clinical practice, and therefore informed consent of patients to be included in this study was not obtained. The study received approval for publication by the Central Adelaide Local Health Network Human Research Ethics Committee (Reference number: 12902).

Demographic data for all patients were obtained from medical records. Additionally, clinical information written on laboratory requests was captured for all patients, including a history of prior *H. pylori* eradication treatment. Where available, information from endoscopy reports and clinical notes was also captured.

Helicobacter pylori culture and antibiotic susceptibility testing

Gastric biopsies were cultured at 37°C under microaerobic conditions (5% O_2; 10% CO_2; 85% N_2) for 3-7days on brain heart infusion plates containing 7% horse blood (Difco Laboratories Pty Ltd). Isolates were tested for susceptibility to amoxicillin, clarithromycin, metronidazole, tetracycline, levofloxacin and rifampicin using E-test strips (AB Biodisk, Sweden). Minimum inhibitory concentrations (MICs) of each antibiotic were determined for each isolate and susceptibility breakpoints used for amoxicillin, tetracycline, clarithromycin, metronidazole, levofloxacin and rifampicin were >0.125, >1, >0.5, >8, >1 and >1 mg/L respectively, based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.[9]

Statistical and subgroup analysis

Analyses of rates of antibiotic resistance, as well as geographic and temporal trends, were undertaken over the study period. Antimicrobial resistance between geographic state (WA and SA) and addition over time were analysed. Rates of multi-resistance, defined as resistance to more than one antibiotic, were also calculated. Incidence rate ratios were calculated between groups, and a p-value of <0.05 was considered statistically significant. SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

RESULTS

Patient demographics

A total of 796 isolates were collected from 707 patients located in WA (520, 65%) and SA (277, 35%) with 81 isolates being from patients undergoing repeat cultures. The mean patient age was 45 years, and 35% were male, which was similar between geographical regions (Table 1).

Prevalence of antibiotic resistance

The rate of resistance to clarithromycin was 82%, metronidazole 68%, amoxicillin 4.4% and tetracycline 0.5%. Resistance to levofloxacin was observed in 22%, and rifampicin 14% (Table 2). Median and geographic mean minimum inhibitory concentrations (MICs) were calculated for all antimicrobials (Appendix 1, Table A1).

Multi-resistance

No anti-microbial resistance was identified in 7% of patients, while 29.6% had resistance to one antimicrobial, and 63.3% were multi-resistant (Table 3). The most common multi-resistant pattern involved resistance to both clarithromycin and metronidazole, which was seen in 89.7% of multi-resistant isolates. Of the 452 isolates with resistance to both clarithromycin and metronidazole, 399 had susceptibility testing to levofloxacin and rifampicin, with 72.6% shown to be sensitive to fluroquinolones and 92% sensitive to rifampicin (Figure 1).

Geographic variation

Rates of resistance to clarithromycin were lower in SA compared to WA (incidence rate ratio 0.69, p=0.0001), but there was no difference between the two states for any other antibiotic (Table 2). There were significantly higher rates of fully sensitive strains in SA (IRR 3.98, p<0.0001), and lower rates of multi-resistant strains (IRR 0.74, p=0.0019), when compared to WA (Table 3).

DISCUSSION

This study represents the first multi-centre, multi-state evaluation of contemporary rates of *H. pylori* antimicrobial resistance to have been undertaken in Australia, and is focussed on patients previously exposed to at least one eradication regimen. The data reveal high rates of clarithromycin and metronidazole resistance, and a high prevalence of multi-resistant *H. pylori*, with 63.3% of isolates resistant to two or more antibiotics. Of note, high rates of resistance to second-line therapies levofloxacin (22%) and rifampicin (13.5%) were observed, which has not previously been reported in peer-reviewed studies in Australasia.[5]

The high rates of resistance to levofloxacin demonstrated in this study raise concern regarding ongoing use of this antibiotic in empiric *H. pylori* eradication therapy for refractory infection. Previous studies conducted between 2014-2016 in Australia demonstrated that levofloxacin-based triple therapy achieved successful eradication in 90% of patients with refractory *H. pylori* infection, with no cases of levofloxacin resistance observed in a subset of 20 patients who had antimicrobial sensitivity testing.[10] The high rates of successful eradication, irrespective of the number of lines of treatment failure, has led to the current Australian recommendation that it be used in refractory infection.[10, 11] As such, its use has increased, which may be contributing to recent emerging isolated reports of levofloxacin resistance in *H. pylori*, which was risen from zero in 2012 to 28% in 2017 in specific parts of Australia.[6] Fluroquinolones have been estimated to be the most prescribed antibiotics worldwide, which has likely contributed to rising levofloxacin resistance, which has been very well described internationally.[3, 12] Meta-analyses from the wider Asia-Pacific region have reported a substantial increase in levofloxacin resistance in recent decades, with the prevalence of levofloxacin resistant *H. pylori* increasing from 7% before 2000 to 21% during 2011-15.[13] Studies in the Middle East [14], Italy[15], Taiwan[16], and Southern Asia[17] have also demonstrated substantial increases in levofloxacin resistance within the last decade. The levels of levofloxacin resistance demonstrated in the current study exceed the limit of 15% recommended by the Maastricht VI/Florence Consensus Report for the use of empiric levofloxacin based eradication therapy for refractory infection, since this antibiotic would be unlikely to achieve adequate (≥80%) eradication rates.[18] However, our study did not capture data on prior eradication regimens, so it is unclear what proportion of patients were treated with levofloxacin previously, potentially contributing to selection bias. Nonetheless, considering levofloxacin resistance rates exceeding 20% in our study, and documented increases in rates of levofloxacin by others, its use in empiric therapy for refractory *H. pylori* infection warrants further study.

The rate of resistance to rifampicin (13.5%) was also considerably higher than in previous reports.[5, 19] A recent meta-analysis comprising 39 studies and 9,721 patients demonstrated a mean *H. pylori* rifabutin resistance rate of 0.13%, and the efficacy of eradication on an intention-to-treat basis in 3,052 patients was 73%.[19] It is recommended that rifabutin may be considered for first-line treatment in regions with high (>15%) resistance to clarithromycin, metronidazole, and levofloxacin, if bismuth is unavailable.[18] Given that resistance rates in the present study approached 15%, rifampicin would be unlikely to achieve adequate eradication rates in our cohort, and may not be appropriate for the wider Australian region. However, whilst the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommends using rifampicin MIC >1.0 mg/L as the rifabutin breakpoint, studies have shown that *H. pylori* isolates with moderate rifampicin resistance were susceptible to rifabutin, so it has been suggested that rifampicin resistance can be used to screen for rifabutin resistance using a breakpoint of 4mg/l.[20] Nevertheless, recent studies have demonstrated that even a breakpoint concentration of 4mg/l may be a poor marker of rifabutin resistance, so the generalisability of rifampicin resistance to rifabutin may be questionable.[21]

The high rate of multi-drug resistance seen in the current study (63%) is comparable to what has been reported in various regions of South East Asia.[17] Of these multi-resistant strains, 90% had resistance to both clarithromycin and metronidazole, while 30% were resistant to levofloxacin. For isolates resistant to clarithromycin, metronidazole and levofloxacin, only 2.8% were sensitive to rifampicin, so the most effective empiric treatment is likely to be bismuth-based quadruple therapy. The reassuringly low rates of tetracycline and amoxicillin resistance support their ongoing use in second or subsequent lines of therapy. Studies have demonstrated varying resistance profiles among regions in the same country and therefore further studies are needed to see if the results are generalisable across Australia.[3] The Maastricht VI/Florence Consensus report recommends bismuth (proton pump inhibitor (PPI), bismuth, tetracycline and metronidazole), or non-bismuth (PPI, amoxicillin, clarithromycin and a nitroimidazole) quadruple therapies as first line treatment in areas of high resistance to clarithromycin, metronidazole and levofloxacin.[18] Ease of access to quadruple therapy is a major limiting factor in Australia, and perhaps increasing availability of combination formulations containing bismuth subcitrate, metronidazole and tetracycline, such as Pylera® – which can achieve eradication rates of more than 90% [22] – may improve this over time. Improved access to bismuth quadruple therapies is needed in Australia, and further studies evaluating successful eradication rates in the Australian cohort are required.

A further issue concerning rising resistance rates is the presence of hetero-resistance of *H. pylori*, where a heterogeneous population of H. pylori may exist within an indvidual, with one or several subpopulations exhibiting increased levels of antibiotic resistance compared to the main population.[23] Recent reviews have demonstrated the presence of hetero-resistance to clarithromycin and/or metronidazole in approximately 7 and 14% of *H. pylori* positive isolates.[24] In our study, 81 isolates were from patients who underwent repeated successful culture. In these cases, prior culture-directed therapy was not successful at eradicating *H. pylori*, and the presence of hetero-resistance is likely to be the cause. Phenotypic methods such as E-test and Agar dilution methods primarily select the dominant strain on culture growth and have limited utility in demonstrating hetero-resistance. More recently, genotypic resistance has been used to improve detection, particularly for clarithromycin resistance.[25] Increasing awareness of hetero-resistance of *H. pylori* multiple is needed, and the use of multiple gastric biopsy sites and/or improved genotyping methods to detect and identify the presence of both multiple strains and resistance profiles may improve detection and eradication success, although further studies are needed.[26] A greater understanding of molecular methods for antibiotic resistance is needed to enable the reliable detection of genotypic resistance for commonly prescribed antimicrobials beyond clarithromycin and levofloxacin.[27]

A limitation of our study was the lack of data capture regarding prior eradication therapy. It is unclear how many lines of antimicrobial therapy, and which specific antibiotics, had previously been prescribed prior to cultures being performed. As many cases can be eradicated using empiric therapy, it is likely that this study captures highly refractory cases. Another limitation is that a minority of isolates did not receive complete extended sensitivity testing due to a change in routine analysis practices in each laboratory. Approximately 80% of isolates were tested for levofloxacin resistance, but only 50% for rifampicin resistance. The reported resistance rates were consistent between states, despite the percentage of Australians born overseas being higher in WA than SA,[28] and recent studies demonstrating that *H. pylori* resistance is clustered in migrant communities.[29]

A major strength of this study was the multi-centre, multi-state capture of data including South Australia and Western Australia, encompassing isolates from a broad geographical area, with the number of isolates making it the largest Australian *H. pylori* antimicrobial sensitivity study to date.[5] The rising rates of *H. pylori* resistance in Australia have largely gone unrecognised until now, as identified in a recent systematic review.[5] The most pertinent issue identified by this study is the need for ongoing monitoring of local resistance levels to guide eradication therapy. Our study also

demonstrates that these changes were generalisable across two states of Australia, although further validation is required across other states for a more accurate nationwide appraisal. Establishment of a national database to track resistance trends, as is done for other pathogens including *E.coli,*[30] should be considered, with a view to updating antimicrobial guidelines on a periodic basis. A recent Australian study demonstrated that currently recommended empiric first line eradication therapy is unlikely to achieve adequate eradication rates, and the present study demonstrates that in the setting of rising resistance rates, the use of empiric levofloxacin for refractory infection should also be re-evaluated in Australasia.[8, 11]

Furthermore, an emphasis on improving access to, and use of, culture-based therapy should be considered for refractory cases. These study data suggest that antibiotic sensitivity testing should be encouraged in Australia, prior to prescribing levofloxacin for refractory *H. pylori* infection, since levofloxacin resistance rates >20% make it unlikely that 'adequate' eradication rates of >80%, as specified in the Maastricht VI/Florence Consensus report, can be achieved in such patients with empiric levofloxacin based therapies.[18] Alternative modalities to assess antimicrobial resistance, such as stool-based polymerase chain reaction (PCR), could be considered as non-invasive and cost effective alternatives to endoscopy and culture-based methods.[31] While genomic studies have identified regions associated with clarithromycin and levofloxacin resistance with reasonable phenotypic correlation, genetic mutations associated with amoxicillin, tetracycline and rifampicin are yet to be fully understood.[32] Further genetic studies evaluating concordance and associations between phenotypic resistance and genetic resistance patterns should be undertaken.

CONCLUSIONS

This first multi-center, multi-state study of *H. pylori* resistance in Australia demonstrated high rates of antimicrobial resistance, including rates of levofloxacin resistance > 20%. This raises concern about the ongoing use of levofloxacin in empirical refractory infection. Improved access to empiric eradication therapies, including bismuth quadruple therapy, is needed to combat the high rates of resistance observed. Increased monitoring and awareness of current *H. pylori* resistance rates in Australia are needed to guide local eradication practices.

REFERENCES

1. Ford AC, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut. 2020;69(12):2113-21.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.
3. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.
4. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Alimentary pharmacology & therapeutics. 2016;43(4):514-33.
5. Schubert JP, Gehlert J, Rayner CK, Roberts-Thomson IC, Costello S, Mangoni AA, et al. Antibiotic resistance of Helicobacter pylori in Australia and New Zealand: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2021;36(6):1450-6.
6. Pateria P CM, Goodheart R, McCullough C, Raby E, Robineron JO, Ingram PR. Increasing secondary resistance to fluoroquinolones amongst Helicobacter pylori in Western Australia. Tasman Medical Journal. 2020;2(1).
7. Khan SF, Teng JC, Holmes JA, Perera C, Prendergast L, Waring L. Helicobacter pylori antimicrobial resistance in Melbourne, Australia. Time to review therapeutic guidelines? Internal medicine journal. 2021;51(11):1919-26.
8. Schubert JP, Warner MS, Rayner CK, Roberts-Thomson IC, Mangoni AA, Costello S, et al. Increasing Helicobacter pylori clarithromycin resistance in Australia over 20 years. Internal medicine journal. 2022;52(9):1554-60.
9. Pan Y, Zheng Y, Yang J, Wei Y, Wu H, Liu S, et al. A new biomarker for the early diagnosis of gastric cancer: gastric juice- and serum-derived SNCG. Future oncology (London, England). 2022;18(28):3179-90.
10. Katelaris PH, Katelaris AL. A prospective evaluation of levofloxacin-based triple therapy for refractory Helicobacter pylori infection in Australia. Internal medicine journal. 2017;47(7):761-6.
11. Therapeutic, Guidelines. Helicobacter pylori infection Melbourne2016 (amended 2019) [Available from: https://www.tg.org.au.
12. Cheng AC, Turnidge J, Collignon P, Looke D, Barton M, Gottlieb T. Control of fluoroquinolone resistance through successful regulation, Australia. Emerging infectious diseases. 2012;18(9):1453-60.

13. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, et al. Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2017;2(10):707-15.

14. Azrad M, Vazana D, On A, Paritski M, Rohana H, Roshrosh H, et al. Antibiotic resistance patterns of Helicobacter pylori in North Israel - A six-year study. Helicobacter. 2022;27(6):e12932.

15. Saracino IM, Fiorini G, Zullo A, Pavoni M, Saccomanno L, Vaira D. Trends in Primary Antibiotic Resistance in H. pylori Strains Isolated in Italy between 2009 and 2019. Antibiotics (Basel, Switzerland). 2020;9(1).

16. Liang CM, Tai WC, Hsu PI, Wu DC, Kuo CH, Tsay FW, et al. Trend of changes in antibiotic resistance in Helicobacter pylori from 2013 to 2019: a multicentre report from Taiwan. Therapeutic advances in gastroenterology. 2020;13:1756284820976990.

17. Sukri A, Lopes BS, Hanafiah A. The Emergence of Multidrug-Resistant Helicobacter pylori in Southeast Asia: A Systematic Review on the Trends and Intervention Strategies Using Antimicrobial Peptides. Antibiotics (Basel, Switzerland). 2021;10(9).

18. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report. Gut. 2022.

19. Gisbert JP. Rifabutin for the Treatment of Helicobacter Pylori Infection: A Review. Pathogens (Basel, Switzerland). 2020;10(1).

20. Boyanova L, Markovska R, Hadzhiyski P, Kandilarov N, Mitov I. Rifamycin use for treatment of Helicobacter pylori infection: a review of recent data. Future microbiology. 2020;15:1185-96.

21. Yang T, Liu B, Zhou J, Shen Y, Song X, Tang X, et al. The Inappropriateness of Using Rifampicin E-Test to Predict Rifabutin Resistance in Helicobacter pylori. The Journal of infectious diseases. 2022;226(Supplement_5):S479-s85.

22. Tursi A, Franceschi M, Allegretta L, Savarino E, De Bastiani R, Elisei W, et al. Effectiveness and Safety of Pylera® in Patients Infected by Helicobacter Pylori: A Multicenter, Retrospective, Real Life Study. Digestive diseases (Basel, Switzerland). 2018;36(4):264-8.

23. Tshibangu-Kabamba E, Yamaoka Y. Helicobacter pylori infection and antibiotic resistance - from biology to clinical implications. Nature reviews Gastroenterology & hepatology. 2021;18(9):613-29.

24. Kouhsari E, Sadeghifard N, Khadiv A, Sayadi H, Amiriani T, Ghafourian S, et al. Heteroresistance to clarithromycin and metronidazole in patients with a Helicobacter pylori infection: a systematic review and meta-analysis. Annals of clinical microbiology and antimicrobials. 2022;21(1):19.

25. Kocsmár É, Kocsmár I, Buzás GM, Szirtes I, Wacha J, Takáts A, et al. Helicobacter pylori heteroresistance to clarithromycin in adults-New data by in situ detection and improved concept. Helicobacter. 2020;25(1):e12670.

26. Andersson DI, Nicoloff H, Hjort K. Mechanisms and clinical relevance of bacterial heteroresistance. Nature reviews Microbiology. 2019;17(8):479-96.

27. Nishizawa T, Suzuki H. Mechanisms of Helicobacter pylori antibiotic resistance and molecular testing. Frontiers in molecular biosciences. 2014;1:19.

28. Australian Bureau of Statistics, Australia's Population by Country of Birth. 2022.

29. Schubert JP, Woodman RJ, Mangoni AA, Rayner CK, Warner MS, Roberts-Thomson IC, et al. Geospatial analysis of Helicobacter pylori infection in South Australia: Should location influence eradication therapy? Journal of gastroenterology and hepatology. 2022;37(7):1263-74.

30. Bell JM, Lubian AF, Partridge SR, Gottlieb T, Iredell J, Daley DA, et al. Australian Group on Antimicrobial Resistance (AGAR) Australian Gram-negative Sepsis Outcome Programme (GnSOP) Annual Report 2020. Communicable diseases intelligence (2018). 2022;46.

31. Beckman E, Saracino I, Fiorini G, Clark C, Slepnev V, Patel D, et al. A Novel Stool PCR Test for Helicobacter pylori May Predict Clarithromycin Resistance and Eradication of Infection at a High Rate. Journal of clinical microbiology. 2017;55(8):2400-5.

32. Arslan N, Yılmaz Ö, Demiray-Gürbüz E. Importance of antimicrobial susceptibility testing for the management of eradication in Helicobacter pylori infection. World journal of gastroenterology. 2017;23(16):2854-69.

TABLES AND FIGURES

Demographic Data	South Australia	Western Australia	Total
Patients*	248	455	707
Isolates	276	520	796
Median Age (years)	45	45	45
Gender (% male)	38%	34%	35%
Documented prior *H. pylori* eradication therapy, n(%)	226 (82%)	370 (71%)	596 (75%)

* derived from de-identified data based on date of birth

Table 6.1: Patient demographic data

	South Australia (SA)		Western Australia (WA)		Total		
Antibiotic	**Isolates**	**Resistant, n(%)**	**Isolates**	**Resistant, n(%)**	**Isolates**	**Resistant, n(%)**	**IRR$^{\cdot}$ (p-value)**
Amoxicillin	274	17 (6.2)	517	18 (3.5)	791	35 (4.4)	1.78 (0.09)
Clarithromycin	275	184 (66.9)	517	465 (89.9)	792	649 (81.9)	0.74 (0.0005)*
Metronidazole	274	172 (62.8)	517	362 (70.0)	791	535 (67.6)	0.90 (0.24)
Tetracycline	276	2 (0.7)	517	2 (0.4)	793	4 (0.5)	1.87 (0.56)
Levofloxacin	138	29 (21)	489	110 (22.5)	627	139 (22.2)	0.93 (0.76)
Rifampicin	136	20 (14.7)	249	32 (12.9)	385	52 (13.5)	0.87 (0.63)

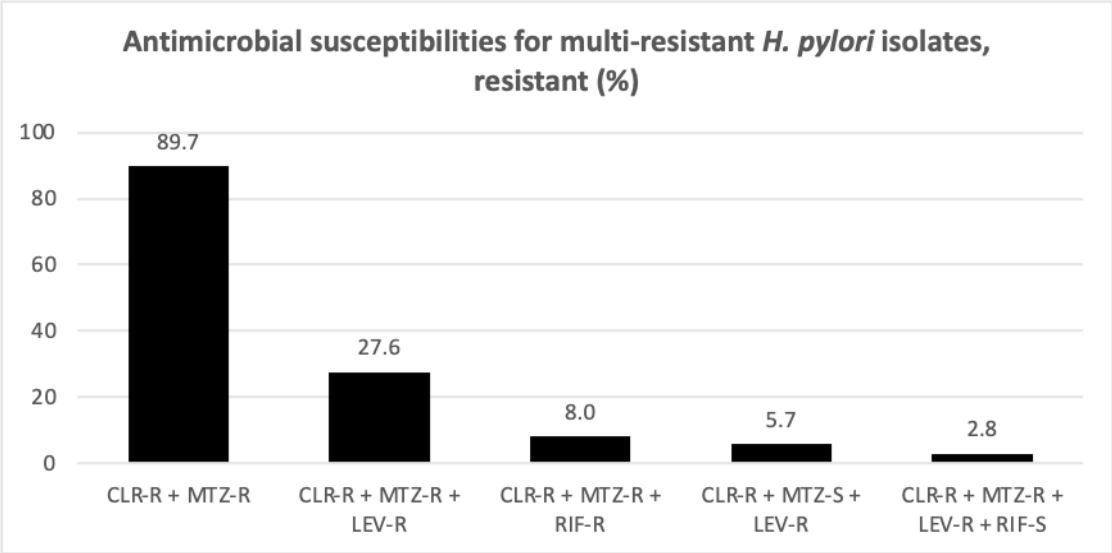
IRR$^{\cdot}$: Incidence Rate Ratio of resistance in South Australia compared to Western Australia, *statistically significant

Table 6.2: *H. pylori* antibiotic sensitivity results between 2018-2022

	South Australia	Western Australia	Total	
Resistance to antimicrobial classes	**Isolates, n(%)**	**Isolates, n(%)**	**Isolates, n(%)**	**IRR[+] (p-value)**
0	38 (13.8)	18 (3.5)	56 (7.0)	3.98 (<0.0001)*
1	96 (34.8)	140 (26.9)	236 (29.6)	1.29 (0.055)
Multi-resistance (≥2)	142 (51.4)	362 (69.6)	504 (63.3)	0.74 (0.0019)*
2	108 (39.1)	252 (48.5)	360 (45.2)	0.81 (0.06)
3	25 (9.1)	96 (18.5)	121 (15.2)	0.49 (0.0008)*
4	8 (2.9)	13 (2.5)	21 (2.6)	1.16 (0.74)
5	1 (0.4)	1 (0.2)	2 (0.3)	1.88 (0.69)
6	0 (0)	0 (0)	0 (0)	-

IRR[+]: Incidence Rate Ratio of isolates with antimicrobial class resistance between South Australia and Western Australia

Table 6.3: Antibiotic class resistance among isolates



CLR clarithromycin, MTZ metronidazole, LEV levofloxacin, RIF rifampicin, -R resistant, -S sensitive

Figure 6.1: Antimicrobial susceptibilities for multi-resistant *H. pylori* isolates

CHAPTER 7: CHARACTERISATION OF EPIDEMOLOGICAL AND GENETIC FACTORS ASSOCIATED WITH LOCAL *H. PYLORI* INFECTION

7.1 Background

Adverse clinical outcomes from *H. pylori* infection vary substantially around the world. Characterisation of factors associated with morbidity and mortality is needed in order to prognosticate risk, identify high risk populations for gastric cancer, and optimise eradication therapy. Genomic and phylogenetic data from whole genome sequencing can be used to identify origins of isolates and identify emerging trends in antimicrobial resistance. A multi-centre, multi-state, retrospective study was performed on isolates undergoing whole genome sequencing in SA and WA. This demonstrated a heterogenous mix of *H. pylori* isolates in Australia, with those from hpEastAsia in particular harbouring isolates with virulence factors associated with an increased risk for gastric cancer, as well as multi-drug resistance and treatment failure.

Presented in this chapter is the manuscript submitted to *Helicobacter*, 2023 [IF 2022 4.4].

7.2 Specific Author Contributions

Title of Paper	Genomic analysis of Helicobacter pylori in Australia – antimicrobial resistance, phylogenetic patterns and virulence factors
Publication Status	Submitted for Publication
Publication Details	Submitted to *Helicobacter,* November 2023

Principal Author

Name of Principal Author (Candidate)	Jonathon P Schubert		
Contribution to the Paper	Conception, Acquiring data, Knowledge, Analysis, Drafting		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/9/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Alfred Tay		
Contribution to the Paper (15%)	Acquiring Data, Analysis, Drafting		
Signature		Date	25/11/2023

Name of Co-Author	Khui Hung (Claire) Lee		
Contribution to the Paper (5%)	Acquiring Data, Analysis, Drafting		
Signature		Date	15/11/2023

Name of Co-Author	Lex Leoung		
Contribution to the Paper (5%)	Acquiring Data, Drafting		
Signature		Date	27/11/2023

Name of Co-Author	Christopher K Rayner		
Contribution to the Paper (2%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Morgyn S Warner		
Contribution to the Paper (2%)	Acquiring Data, Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Ian C Roberts-Thomson		
Contribution to the Paper (2%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Samuel P Costello		
Contribution to the Paper (2%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Robert V Bryant		
Contribution to the Paper (2%)	Knowledge, Drafting		
Signature		Date	28/11/2023

[Manuscript 5] Genomic analysis of Helicobacter pylori in Australia – antimicrobial resistance, phylogenetic patterns and virulence factors

Jonathon P Schubert[1,2], Alfred Tay[3], Khui Hung (Claire) Lee[3], Lex Leong[4], Christopher K Rayner[1,5], Morgyn S Warner[1,4], Ian C Roberts-Thompson[1], Samuel P Costello[1,6], Robert V Bryant[1,6]

Author Affiliations

[1] Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia

[2] Department of Gastroenterology, Royal Brisbane and Women's Hospital, Brisbane, Australia

[3] The Marshall Centre for Infectious Diseases, Research and Training, University of Western Australia, Perth, Australia

[4] Microbiology and Infectious Diseases Directorate, SA Pathology, Adelaide, Australia

[5] Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia

[6] Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, South Australia, Australia

Corresponding author

Dr Jonathon P Schubert

Faculty of Health Sciences, Adelaide Medical School, University of Adelaide, Adelaide, Australia

Email: jonathon.schubert@adelaide.edu.au

Conflict of interests

JPS, AT, KL, LL, IRT none to disclose. CKR has received research funding from AstraZeneca, Merck Sharp & Dohme, Eli Lilly and Company, Novartis, and Sanofi and has participated in advisory boards for Allergan (now AbbVie). SPC has received advisory, speaking fees, or research support from Ferring, Microbiotica, Janssen. Shareholder in BiomeBank. RVB has received grant/research support/speaker fees (all paid to employer for research support): AbbVie, Ferring, Janssen, Shire, Takeda, Emerge Health. Shareholder in BiomeBank.

Acknowledgements

We wish to thank SA Pathology staff, especially Casey Moore for their assistance with collecting, storing and sequencing isolates for whole genome analysis.

ABSTRACT

Background and Aim: Rates of antimicrobial-resistant *Helicobacter pylori* infection are rising in Australia and globally. Little is known, however, about local resistance patterns, virulence factors and phylogenetic patterns of isolates within Australia.

Methods: Whole genome sequencing (WGS), culturing and antibiotic sensitivity analysis of refractory *H. pylori* isolates collected at multiple centres was performed between 2014-22. Phylogenetic origins of isolates were determined using core genome analysis. Mutations associated with antibiotic resistance were characterised and rates of concordance with phenotypic culture resistance profiles were determined, in addition to known virulence factors.

Results: Of the 135 isolates, 109 underwent WGS. 43 were isolated from patients in South Australia, and 66 from Western Australia. Isolates originated primarily from hpEurope (59.6%), hpEastAsia (25.7%), and hpNEAfrica (6.4%). Antimicrobial resistance to clarithromycin was seen in 85% of isolates, metronidazole in 52%, levofloxacin in 18%, rifampicin in 14%, and amoxicillin in 9%. Most isolates (59%) were multi-drug resistant. Resistance concordance between genetically determined resistance and phenotypic resistance was 92% for clarithromycin and 94% for levofloxacin. Analysis of virulence factors demonstrated Cag Pathogenicity Island (*cag PAI*) was present in 67% of isolates, with *cagA* in 61%, correlating with isolate genetic origin. Vacuolating cytotoxin A analysis revealed that 26% of isolates contained the most virulent s1m1 genotype. For 9 patients with gastric intestinal metaplasia, 8 (89%) of *H. pylori* isolates contained *cagA*, with the majority (67%) originating from East Asia.

Conclusion: Refractory *H. pylori* isolates in Australia emanate from multiple global origins. Concordance between genetic and phenotypic antibiotic resistance raises the possibility of region-specific polymerase chain reaction being a useful tool in clinical practice. The dynamic landscape of *H. pylori* in Australia warrants the establishment of a national database to monitor *H. pylori* resistance alongside genome sequencing to evaluate evolving virulence.

Keywords: *Helicobacter pylori*, antibiotics, antimicrobial, resistance, antimicrobial resistance, whole genome sequencing

INTRODUCTION

Helicobacter pylori (H. pylori) colonises the gastric mucosa of more than half of the world's population[1], and results in adverse outcomes in 10–20% of infected people.[2, 3] *H. pylori* strains display substantial diversity in genetic profiles, structure of genes and clinical outcomes between human populations, having co-evolved with people over tens of thousands of years.[4-6] Seven main *H. pylori* genetic ancestral populations have been identified, including hpEurope, hpAfrica1, hpAfrica2, hpEastAsia, hpNEAfrica, hpAsia2 and hpSahul, with the latter present only in the Australian Indigenous community and Papua New Guinea.[7, 8] Considerable genetic recombination and exchange occurs between strains, providing significant adaptive benefits.[9, 10] Moreover, *H. pylori* can incorporate genetic material from other closely related organisms, allowing the pathogen to adapt to its host.[11, 12]

H. pylori antibiotic resistance varies substantially between geographic zones, and has been increasing globally over time, with a corresponding fall in eradication rates.[13-15] As such, the World Health Organization has listed antibiotic-resistant *H. pylori* as a high priority for antibiotic research and development.(16) In Australia, there is a paucity of recent data on antimicrobial resistance, despite studies showing rising resistance in the last two decades.[17, 18] Genomic profiles may be used to predict antimicrobial resistance, with resistance to clarithromycin being associated with mutations in the 23S ribosomal RNA gene, fluroquinolone resistance with gyrA mutations[15, 19, 20], and tetracycline resistance with 16S rRNA mutations.[21]

Gastric cancer is the third leading global cause of cancer related mortality, with up to 90% of cases attributable to *H. pylori* infection. *H. pylori* eradication has been shown to reduce the incidence of gastric cancer in high risk populations.[22] However, there is marked geographical variation in both the prevalence of *H. pylori* infection and gastric cancer risk. This is highlighted by regions in Africa where infection rates approach 100%, but rates of gastric cancer are very low.[23] The pathogenesis of gastric cancer is likely multifactorial and dependent on *H. pylori* genotype, in addition to diet, lifestyle, host genetics and co-infection.[24, 25]

The genotype of the *H. pylori* strain is a determinant of the risk of development of gastric cancer. Virulence factors, including cag pathogenicity island (*cagPAI*) and the vacuolating cytotoxin gene A (*vacA*), are associated with gastric cancer pathogenesis.[26-28] Cytotoxin-associated gene A (*cagA*) is the most investigated oncoprotein of *cagPAI* operon and is highly prevalent in regions of Southeast Asia where rates of gastric cancer are high.[29, 30] The *cagA* gene contains Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs (including four classes, A, B, C, and D) based on the presence of repeating regions at the C-terminal, and the motif ABD, highly prevalent in East Asia, which has been associated with an increased risk of gastric cancer.[31] The *VacA* protein has a variable structure in the signal region with s1 or s2 allele types, and middle region with m1 and m2 allele types, and the combination of these alleles determines the level of cytotoxin produced. *VacA* s1m1 variants produce high levels of cytotoxin and have been associated with an increased risk of peptic ulcer disease, chronic gastritis and gastric cancer.[32, 33]

Characterising the phylogenetic origins of *H. pylori* isolates through core genome analysis enables recognition of transmission patterns and the geographical origins of isolates, and may help prognosticate on the risk of adverse clinical outcomes.[34] In this study, our aim was to characterise antimicrobial resistance and genetic mutations associated with adverse clinical outcomes of *H. pylori* isolates, and their relationship with phylogenetic origins of refractory isolates encountered in routine clinical practice at two laboratories that each receive specimens from multiple hospitals across Australia.

METHODS

Gastric biopsy specimens collected from sequential patients referred with refractory *H. pylori* infection in South Australia (SA) between 2020-2022, and Western Australia (WA) between 2013-2018, underwent culture and antibiotic sensitivity analysis, as well as bacterial whole genome sequencing. SA and WA have multicultural populations of approximately 1.4 million and 1.9 million people respectively, including a substantial number of migrants from Europe and Asia. Demographic data including age, gender, endoscopy and histology findings, and prior treatment were obtained from patient records, when available, following approval by the Central Adelaide Human Research Ethics Committee (Reference number: 12902) and Government of WA, Department of Health (PRN:RGS0000001633).

H. pylori culture and antibiotic susceptibility testing

Gastric biopsies were cultured at 37°C on brain heart infusion plates containing 7% horse blood (Difco Laboratories Pty Ltd, Detroit, USA) under microaerobic conditions (5% O_2; 10% CO_2; 85% N_2) for 3-10 days. Positive *H. pylori* isolates were tested for susceptibility to amoxicillin, clarithromycin, metronidazole, tetracycline, rifampicin and levofloxacin using E-Test strips (AB Biodisk, Sweden), from which minimum inhibitory concentrations were determined. Consistent with European Committee on Antimicrobial Susceptibility Testing guidelines, breakpoints for amoxicillin, clarithromycin, tetracycline, metronidazole, rifampicin and levofloxacin were >0.125, >0.25, >1, >8, >1 and 1mg/L respectively.[35]

Genome Sequencing

Due to funding limitations, only 109 (81%) of the 135 isolates underwent whole genome sequencing. The sequencing protocol has been described previously.[36] Isolates from SA were sequenced by the Pathogen Genomics group in SA Pathology, while isolates from WA were sequenced by the Marshall Centre for Infectious Diseases. In brief, amplicon libraries were performed using 1 ng of genomic DNA with Illumina Nextera XT Library Preparation kit. DNA Libraries were sequenced using the Nextseq 500/550 platforms (Illumina Inc., San Diego, CA). All bioinformatics analyses were completed using a Pawsey supercomputer. Each genome was planned to have at least 100-150x coverage. The raw sequencing data were trimmed and cleaned using bbduk package (version 39.0),

assembled into contigs using shovill (version 0.9.0), assembler (version 3.14.1), annotated using dfast (version 1.2.13), and core genome analysed using proteinortho (version 6.0.18).

Phenotypic antimicrobial resistance from susceptibility testing was compared to genomically predicted resistance profiles. Genetic screening for clarithromycin resistance was conducted using a two-point mutation approach in the 23S rRNA gene (A2142 and A2143). Levofloxacin resistance was screened for using a single gene, two-point mutation approach in the GyrA (QRDR) gene (N87 and D91). Tetracycline resistance was screened for by point mutations in 16S rRNA (AGA926-928TTC). Core genome analyses for isolates were plotted on a phylogenetic tree. As the cohort lacked sufficient population diversity to encompass all MLST population types, an additional 95 established reference genomes were utilised to generate the phylogenetic tree (Supplementary Table 1). Genetic mutations associated with antibiotic resistance were described and contrasted between geographic origins of the isolates. Mutations in genomic regions associated with virulence factors, including *cagPAI* and *vacA*, were described and compared between different phylogenetic origins using FastTree (version 2.1).

Statistical Analysis

Rates of *H. pylori* infection were assessed, as well as antibiotic resistance of *H. pylori* to clarithromycin, metronidazole, amoxicillin, tetracycline, rifampicin and levofloxacin. Multi-resistance was defined as resistance to two or more classes of antimicrobials. Genomic profiles of resistance were compared to phenotypic profiles and assessed for concordance. Sub analyses were conducted for patients whose country of birth was known, and correlation with country of birth was described. Descriptive statistics and linear regression were performed using Stata version 16.0 (StataCorp, USA, Texas), and a 2-sided type 1 error rate of α=0.05 was used for all tests.

RESULTS

Patient demographics

The study population consisted of 135 subjects, 44 (33%) from South Australia and 91 (67%) from Western Australia. 59 (44%) were male and the mean (SD) age was 44 (15) years, which was similar between SA and WA. Of the 135 *H. pylori* isolates, 109 (81%) underwent whole genome sequencing.

Antimicrobial resistance

Among the 135 isolates, 115 (85.2%) demonstrated clarithromycin resistance, 70 (51.9%) metronidazole resistance, 24 (17.8%) levofloxacin resistance, 19 (14.1%) rifampicin resistance, 12 (8.9%) amoxicillin resistance, and none tetracycline resistance. Ten (7.4%) isolates were sensitive to all antimicrobials, while 125 (92.6%) were resistant to at least one antimicrobial and 79 (58.5%) were resistant to two or more antimicrobials (i.e. multi-resistant) (Table 1). There were no differences in antimicrobial resistance profiles between isolates collected in SA and WA.

Genotypic resistance

Clarithromycin

Genetically determined clarithromycin resistance, screened for using a two-point mutation approach in the 23S rRNA gene (A2142 and A2143), demonstrated concordance with phenotypic resistance in 94 of 102 cases (92%). Seven of the 109 isolates did not have phenotypic clarithromycin resistance reported and were excluded. Seven isolates had mutations in A2142 and 75 isolates had mutations in A2143.Of the 8 non-concordant cases, 6 had phenotypic resistance without a mutation in A2142 or A2143, and 2 had an isolated A2143 point mutation without phenotypic resistance (Table 2).

Levofloxacin

Mutations associated with levofloxacin resistance, screened for using a two- point mutations approach in the GyrA (QRDR) gene (N87 and D91), demonstrated concordance with phenotypic resistance in 95 of 101 cases (94%). Eight of the 109 isolates did not have phenotypic clarithromycin resistance reported and were excluded. 16 isolates had mutations in N87 and eight had mutations in D91. Of the 6 non-concordant cases, all had point mutations without phenotypic resistance, while all cases of phenotypic resistance had point mutations in N87 and/or D91.

Tetracycline

There were no cases of tetracycline resistance, although point mutations in 16S rRNA (AGA926-928TTC) were seen in 14/109 (12.8%) of isolates. Thirteen isolates harboured the A926G mutation, one with the A926T mutation, and one with the A928C mutation. However, none of these mutations corresponded to phenotypic resistance to tetracycline.

Core genome analysis

H. pylori strains from six of the seven genetic ancestral populations were observed in our cohort. The highest prevalence was from hpEurope (65, 59.6%) followed by hpEastAsia (28, 25.7%) (Table 3). The core genome for isolates was plotted comparatively with 95 established reference genomes from the National Centre for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/refseq/) (Figure 1)(Supplementary Table).

Characterisation of virulence factors and genetic origin

Of the 109 *H. pylori* isolates that underwent whole genome sequencing, *cagPAI* was seen in 73 isolates (67%), with *cagA* present in 66 (61%). Either partial or complete presence of *cagPAI* was seen in all isolates of Asian origin (Table 4). For 9 patients (8%) with gastric intestinal metaplasia on biopsies, 8 isolates (89%) contained the *cagA* gene, with 6 of these (67%) originating genetically from hpEastAsia. EPIYA ABD, which has been associated with increased risk of gastric cancer, was present in 27 of 66 isolates (44%).

Analysis of VacA demonstrated the most virulent s1m1 genotype in 28 isolates (26%), with the least virulent s2m2 genotype in 26 (24%). Half of the isolates (55, 50%) were s1m2, with moderate production of the cytotoxin (Table 4).

Correlation of endoscopic and histological findings with virulence factors

Endoscopy reports corresponding to 95 of the isolates were available. Gastritis was reported in 68 (71.6%) and duodenitis in 14 (14.7%) of reports. There were no cases of gastric cancer or dysplasia, however there were 9 cases of gastric intestinal metaplasia confirmed on histology. Of the 9 histological cases of gastric intestinal metaplasia, eight infecting isolates had *cagA* mutations, and six had the s1m2 *vacA* genotype, two had s1m1, and one had the least virulent s2m2 genotype. The genetic origins of these isolates demonstrated that the majority (six, 67%) were of hpEastAsia ancestry, all of which had the *cagA* EPIYA ABD motif.

DISCUSSION

This study demonstrates the heterogeneous population of *H. pylori* colonising patients with refractory infection in Australia, revealing variable resistance profiles between isolates, but overall high rates of both antimicrobial resistance and multi-drug resistance. Genetically determined resistance profiles using a single gene approach correlated strongly with phenotypic resistance for clarithromycin and levofloxacin in this cohort. This illustrates the potential role of non-invasive PCR faecal analysis as a screening test for *H. pylori* antimicrobial resistance. The majority of strains identified were of European (60%) or East Asian (26%) ancestry, reflecting Australian population migration trends over recent decades.[37] The known virulence factor *cagPAI* was present in the majority of strains encountered, and approximately a quarter of patients had the highest virulence s1m1 *vacA* genotype.

In our cohort of patients with refractory *H. pylori* infection, the majority of isolates (almost 60%) were multi-drug resistant, highlighting the importance of clinician access to contemporary rates of antimicrobial resistance for best selection of eradication regimens. Rates of resistance to clarithromycin, metronidazole and levofloxacin were all above 15%, validating recommendations against their empiric use for eradication of refractory *H. pylori* infection, especially in patients who have experienced failure with multiple lines of prior treatment.[38] Excellent concordance between genetically determined antibiotic resistance and phenotypic resistance from culture and sensitivity testing was demonstrated for both clarithromycin (92%) and levofloxacin (94%) using a single gene approach. Of note, all isolates with levofloxacin resistance contained *gyrA N87/D91* mutations, while the absence of these mutations had a 100% negative predictive value, suggesting that PCR of this region may be a useful screening tool to exclude resistance prior to empiric treatment. This may be possible using less invasive, non-endoscopic approaches, such as PCR of *H. pylori* isolates from stool samples.[39] The prevalence of 16S rRNA (AGA926-928TTC) mutations was 13% in our cohort, but no phenotypic tetracycline resistance was demonstrated, suggesting that these particular mutations have limited predictive value for tetracycline resistance. Further studies are needed to improve the characterisation of mutations associated with phenotypic resistance to amoxicillin, tetracycline, rifampicin and metronidazole if region specific PCR is to be a viable tool for predicting phenotypic resistance to these antimicrobials.

Globally, there are high rates of antimicrobial resistance to *H. pylori*, both in Europe and East Asia.[15] It is likely that migration patterns will influence ongoing changes in antimicrobial resistance rates in Australia, and this is important when considering empiric treatment options. Establishment of a national database should ideally be pursued to track antimicrobial resistance trends in order to assist clinicians in understanding local resistance patterns and guide eradication therapy. Multiple recent studies have highlighted rising rates of clarithromycin resistance, and current antimicrobial guidelines suggesting that clarithromycin should be part of first line empiric therapy ought to be revisited, as its empiric use is unlikely to achieve adequate eradication rates.[17, 18] Globally, rates of levofloxacin resistance are rising, and we found resistance to this antibiotic exceeded 15%, consistent with other recent Australian studies, suggesting that the use of empiric second line regimens containing levofloxacin should also be reconsidered.[40, 41]

The genetic origins of the isolates in this study, with most strains being of European or East Asian ancestry, were not surprising based on Australian immigration patterns. Virulence factors were present in the majority of isolates, with around 60% containing cagA. Prior studies have demonstrated that virulence factors, including *cagA*, are present at higher rates in geographic regions with a high prevalence of diffuse gastric cancer.[42] All strains from hpEastAsia origin, where rates of gastric cancer are among the highest in the world, were *cagA* positive. Knowledge of strain factors may therefore play a role in risk stratification, but more research is needed in this area. Furthermore, all cases of *cagA* originating from East Asia who had intestinal metaplasia on biopsies had the EPIYA ABD motif, which has been associated with an increased risk of gastric cancer.[31] Migrant populations are frequently burdened by both the risk of resistance as well as the complications of *H. pylori* infection, and risk factors beyond gastric metaplasia and a family history of gastric cancer should be considered when assessing the long term risk of adverse clinical outcomes.[43] A better understanding of pathogen factors, including resistance profiles, global origins and virulence factors and distributions, combined with patient-specific risk factors, may allow clinicians to provide comprehensive, individualised management for *H. pylori* infection.

The differing long term clinical outcomes in patients infected with *H. pylori* remain poorly characterised, and multiple factors including diet, lifestyle, and host and pathogen characteristics are likely to play a role.[44] For example, particular *H. pylori* strains that associate with gastric cancer risk may be of assistance to clinicians in guiding counselling and endoscopic surveillance. However, further individualised host and pathogen data are needed before management can be truly

individualised, including identifying those at risk of eradication treatment failure. It may be possible in the future to create individualised risk scores, incorporating such environmental factors as alcohol and smoking, host factors such as a family history of gastric cancer or dysplasia, and pathogenic factors including the strain of *H. pylori* infection, virulence factors and duration of infection. In patients without clinical risk factors and with benign *H. pylori* pathogen factors, there may be a role for not attempting *H. pylori* eradication, especially considering the potential, but overlooked, benefits of *H. pylori* infection which include immune modulation and an inverse association with gastro-oesophageal reflux disease and development of oesophageal adenocarcinoma.[45]

Strengths of this study include the multicentre approach, with data collected from multiple hospitals in two Australian states. The comprehensive whole genome approach and large number of *H. pylori* isolates sequenced further strengthen the study. A limitation was the lack of information on the migration status of individual patients, nor detailed clinical information regarding prior antimicrobial exposure. Data capture was limited to two states in Australia and further studies are recommended to confirm whether these associations are generalisable to other regions. Further limitations include inclusion of only patients who had refractory *H. pylori* who underwent endoscopy, biopsy and culture, so this cohort likely represents a subset of patients with highly refractory *H. pylori* infection, although it does encapsulate culture results encountered in "real world" clinical practice. Furthermore, only a subset of outcomes available from whole genome sequencing were explored in this study, and further studies including antimicrobial resistance linkage analysis to point mutations is needed.

CONCLUSIONS

The refractory *H. pylori* seen in Australian clinical practice is a heterogeneous mix of strains with origins throughout the world. Whilst resistance profiles vary, there is strong concordance between genetically determined clarithromycin and levofloxacin resistance and phenotypic resistance from culture, suggesting region-specific polymerase chain reaction as a viable method for predicting resistance in clinical practice. More than half of the isolates tested contained virulence factors associated with increased risk of gastric cancer, and further studies are needed in order to identify patients at risk of adverse outcomes so as to individualise their management. The establishment of a national database to monitor *H. pylori* resistance is recommended, with genome sequencing having a potential role in monitoring established risk factors for adverse outcomes in the dynamic landscape of *H. pylori* in Australia.

REFERENCES

1. Suerbaum S, Michetti P. Helicobacter pylori infection. The New England journal of medicine. 2002;347(15):1175-86.

2. Pohl D, Keller PM, Bordier V, Wagner K. Review of current diagnostic methods and advances in Helicobacter pylori diagnostics in the era of next generation sequencing. World J Gastroenterol. 2019;25(32):4629-60.

3. Attila T, Zeybel M, Yigit YE, Baran B, Ahishali E, Alper E, et al. Upper socioeconomic status is associated with lower Helicobacter pylori infection rate among patients undergoing gastroscopy. Journal of infection in developing countries. 2020;14(3):298-303.

4. Maixner F, Krause-Kyora B, Turaev D, Herbig A, Hoopmann MR, Hallows JL, et al. The 5300-year-old Helicobacter pylori genome of the Iceman. Science (New York, NY). 2016;351(6269):162-5.

5. Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. The Journal of clinical investigation. 2004;113(3):321-33.

6. Kodaman N, Pazos A, Schneider BG, Piazuelo MB, Mera R, Sobota RS, et al. Human and Helicobacter pylori coevolution shapes the risk of gastric disease. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(4):1455-60.

7. Correa P, Piazuelo MB. Evolutionary History of the Helicobacter pylori Genome: Implications for Gastric Carcinogenesis. Gut and liver. 2012;6(1):21-8.

8. Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, et al. Traces of human migrations in Helicobacter pylori populations. Science (New York, NY). 2003;299(5612):1582-5.

9. Suerbaum S, Josenhans C. Helicobacter pylori evolution and phenotypic diversification in a changing host. Nature reviews Microbiology. 2007;5(6):441-52.

10. Falush D, Kraft C, Taylor NS, Correa P, Fox JG, Achtman M, et al. Recombination and mutation during long-term gastric colonization by Helicobacter pylori: estimates of clock rates, recombination size, and minimal age. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(26):15056-61.

11. Kang J, Blaser MJ. Bacterial populations as perfect gases: genomic integrity and diversification tensions in Helicobacter pylori. Nature reviews Microbiology. 2006;4(11):826-36.

12. Didelot X, Nell S, Yang I, Woltemate S, van der Merwe S, Suerbaum S. Genomic evolution and transmission of Helicobacter pylori in two South African families. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(34):13880-5.

13. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.

14. Graham DY, Shiotani A. New concepts of resistance in the treatment of Helicobacter pylori infections. Nature clinical practice Gastroenterology & hepatology. 2008;5(6):321-31.

15. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016;43(4):514-33.

16. Tacconelli E. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development. 2017.

17. Schubert JP, Warner MS, Rayner CK, Roberts-Thomson IC, Mangoni AA, Costello S, et al. Increasing Helicobacter pylori clarithromycin resistance in Australia over 20 years. Intern Med J. 2022;52(9):1554-60.

18. Schubert JP, Gehlert J, Rayner CK, Roberts-Thomson IC, Costello S, Mangoni AA, et al. Antibiotic resistance of Helicobacter pylori in Australia and New Zealand: A systematic review and meta-analysis. J Gastroenterol Hepatol. 2021;36(6):1450-6.

19. Cattoir V, Nectoux J, Lascols C, Deforges L, Delchier JC, Megraud F, et al. Update on fluoroquinolone resistance in Helicobacter pylori: new mutations leading to resistance and first description of a gyrA polymorphism associated with hypersusceptibility. International journal of antimicrobial agents. 2007;29(4):389-96.

20. Nishizawa T, Suzuki H. Mechanisms of Helicobacter pylori antibiotic resistance and molecular testing. Frontiers in molecular biosciences. 2014;1:19.

21. Gerrits MM, Berning M, Van Vliet AH, Kuipers EJ, Kusters JG. Effects of 16S rRNA gene mutations on tetracycline resistance in Helicobacter pylori. Antimicrobial agents and chemotherapy. 2003;47(9):2984-6.

22. Lee YC, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, et al. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. Gastroenterology. 2016;150(5):1113-24.e5.

23. Holcombe C. Helicobacter pylori: the African enigma. Gut. 1992;33(4):429-31.

24. Whary MT, Sundina N, Bravo LE, Correa P, Quinones F, Caro F, et al. Intestinal helminthiasis in Colombian children promotes a Th2 response to Helicobacter pylori: possible implications for gastric carcinogenesis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2005;14(6):1464-9.

25. Correa P, Malcom G, Schmidt B, Fontham E, Ruiz B, Bravo JC, et al. Review article: Antioxidant micronutrients and gastric cancer. Aliment Pharmacol Ther. 1998;12 Suppl 1:73-82.

26. Peek RM, Jr., Crabtree JE. Helicobacter infection and gastric neoplasia. The Journal of pathology. 2006;208(2):233-48.

27. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer research. 1995;55(10):2111-5.

28. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40(3):297-301.

29. Kim SY, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, et al. Genotyping CagA, VacA subtype, IceA1, and BabA of Helicobacter pylori isolates from Korean patients, and their association with gastroduodenal diseases. Journal of Korean medical science. 2001;16(5):579-84.

30. Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, et al. Structure of cag pathogenicity island in Japanese Helicobacter pylori isolates. Gut. 1999;44(3):336-41.

31. Sahara S, Sugimoto M, Vilaichone RK, Mahachai V, Miyajima H, Furuta T, et al. Role of Helicobacter pylori cagA EPIYA motif and vacA genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC infectious diseases. 2012;12:223.

32. Gangwer KA, Shaffer CL, Suerbaum S, Lacy DB, Cover TL, Bordenstein SR. Molecular evolution of the Helicobacter pylori vacuolating toxin gene vacA. Journal of bacteriology. 2010;192(23):6126-35.

33. Román-Román A, Martínez-Carrillo DN, Atrisco-Morales J, Azúcar-Heziquio JC, Cuevas-Caballero AS, Castañón-Sánchez CA, et al. Helicobacter pylori vacA s1m1 genotype but not cagA or babA2 increase the risk of ulcer and gastric cancer in patients from Southern Mexico. Gut pathogens. 2017;9:18.

34. Achtman M, Azuma T, Berg DE, Ito Y, Morelli G, Pan ZJ, et al. Recombination and clonal groupings within Helicobacter pylori from different geographical regions. Molecular microbiology. 1999;32(3):459-70.

35. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 13.0, 2023. [Available from: http://www.eucast.org/clinical_breakpoints/.

36. Perkins TT, Tay CY, Thirriot F, Marshall B. Choosing a benchtop sequencing machine to characterise Helicobacter pylori genomes. PloS one. 2013;8(6):e67539.

37. Statistics ABo. Australian Census Data 2021 [Available from: https://www.abs.gov.au/statistics.

38. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report. Gut. 2022.

39. Beckman E, Saracino I, Fiorini G, Clark C, Slepnev V, Patel D, et al. A Novel Stool PCR Test for Helicobacter pylori May Predict Clarithromycin Resistance and Eradication of Infection at a High Rate. Journal of clinical microbiology. 2017;55(8):2400-5.

40. Pateria P CM GR, McCullough C, Raby E, Robineron JO, Ingram PR. . Increasing secondary resistance to fluoroquinolones amongst Helicobacter pylori in Western Australia. Tasman Medical Journal. 2020;2.

41. Schubert JP, Ingram PR, Warner MS, Rayner CK, Roberts-Thomson IC, Costello SP, et al. Refractory Helicobacter pylori infection in Australia: updated multicentre antimicrobial resistance. Internal medicine journal. 2023.

42. Dominguez RL, Cherry CB, Estevez-Ordonez D, Mera R, Escamilla V, Pawlita M, et al. Geospatial analyses identify regional hot spots of diffuse gastric cancer in rural Central America. BMC cancer. 2019;19(1):545.

43. Schubert JP, Woodman RJ, Mangoni AA, Rayner CK, Warner MS, Roberts-Thomson IC, et al. Geospatial analysis of Helicobacter pylori infection in South Australia: Should location influence eradication therapy? J Gastroenterol Hepatol. 2022;37(7):1263-74.

44. Kumar S, Patel GK, Ghoshal UC. Helicobacter pylori-Induced Inflammation: Possible Factors Modulating the Risk of Gastric Cancer. Pathogens (Basel, Switzerland). 2021;10(9).

45. Schubert JP, Rayner CK, Costello SP, Roberts-Thomson IC, Forster SC, Bryant RV. Helicobacter pylori: Have potential benefits been overlooked? JGH Open. 2022;6(11):735-7.

TABLES AND FIGURES

Antimicrobial	Resistant isolates n (%)
Clarithromycin	115 (85.2%)
Metronidazole	70 (51.9%)
Levofloxacin	24 (17.8%)
Rifampicin	19 (14.1%)
Amoxicillin	12 (8.9%)
Tetracycline	0 (0%)
No resistance	10 (7.4%)
Single drug resistance	46 (34.1%)
Multi-drug resistance	79 (58.5%)

Table 7.1: Antimicrobial resistance of 135 *H. pylori* isolates

	Clarithromycin resistance*	Levofloxacin resistance^
Concordance	92.2%	94.1%
Positive predictive value (PPV)	97.6%	71.4%
Negative predictive value (NPV)	70.0%	100%
Sensitivity	93.0%	100%
Specificity	87.5%	93.0%

* based on single gene, two-mutation S23 A2142 & A2143 analysis

^ based on single gene, two mutation GyrA N87 & D91 analysis

Table 7.2: Comparison between phenotypic resistance and genetically predicted resistance profiles for clarithromycin and levofloxacin

Genetic ancestral population	Geographic/ethnic distribution	Isolates, n (%)
hpEurope	Europe, Middle East, India and Iran	65 (59.6%)
hpAfrica1	Western Africa, South Africa	4 (3.7%)
hpAfrica2	South Africa	1 (0.9%)
hpNEAfrica	Ethiopia, Somalia, Sudan, Northern Nigeria	7 (6.4%)
hpEastAsia	East Asians, Taiwan Aboriginals, Melanesians, Polynesians, Native Americans	28 (25.7%)
hpAsia2	Northern India, Bangladesh, Thailand, Malaysia	4 (3.7%)
hpSahul	Australia Aboriginals and Papua New Guineans	0 (0%)

Table 7.3: Ancestral genetic origins of the 109 isolates sequenced in this study

Virulence Factor		Isolates, n (%)
cagPAI	Complete	61 (56%)
	Partial	12 (11%)
	Absent	36 (33%)
	cagA	66 (61%)
cagA genotypes (EPIYA)	AB	4 (6%)
	ABC	24 (39%)
	ABD	27 (44%)
	BC	3 (5%)
	Other	8 (12%)
vacA	s1m1	28 (26%)
	s1m2	55 (50%)
	s2m1	0 (0%)
	s2m2	26 (24%)

Table 7.4: Virulence factors among the 109 isolates sequenced in this study

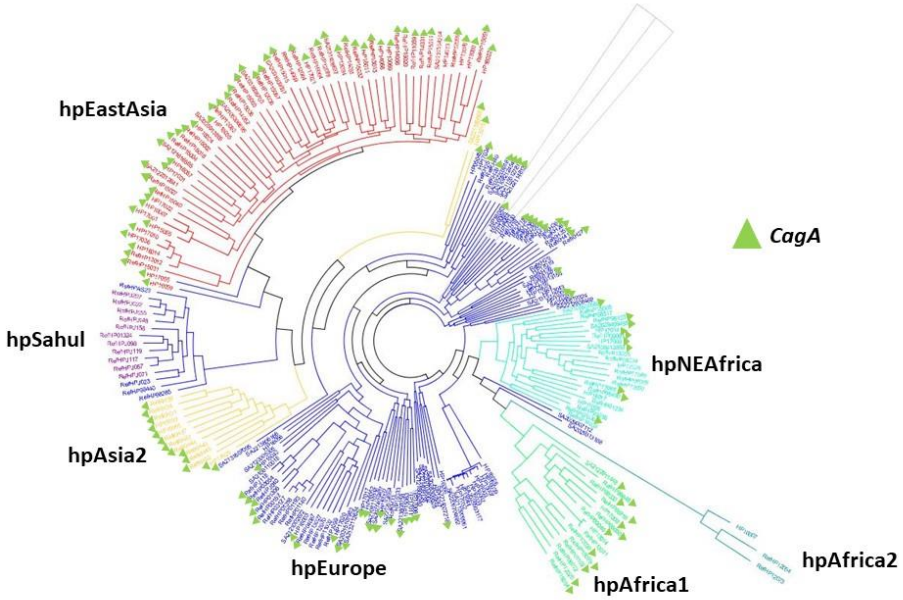


Figure 7.1: Phylogenetic analysis of the genetic origins of H. pylori isolates and the CagA distribution (109 strains from the current study and 95 strains from published genomes)

CHAPTER 8: *H. PYLORI*: HAVE POTENTIAL BENEFITS BEEN OVERLOOKED?

8.1 Background

H. pylori has co-evolved with humans over more than 50,000 years, but despite this its function as a commensal organism remains poorly understood. Its role in the pathogenesis of peptic ulcer and gastric cancer is widely acknowledged, with current guidelines recommending eradication if detected. However, less publicised is the multi-faceted physiological function that *H. pylori* plays as a commensal in the human gastric microbiome. There is increasing evidence regarding the role of *H. pylori* as an immune modulator, with evidence of an inverse association between *H. pylori* colonisation and immune-mediated disorders including asthma and inflammatory bowel disease.

Furthermore, *H. pylori* has been implicated in the modulation of satiety hormones, including leptin and ghrelin, that may influence appetite and contribute to weight control. With the rapidly rising incidence of oesophageal adenocarcinoma in the Western world, the relationship of *H. pylori* to gastric acid secretion, gastroesophageal reflux disease, and the rising incidence of adenocarcinoma of the lower oesophagus, warrants further characterisation. This paper aims to explore the positive associations of *H. pylori* colonisation, which are largely overlooked in current clinical practice.

8.2 Specific Author Contributions

Statement of Authorship

Title of Paper	Helicobacter pylori: Have potential benefits been overlooked?
Publication Status	Published
Publication Details	Schubert JP, Rayner CK, Costello SP, Roberts-Thomson IC, Forster SC, Bryant RV. Helicobacter pylori: Have potential benefits been overlooked?. JGH Open. 2022;6(11):735-737. Published 2022 Nov 16. doi:10.1002/jgh3.12842

Principal Author

Name of Principal Author (Candidate)	Jonathon P Schubert		
Contribution to the Paper	Conception, Acquiring data, Knowledge, Analysis, Drafting		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/9/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

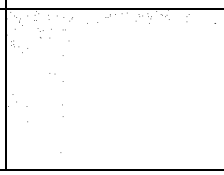
i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Christopher K Rayner
Contribution to the Paper (5%)	Knowledge, Drafting

Signature		Date	28/11/2023

Name of Co-Author	Ian C Roberts-Thomson		
Contribution to the Paper (5%)	Conception, Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Samuel P Costello		
Contribution to the Paper (5%)	Knowledge, Drafting		
Signature		Date	28/11/2023

Name of Co-Author	Samuel C Forster		
Contribution to the Paper (5%)	Knowledge, Drafting		
Signature		Date	28/9/2023

Name of Co-Author	Robert V Bryant		
Contribution to the Paper (5%)	Conception, Knowledge, Drafting		
Signature		Date	

[Manuscript 6] *H. pylori*: have potential benefits been overlooked?

Jonathon P Schubert[1,2,3], Christopher K Rayner[1,3], Samuel P Costello[1,2], Ian C Roberts-Thomson[1], Samuel C Forster[4], Robert V Bryant[1,2]

[1]Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia

[2]Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, Australia

[3]Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia

[4]Microbiota and Systems Biology, Hudson Institute of Medical Research, Clayton, Australia

Corresponding author

Dr Jonathon P Schubert

Faculty of Health Sciences, Adelaide Medical School, University of Adelaide, Adelaide, Australia

Email: jonathon.schubert@adelaide.edu.au

Conflict of interests

Dr. Ian Roberts-Thomson is an Editorial Board member of JGH Open and the corresponding author of this article. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication.

Helicobacter pylori (H. pylori) has co-evolved with humans over tens of thousands of years.[1] While its role in the pathogenesis of peptic ulcer and gastric cancer is widely acknowledged, less publicised is the multi-faceted physiological function that *H. pylori* plays as a commensal in the human gastric microbiome. Recent evidence has highlighted the role of *H. pylori* as an immune modulator with increasing evidence of an inverse association between *H. pylori* colonisation and immune mediated disorders including asthma and inflammatory bowel disease (IBD). In addition, there is burgeoning evidence to suggest that *H. pylori* modulates satiety hormones, including leptin and ghrelin, that may influence appetite and contribute to weight control. Yet another issue is the relationship of *H. pylori to* gastric acid secretion, gastro-oesophageal reflux disease (GERD) and the rising incidence of adenocarcinoma of the lower oesophagus.

The role of *H. pylori* in chronic gastric inflammation is well established. Almost all patients infected with *H. pylori* exhibit histological chronic active inflammation, even those who are asymptomatic.[2] The association of *H. pylori* with peptic ulceration, particularly duodenal ulceration, is also clear. Whereas peptic ulceration was estimated to affect at least 10% of the human population in the mid-20th century, current prevalence rates have substantially decreased because of falling rates of *H. pylori* infection, eradication regimens, fewer smokers and the widespread use of medication to reduce gastric acid secretion. Gastric cancer is mostly associated with *H. pylori* (80%-90%) and will develop in approximately 1% of infected individuals over their lifetime.[3,4] However, the risk of gastric malignancy varies widely between different populations with a greater incidence of *H. pylori*-associated cancer in Eastern and Central Asia [approximately 30 per 100,000 males], compared to regions of North and East Africa (approximately 5 per 100,000 males).[5] Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a rare disease but is almost always associated with *H. pylori* and may resolve with eradication of the infection.[4]

The 2015 Kyoto global consensus report on *H. pylori* gastritis recommends that all patients with this condition should receive eradication therapy, regardless of the presence of peptic ulcer or the background risk of gastric cancer.[6] This recommendation promotes the belief that "the only good *H. pylori* is a dead *H. pylori"* and includes the assumption that the infection is associated with few, if any, beneficial effects. With the passage of time, this recommendation may need to be re-examined in the light of new epidemiological data that suggests a relationship between *H. pylori* infection and lower risks for obesity and important gastrointestinal diseases such as IBD, GERD and oesophageal cancer.

Arguably, the management of obesity and its complications poses the greatest challenge to health care in the current era. Clearly, the most important risk factors relate to diet and lifestyle. Whether *H. pylori* infection is associated with a lower body weight is less clear but appears to apply in some lower income countries.[7] This effect may be related to changes in the intestinal microbiome or to the effects of gastric infection on levels of appetite-related hormones such as leptin and ghrelin. Although the main source of leptin is adipose tissue, leptin is also produced by gastric chief and parietal cells and released in response to meals and hormonal signals.[8, 9] Leptin signals satiety to the hypothalamus and is followed by diverse effects including increased energy expenditure and reduced gastric acid secretion.[10] In contrast, ghrelin is released from oxyntic cells during fasting and reduces energy expenditure while increasing appetite and gastric acid secretion.[11, 12]

In individuals colonised with *H. pylori,* leptin levels are higher than in uninfected controls. Conversely, ghrelin levels substantially increase with eradication of *H. pylori*. Both effects have the potential to stimulate hunger, adipose tissue deposition and growth hormone release.[13] This is supported by clinical studies showing slower weight gain in infected compared to uninfected children and weight gain after the eradication of *H. pylori.*[13, 14] Whether these metabolic effects of *H. pylori* are beneficial in low socioeconomic areas with marginal food availability remains unclear.[7] However, rising rates of obesity in developed countries [where the *H. pylori* prevalence is falling], support the possibility of a link between *H. pylori* and weight control, perhaps mediated by hormonal factors.

Several studies have shown a lower than expected frequency of *H. pylori* in patients with a variety of allergic disorders such as asthma and allergic rhinitis and immunologic disorders such as IBD. One possibility is that *H. pylori* has a specific effect on immune tolerance while another is that the presence of *H. pylori* is a non-specific marker of exposure to a more contaminated environment. The latter is widely recognized as the “hygiene hypothesis”. Recent meta-analyses indicate that *H. pylori* colonisation reduces the risk of IBD by 38%-52%.[15, 16] This may be related to an effect of *H. pylori* on systemic immune homeostasis with the induction of tolerant dendritic cells and immunosuppressive regulatory T cells. These ideas are supported by murine models of colitis showing suppression of systemic inflammation in the presence of *H. pylori* infection.[17] Whether the beneficial effects of *H. pylori* are lost after eradication of the infection remain unclear. Of interest was a recent study

showing that regulatory T cells induced by *H. pylori* were able to skew the adaptive immune response towards immune tolerance with effects on T cell responses to other allergens and auto-allergens.[18] These effects have the potential to reduce the risk of asthma and other allergic disorders.

For clinical gastroenterologists, the final potential benefit of *H. pylori* lies in the links between gastric infection with *H. pylori*, GERD, Barrett's oesophagus and the risk of oesophageal adenocarcinoma. In several countries, increasing rates of reflux oesophagus at upper GI endoscopy have been accompanied by decreasing rates of positive urease tests.[19] In Singapore (with a higher prevalence of *H. pylori*), the frequency of reflux oesophagitis (3.3%) is less than one-third of that observed in the USA.[20] Furthermore, there is now persuasive evidence that eradication of *H. pylori* is followed by an increase in the frequency of both reflux symptoms and oesophagitis at endoscopy.[19] For oesophageal adenocarcinoma in the USA, rates have increased more than three-fold in the last 50 years, making it the fastest rising malignancy in that country.[21] An increase of this magnitude has not been observed in Singapore.[20] A prospective study of the influence of *H. pylori* on Barrett's oesophagus and oesophageal cancer found that patients infected with *H. pylori* had a lower frequency of both Barrett's high-grade dysplasia and Barrett's adenocarcinoma.[22] These conclusions have been supported by other studies.[23]

Reasons for the above observations appear to rely on the complex relationship between *H. pylori* and the secretion of gastric acid. In the majority of infected individuals, gastric acid secretion is lower than in uninfected controls, presumably because of gastritis [sometimes associated with atrophic gastritis] involving the body of the stomach. In this group, eradication of infection is usually associated with an increase in acid secretion. A separate but smaller group have an increase in acid secretion with *H. pylori* with minimal inflammation in the body of the stomach and are at higher risk for duodenal ulceration. In this group, eradication of infection results in a fall in acid secretion, largely because of a fall in serum gastrin.[24] As eradication of infection increases acid secretion in a substantial majority, the overall effect is an increase in acid secretion that increases the risk of GERD. By extension, more prominent oesophageal inflammation could increase the risk of Barrett's oesophagus and the subsequent risk of adenocarcinoma. This hypothesis could explain the inverse relationship of *H. pylori* with Barrett's dysplasia and adenocarcinoma but other explanations are possible involving changes in the gastric microbiome or changes in the composition of refluxed fluid.

The epidemiologic evidence outlined above raises the possibility that the beneficial effects of *H. pylori* have been underestimated. Absence of infection has been associated with higher risks for obesity, IBD and reflux oesophagitis and with the uncommon but important complications of reflux, namely Barrett's oesophagus and oesophageal adenocarcinoma. However, these risks need to be compared to the risk of gastric cancer and peptic ulceration. Policies that favour eradication regimens in infected individuals may well be appropriate in countries with a high burden of gastric cancer. However, similar policies in countries with a lower burden of gastric cancer may need to be re-examined in the light of newer epidemiologic information. One day, perhaps, studies will examine the use of *H. pylori* for the treatment of human disease.

REFERENCES

1. Maixner F, Krause-Kyora B, Turaev D, Herbig A, Hoopmann MR, Hallows JL, et al. The 5300-year-old Helicobacter pylori genome of the Iceman. Science. 2016; 351(6269): 162-5.
2. Dooley CP, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, et al. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. N Engl J Med. 1989; 321(23): 1562-6.
3. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med. 1991; 325(16): 1127-31.
4. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, et al. Helicobacter pylori infection and gastric lymphoma. N Engl J Med. 1994; 330(18): 1267-71.
5. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. Prz Gastroenterol. 2019; 14(1): 26-38.
6. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut. 2015; 64(9): 1353-67.
7. Moayyedi P, Axon AT, Feltbower R, Duffett S, Crocombe W, Braunholtz D, et al. Relation of adult lifestyle and socioeconomic factors to the prevalence of Helicobacter pylori infection. International Journal of Epidemiology. 2002; 31(3): 624-31.
8. Sobhani I, Buyse M, Goïot H, Weber N, Laigneau JP, Henin D, et al. Vagal stimulation rapidly increases leptin secretion in human stomach. Gastroenterology. 2002; 122(2): 259-63.
9. Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. J Clin Invest. 2004; 113(3): 321-33.
10. Goïot H, Attoub S, Kermorgant S, Laigneau JP, Lardeux B, Lehy T, et al. Antral mucosa expresses functional leptin receptors coupled to STAT-3 signaling, which is involved in the control of gastric secretions in the rat. Gastroenterology. 2001; 121(6): 1417-27.
11. Lee HM, Wang G, Englander EW, Kojima M, Greeley GH, Jr. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. Endocrinology. 2002; 143(1): 185-90.
12. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology. 2001; 120(2): 337-45.
13. Azuma T, Suto H, Ito Y, Ohtani M, Dojo M, Kuriyama M, et al. Gastric leptin and Helicobacter pylori infection. Gut. 2001; 49(3): 324-9.
14. Nwokolo CU, Freshwater DA, O'Hare P, Randeva HS. Plasma ghrelin following cure of Helicobacter pylori. Gut. 2003; 52(5): 637-40.

15. Wu XW, Ji HZ, Yang MF, Wu L, Wang FY. Helicobacter pylori infection and inflammatory bowel disease in Asians: a meta-analysis. World J Gastroenterol. 2015; 21(15): 4750-6.
16. Rokkas T, Gisbert JP, Niv Y, O'Morain C. The association between Helicobacter pylori infection and inflammatory bowel disease based on meta-analysis. United European Gastroenterol J. 2015; 3(6): 539-50.
17. Yu Y, Zhu S, Li P, Min L, Zhang S. Helicobacter pylori infection and inflammatory bowel disease: a crosstalk between upper and lower digestive tract. Cell Death Dis. 2018; 9(10): 961.
18. Arnold IC, Hitzler I, Müller A. The immunomodulatory properties of Helicobacter pylori confer protection against allergic and chronic inflammatory disorders. Front Cell Infect Microbiol. 2012; 2:10.
19. Ho KY, Chan YH, Kang JY. Increasing trend of reflux esophagitis and decreasing trend of Helicobacter pylori infection in patients from a multiethnic Asian country. Am J Gastroenterol. 2005; 100(9): 1923-8.
20. Kang JY, Tay HH, Yap I, Guan R, Lim KP, Math MV. Low frequency of endoscopic esophagitis in Asian patients. J Clin Gastroenterol. 1993; 16(1): 70-3.
21. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer. 1998; 83(10): 2049-53.
22. Weston AP, Badr AS, Topalovski M, Cherian R, Dixon A, Hassanein RS. Prospective evaluation of the prevalence of gastric Helicobacter pylori infection in patients with GERD, Barrett's esophagus, Barrett's dysplasia, and Barrett's adenocarcinoma. Am J Gastroenterol. 2000; 95(2): 387-94.
23. Islami F, Kamangar F. Helicobacter pylori and esophageal cancer risk: a meta-analysis. Cancer Prev Res (Phila). 2008; 1(5): 329-38.
24. Roberts-Thomson IC. How did the ancient bacterium, Helicobacter pylori, cause an epidemic of chronic duodenal ulceration? JGH Open. 2021; 5(6): 636-42.

CHAPTER 9: DISCUSSION

The body of research presented in this thesis explores and characterises *H. pylori* infection across Australia. It characterises antimicrobial resistance, population, genetic and environmental factors associated with adverse clinical outcomes, which are fundamental to improve care of patients infected by this pathogen. Treatment directed at this organism needs to reflect updated patterns of antimicrobial resistance and should be individualised to address both individual and host risk factors. Risk stratification and improved awareness of risk factors associated with adverse clinical outcomes are needed to minimise the burden of disease.

Eradication therapy needs to be based on knowledge of contemporary antibiotic resistance to ensure appropriate antimicrobial stewardship and achieve adequate eradication rates. In the setting of evolving resistance and an increasing prevalence of multi-drug resistant strains, eradication therapy needs to be appropriate and targeted. Factors associated with multi-resistant strains of *H. pylori*, including migration status and prior antimicrobial exposure, need to be considered along with recent patterns of local antimicrobial resistance. Currently recommended antimicrobial eradication therapy in Australia is based on historic data which are now outdated. These regimens are unlikely to achieve adequate eradication rates, will likely contribute to the burden of growing antimicrobial resistance, and need to be revised.

Management of *H. pylori* needs to be individualised to both the host and pathogen, and individual risk factors need to be considered. Knowledge of virulence mutations and their associations within populations and communities should be considered as part of eradication regimens. Stratification of risk should be a routine part of quality care, but this requires informed and contemporary data to guide clinical management. Further work is needed to enable phenotypic prediction of antimicrobial resistance from genomic analysis, including further understanding of mutations conferring resistance. Virulence factors, while increasingly understood, need further characterisation regarding their association with adverse clinical outcomes.

The work performed in this thesis illustrates that management of *H. pylori* infection requires an individualised and multifaceted approach. Both pathogen factors, including antimicrobial resistance and virulence mutations, as well as host factors, including comorbidities, individualised risk of adverse clinical outcomes and geographic region need to be addressed, so as to improve quality of care in *H. pylori* infection.

9.1 Key outcomes, significance and limitations

Inadequate monitoring of antimicrobial resistance

The Maastricht VI *H. pylori* Consensus Report recommends that clinicians understand the local prevalence of *H. pylori* resistance so that they can select the most appropriate first- and second-line eradication regimens.[1] Despite years of widespread community antibiotic use, and population changes due to migration from countries with a high prevalence of *H. pylori* resistance, there has been little attention paid to rising *H. pylori* resistance in Australia, as demonstrated in this body of research. In particular, there has been a paucity of local data to identify rates of clarithromycin resistant *H. pylori* since the turn of the century.[2]

Informed and appropriate prescribing requires up to date data on antimicrobial resistance trends. The results of the current study fill a gap in the literature, raise substantial concern about recent changes in resistance, and highlight the need for improved antimicrobial monitoring at a national level. The systematic review and meta-analysis presented highlights the lack of contemporary data and the need for contemporary data to guide clinical decisions on eradication therapy.

Empiric first line antimicrobial eradication therapy needs revision

Over the last 20 years there has been a marked rise in the rate of *H. pylori* resistance to clarithromycin in our Australian centre studied in Chapter 4. Reassuringly, the rate of metronidazole resistance has been stable, and rates of amoxicillin and tetracycline resistance have remained low. The results of the study presented in Chapter 4 fill a void in data over this period and demonstrate a consistent local rise in clarithromycin resistance since 1998, which continued until the end of data capture in 2017. Further Australian studies are warranted to provide insight as to whether these trends are evident nationally. The outcomes suggest that the current first line *H. pylori* eradication therapy may fail to achieve adequate eradication rates, and that further research to inform recommendations as to the optimal empiric eradication therapy in Australia should be a priority at a national level, in line with guidelines by the WHO placing it as a high priority for research internationally.

Antimicrobial resistance is clustered in migrant populations

The population-based geospatial study presented in Chapter 5 provides evidence for a heterogeneous distribution of *H. pylori* resistance within a city. These data also reveal an association between the migrant density of a suburb and the rate of resistant *H. pylori* isolates. These findings illustrate that a patient's geospatial location may be a useful surrogate marker for risk of *H. pylori* resistance, which may help to guide clinicians in prescribing appropriate empirical *H. pylori* eradication therapy.

This study illustrates the role of risk factor stratification for *H. pylori* resistance to include geospatial location alongside previously established risk factors. Prior studies have demonstrated that migrants have higher rates of *H. pylori* infection as a consequence of emigrating from regions of higher antibiotic resistance than the general Australian population.[3, 4] Clustering of resistant *H. pylori* strains is likely to relate to both migration status and local transmission of *H. pylori* among close contacts within the suburb. Recognition of a patient's individual migrant status as well as a geospatial regions associated with higher migrant density, should alert a clinician to a higher risk of harbouring a resistant *H. pylori* strain. Prompt consideration should then be given to additional culture and sensitivity analyses, as well as alternative (even second-line) empirical eradication therapy.

Refractory infection guidelines need revision based on emerging resistance

The study presented in Chapter 6 represents the only multi-centre, multi-state evaluation of contemporary rates of *H. pylori* antimicrobial resistance to have been undertaken in Australia. The data reveal high rates of clarithromycin and metronidazole resistance, and a high prevalence of multi-resistant *H. pylori*, with 63.3% of isolates resistant to two or more antibiotics. Perhaps more alarmingly, high rates of resistance to second-line therapies levofloxacin (22%) and rifampicin (13.5%) were observed, which has not previously been reported in peer-reviewed studies in Australasia.[5] The high rate of levofloxacin resistance (>20%) raises concern as to the current empirical recommendation for use of levofloxacin in second-line eradication regimens. Increased awareness of current *H. pylori* resistance rates in Australia is needed to guide local eradication practices, and current antimicrobial prescribing guidelines for second line therapy should be revisited.

Potential benefits of *H. pylori* have been overlooked in clinical practice

The 2015 Kyoto global consensus report on *H. pylori* gastritis recommends that all patients with this condition should receive eradication therapy, regardless of the presence of peptic ulceration or their background risk of gastric cancer.[6] This recommendation may need to be re-examined in the light of new epidemiological data that suggest a relationship between *H. pylori* infection and lower risks for obesity and other gastrointestinal diseases that have recently been rising in prevalence, including IBD, GORD and oesophageal cancer. Absence of infection has been associated with higher risks for obesity, IBD and reflux oesophagitis along with Barrett's oesophagus and oesophageal adenocarcinoma. However, these risks need to be compared to the risk of morbidity and mortality from adverse clinical outcomes including gastric cancer and peptic ulceration. Policies that favour eradication in infected individuals may be appropriate in countries with a high burden of gastric cancer, where population benefits of eradication outweigh the risks. However, similar policies in countries with a lower burden of gastric cancer may need to be re-examined in the light of newer epidemiologic information. An in-depth understanding is needed of host and pathogen factors to enable appropriate risk and benefit analyses, and will allow for risk factor stratification to be part of therapeutic algorithms in clinical practice. Attempts at global eradication of *H. pylori* will only lead to ongoing rising rates of antimicrobial resistance, and a point may be reached where surviving *H. pylori* species become resistant to all currently available antimicrobials. Individualised, selective eradication is likely to form the basis for future *H. pylori* management; however, a greater understanding of the genetic and molecular factors is needed to guide clinical practice.

Refractory *H. pylori* encountered in Australian clinical practice is highly heterogenous mix of strains with global origins

The populations of refractory *H. pylori* encountered in clinical practice in Australia are diverse and originate from all continents. The isolates seen are a heterogeneous mix, with substantial variation in antimicrobial resistance profiles, virulence factors and malignant potential. For tens of thousands of years, *H. pylori* has co-evolved with humans and followed human migration patterns, and the study presented in Chapter 7 demonstrates that this phenomenon continues to the present day. An understanding of the origins of the isolates found in a community helps to predict risk of adverse clinical outcomes, including treatment failure as well as peptic ulceration and gastric cancer.

There are known substantial variations in clinical outcomes between patients infected with *H. pylori*, ranging from asymptomatic carriage of the organism, to recurrent peptic ulceration and early death from gastric cancer, yet the mechanisms contributing to this variation have not been well understood. Factors known to relate to these adverse clinical outcomes outlined in Chapter 7 need to be considered by clinicians when providing quality care for patients with *H. pylori* infection. Individualised management plans need to be considered, involving an informed understanding of individual risk factors for adverse clinical outcomes.

Whole genome sequencing is a viable method of improving characterisation of *H. pylori* isolate origin, antimicrobial resistance and virulence factors

The study presented in Chapter 7 demonstrates the highly heterogeneous origins of refractory *H. pylori* encountered in "real world" practice. The patterns demonstrated reflect migration trends, and the highly heterogeneous mix of resistance demonstrated means that individualised approaches are needed, given the substantial variation in isolates encountered in Australian clinical practice. Current Australian guidelines for empiric treatment of refractory infection are not recommended based on the resistance rates demonstrated.

Genetically predicted resistance profiles have high (>92%) concordance with phenotypic resistance for both levofloxacin and clarithromycin, using a two-gene approach. Utilisation of PCR techniques to the antimicrobial carrying region of *H. pylori*, which could be collected from non-invasive means such as stool samples, may be appropriate for screening of patients for antimicrobial resistance. This would enable targeted therapy for patients and avoid treatment with antibiotics that isolates are already resistant to, compounding the formation of antimicrobial resistance amongst other host organisms. The origins of *H. pylori* documented in Chapter 7 reflect migration trends, and it is likely that antimicrobial resistance is in part generated by migration of resistant strains to Australia. This was supported by the findings in Chapter 5, that resistance is clustered in suburbs harbouring increased migrant populations.

Known virulence factors were examined in Chapter 7, demonstrating that the majority of isolates in clinical practice harboured mutations and genotypes that have been associated with an increase in rates of gastric cancer. While available endoscopic histopathology data were limited, approximately 90% of strains with pre-malignant gastric intestinal metaplasia had *cagA* mutations, demonstrating this link. Characterisation and understanding of virulence mutations has the potential to provide clinicians with individualised pathogen-specific risk profiles, which when combined with patient

information, can be used to prognosticate on the risk of long-term adverse outcomes such as gastric cancer.

Our study demonstrates that the current 'one size fits all' approach to eradication therapy is suboptimal for managing patients with *H. pylori*. Individual factors need to be considered in order to tackle the burden of disease and emergence of multi-resistant strains.

Screening for antimicrobial resistance is needed in the context of rising resistance

Empiric first-line and refractory therapy currently recommended in Australia is inadequate for the South and Western Australian cohorts studied. In the cohorts studies in Chapters 3,4,6 and 7, the common theme in all studies was the rising rates of antimicrobial resistance which have now reached high levels. Screening with either invasive testing such as endoscopy, biopsy, culture and determination of antibiotic sensitivities, or with PCR based methods on *H. pylori* from stool samples needs to be considered to guide therapy. Otherwise, empirical use of therapy with higher eradication potential needs to be considered as a first-line option, including bismuth quadruple therapy. However, the availability of medications, which are not covered by the Pharmaceutical Benefits Scheme, needs to be improved to handle the emerging burden of multi-resistant disease. The World Health Organisation (WHO) designating clarithromycin-resistant *H. pylori* a high priority for antibiotic research and development in 2017 and the results of studies presented only highlight further the need for research and development in this area.[7] Improved recognition, detection and monitoring of resistance is needed.

Optimal management of *H. pylori* involves individualised management, based on risk stratification of adverse clinical outcomes

Historically, host factors including a family history of gastric cancer, or other genetic syndromes with increased gastric cancer risk were the main focus of surveillance approaches that aimed to reduce gastric cancer incidence. The evidence presented here highlights the need to individualise management according to risk. Beyond host factors, pathogenic factors of *H.pylori* also need to be considered. A binary approach to *H. pylori* presence or absence if insufficient to prognosticate gastric cancer risk and further characterisation of virulence factors is vital in clinical practice.

The optimal management of *H. pylori* must be individualised. A suggested approach to indivudalsied management is shown in Figure 9.1. Factors that should be considered include:

1. **Patient factors**
    a. Family history of gastric cancer
    b. Smoking
    c. Lifestyle factors – diet, BMI
    d. Endoscopic history, including the presence of intestinal metaplasia, atrophic gastritis
    e. Age of acquisition of *H. pylori*
    f. Risk factors associated with both successful eradication (e.g. GORD, autoimmune disease) and unsuccessful eradication (e.g. peptic ulceration, gastric cancer)

2. **Pathogen factors**
    a. Ancestral origin isolates
    b. Presence of antimicrobial resistance
    c. Virulence factors
    d. Prior antimicrobial exposure

Prognosticating on the risk of *H. pylori* infection is also multifaceted. Multiple considerations need to be made:

1. **Risk of eradication treatment failure**
    a. Prior eradication therapy, place of birth, migration history, family history
2. **Clinical outcomes from chronic infection**
    a. Probability of adverse outcomes: gastric cancer, peptic ulceration, atrophic gastritis, MALT
    b. Probability of beneficial outcomes: immune regulation, reduction in GORD, Barrett's oesophagus and development of oesophageal adenocarcinoma

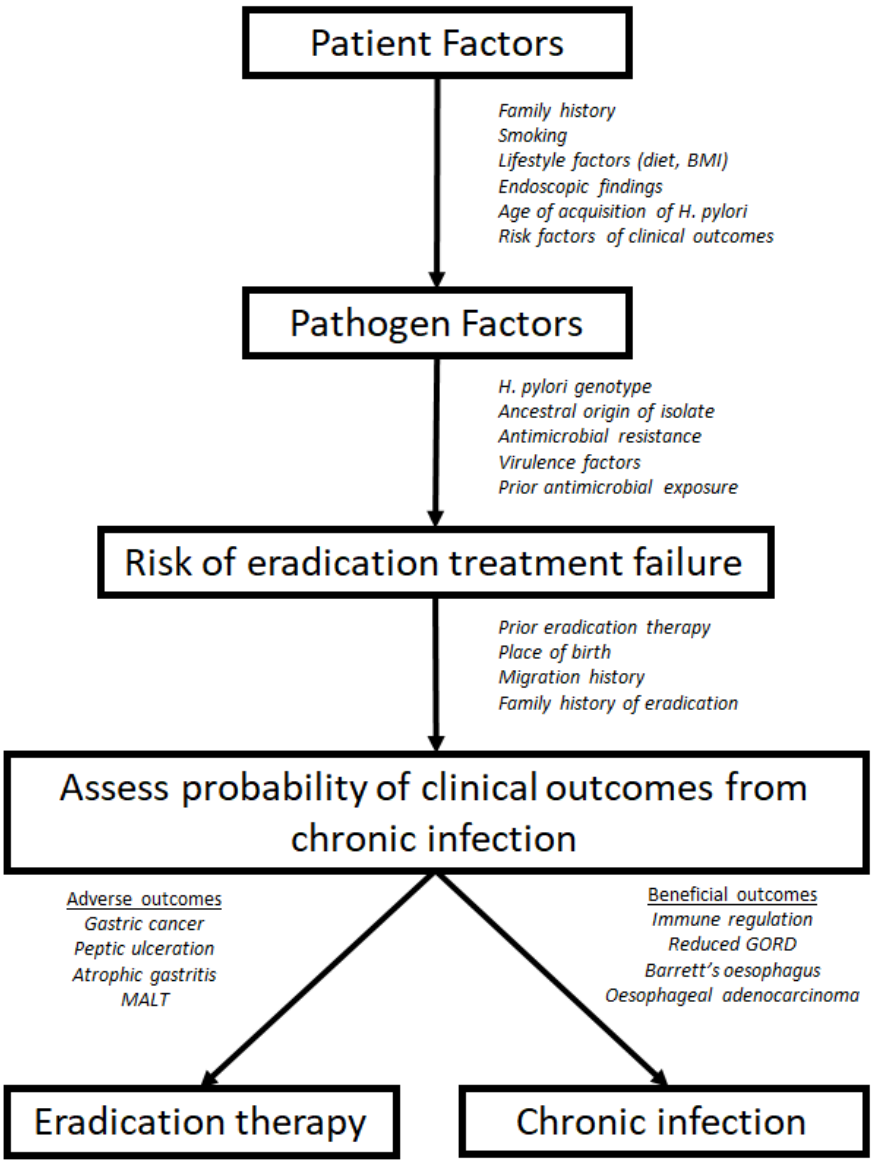


Figure 9.1: Individualised management flowchart of *H. pylori* infection

Optimal management will balance both host and pathogen factors, and eradication should only be pursued in cases where the benefits of eradication are likely to outweigh the risks. This is an individualised decision and requires a holistic clinical approach. The formerly mentioned concept "the only good *H. pylori* is a dead *H. pylori*" is outdated in view of the literature presented, and clinical practice needs to be updated to account for emerging evidence in this field.

Further studies are needed to define clinical endpoints related to host and pathogen factors. Epidemiological studies are currently insufficient, and improved data capture is needed to identify meaningful long-term associations and allow accurate prognostication.

9.2 Implications for clinical practice

Current guidelines for the management of *H. pylori* are outdated. They reflect data from decades ago and the eradication regimen recommended is unlikely to achieve adequate eradication rates (>80%) in the Australian population. Guidelines need to be updated to ensure quality care of patients, including appropriate antimicrobial stewardship to prevent the further development of multi-resistant strains, and to ensure that adequate eradication rates are achieved, especially with empiric therapy.

Both primary and secondary antimicrobial eradication therapy needs to be updated to reflect contemporary patterns of *H. pylori* resistance, as outlined in Chapter 6. In particular, given the heterogeneous distribution of antimicrobial resistance, therapy needs to be individualized to each geographical region, and this should be considered as part of routine clinical practice.

Ongoing monitoring of patterns of *H. pylori* antimicrobial resistance nationally is needed to provide clinicians with updated resistance information on which to base antimicrobial therapies. Establishment of an H. pylori antimicrobial resistance registry is recommended, to enable real-time tracking of resistance profiles and guide eradication therapy.

Update guidelines for empiric therapy

The use of clarithromycin, based on best available evidence, is not appropriate for empiric first line eradication therapy. While amoxicillin resistance remains rare and therefore its use is appropriate for empiric therapy, combination therapy with tetracycline, rifampicin or levofloxacin could be considered for empiric first line therapy. Further, prospective studies are needed to ensure that it will achieve adequate eradication rates locally in the context of rising antimicrobial resistance, especially for levofloxacin. Second line therapy involving levofloxacin should be avoided given the high rates of levofloxacin resistance demonstrated in this body of work.

Bismuth quadruple therapy should be considered as the preferred choice for empiric second line eradication therapy. However, to meet local clinical demands, access needs to be improved via the PBS given that this is a current barrier to treatment. Access to formulations of 'all-in-one' tablets such as Pylera® (containing a combination of bismuth subcitrate potassium, metronidazole, and tetracycline taken 4 times daily for 10 days), available in Europe, and recommended for second line eradication therapy in the recently updated Italian guidelines, would likely address this issue.[8]

Improved ease of access needs to be explored for the Australian and New Zealand markets, as there is likely to be growing demand as rising rates of resistance are better characterised with current eradication therapy likely failing to achieve adequate (>80%) eradication rates.

9.3 Future research directions

Based on the work performed in this thesis, future research directions to improve treatment outcomes for *Helicobacter pylori* infection are presented below.

1. **Establishment of a national registry to track antimicrobial resistance**

The rising rates of antimicrobial resistance identified in the work performed demonstrate a need for monitoring and distribution of updated of antimicrobial resistance in *H. pylori*. Updated resistance profiles will allow for the use of optimal empiric antimicrobial eradication regimens, and over time will reduce the probability of further increasing antibiotic resistance. In addition, updated resistance profiles are also expected to reduce rates of treatment failure and therefore minimise resource utilisation, while improving clinical outcomes. Future research and development would include:

a. Establish a national, centralised database to monitor antimicrobial resistance
b. National, multi-centre pathology engagement, enabling tracking of resistance trends locally and broadly across Australia
c. Regular dissemination (e.g. annually) of antimicrobial resistance trends locally and nationally

2. **Identify risk factors associated with antimicrobial resistance**

Improved characterisation of risk factors associated with treatment failure is needed. Migration from countries with high rates of antimicrobial resistance is likely to be driving rising resistance. Improved screening for antibiotic resistance prior to treatment, and identification of factors associated with failed eradication, is required. Tracking of antimicrobial resistance nationally and internationally is needed in order to provide accurate real time information.

a. Further research into identifying and tracking the genomic basis of antimicrobial resistance.
b. Establishment and integration of phylogenetic origins into a centralised antimicrobial resistance registry to monitor risk and emergence of new strains
c. Monitoring of migration trends associated with imported antimicrobial resistance

3. **Improve genotypic characterisation of antimicrobial resistance**

In order to accurately characterise antimicrobial resistance, improved correlation between phenotypic and genomic resistance profiles are needed. In particular, improved characterisation of mutations associated with amoxicillin, tetracycline and rifampicin resistance is needed, as presently phenotypic resistance can not be accurately predicted from genomic profiles. Improving genotypic

characterisation of antimicrobial resistance profiles may allow for less invasive targeted PCR testing of stool samples, allowing for a less invasive means of screening for resistance prior to eradication therapy. Suggested steps include:

a. Multi-centre collaboration to perform whole genome sequencing on isolates, combined with phenotypic resistance profiles, with linkage analysis to identify genes associated with resistance
b. Due to the rarity of amoxicillin and/or tetracyciline resistant *H. pylori*, a multi-centre and ideally international approach is needed to identify sufficient isolates for this study

4. Develop improved technologies for eradication therapy

In view of rising rates of antimicrobial resistance and limited antimicrobial options, further technological advances in eradication therapy are needed. Improved ease of access to medications is required, e.g. the three-in-one (bismuth, metronidazole, tetracycline) combination bismuth therapy, Pylera®.[9] In the setting of universal eradication, resistance will rise over time, and measures will be needed to develop new therapy to combat this rise. Regarding acid suppression, which Is a core component of *H. pylori* eradication therapy, this may be aided by improved access to potassium-competitive acid blockers (P-CABs) (e.g. vonoprazan), delivering more consistent results and lower rates of treatment failure. Furthermore, improved formulations are likely to make regimens simpler to use and help ensure that lack of compliance is not a cause for treatment failure. Simple regimens consisting of combined medications in a single tablet or capsule seem likely to improve compliance and reduce treatment failure. Further studies of emerging therapeutics are needed to assist with the growing problem of increasing antimicrobial resistance.

Improved technology to detect antimicrobial resistance prior to eradication therapy is also needed. This involves ongoing tracking of resistance trends, which may enable identification of risk factors associated with resistance, including those explored in Chapter 5, and the integration of these into clinical practice. Furthermore, improved technology, such as PCR stool screening for *H. pylori* mutations causing antimicrobial resistance, would enable clinicians to individualise management and potentially avoid multiple courses of inappropriate empiric eradication therapy.[10] This would also likely lead to better antibiotic stewardship with a view to the development of less multi-resistant isolates, including bacteria that colonise the gastrointestinal tract and other systems of the body.

5. **Improve stratification of patients most likely to benefit from eradication therapy**.

The current "one size fits all" approach to *H. pylori* eradication therapy is outdated and needs revision. Patients need risk stratification to ensure that pursuing eradication therapy is worthwhile. While adverse long term clinical outcomes of *H. pylori* infection are well recognised, the benefits of colonisation with this organism are often overlooked. Not all *H. pylori* infections are the same, and clinical outcomes vary enormously. While the literature presented explores important concepts in individualising management, further research is needed to develop refined, individualised treatment approaches.

Studies assessing long term outcomes, controlled for both host and pathogen factors, are needed in order to prognosticate the risk of *H. pylori* infection accurately. Over time, better identification of these factors will likely allow for individualised management. In many cases, the potential harms of *H. pylori* eradication will outweigh the benefits, and the ongoing presence of *H. pylori* as an important organism in the upper GI microbiome is likely to be recognised. With *H. pylori* being present in half of the world's population, large scale eradication is unlikely to be effective and would probably perpetuate the trend for growing resistance, to the point where strains of this organism may become refractory to all available therapies. Further development of individualised *H. pylori* management algorithms are needed to combat the burden of disease. Whole genome sequencing represents a comprehensive modality for assessing pathogen factors, and when combined with linkage analysis and prospective observation cohort studies, risk factor identification and stratification can be improved. Suggested research directions include:

a. Assess long term outcomes from *H. pylori* infection, controlled for both host and pathogen factors to understand and prognosticate risk

b. Development of individualised *H. pylori* management algorithms to combat the burden of disease and identify patients most likely to benefit from eradication therapy

9.4 Conclusion

Revised antimicrobial guidelines and recognition of risk factors associated with resistance are needed to improve the quality of care in patients with *H. pylori* infection.

Updates to recommended therapy based on objective and up-to-date data are needed. Currently recommended eradication regimens are outdated and do not take into account the rising resistance to antimicrobial therapy.

The work performed in this thesis has identified opportunities for improving quality of care for patients with *H. pylori* infection via:

a) Development of a national registry to report of updated antimicrobial resistance trends to guide clinicians in clinical management of *H. pylori* eradication therapy
b) Characterisation of risk factors associated with antimicrobial resistance to reduce the probability of unsuccessful eradication therapy
c) Greater understanding of associations of *H. pylori* infection with both adverse and beneficial clinical outcomes through studies assessing long term outcomes combined with genomic profiles
d) Improved prognostication of patient risk from long term infection, based on both host and isolate factors, to enable risk factor stratification and optimal eradication management
e) Further research into the role of genetically determined profiles and individualised risk factor stratification to develop algorithms for individualised *H. pylori* management

REFERENCES

1. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report. Gut. 2022.
2. Schubert J, Gehlert, J, Rayner, C, Costello, S, Mangoni, AA, Bryant, RV. Antibiotic Resistance of Helicobacter pylori in Australia and New Zealand: A Systematic Review and Meta-analysis. Accepted for publication by the Journal of Gastroenterology and Hepatology. 2020.
3. Pandeya N, Whiteman DC. Prevalence and determinants of Helicobacter pylori sero-positivity in the Australian adult community. Journal of gastroenterology and hepatology. 2011;26(8):1283-9.
4. Mitchell H, Katelaris P. Epidemiology, clinical impacts and current clinical management of Helicobacter pylori infection. Med J Aust. 2016;204(10):376-80.
5. Schubert JP, Gehlert J, Rayner CK, Roberts-Thomson IC, Costello S, Mangoni AA, et al. Antibiotic resistance of Helicobacter pylori in Australia and New Zealand: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2021;36(6):1450-6.
6. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut. 2015;64(9):1353-67.
7. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-82.e17.
8. Romano M, Gravina AG, Eusebi LH, Pellegrino R, Palladino G, Frazzoni L, et al. Management of Helicobacter pylori infection: Guidelines of the Italian Society of Gastroenterology (SIGE) and the Italian Society of Digestive Endoscopy (SIED). Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2022;54(9):1153-61.
9. Nyssen OP, McNicholl AG, Gisbert JP. Meta-analysis of three-in-one single capsule bismuth-containing quadruple therapy for the eradication of Helicobacter pylori. Helicobacter. 2019;24(2):e12570.
10. Beckman E, Saracino I, Fiorini G, Clark C, Slepnev V, Patel D, et al. A Novel Stool PCR Test for Helicobacter pylori May Predict Clarithromycin Resistance and Eradication of Infection at a High Rate. Journal of clinical microbiology. 2017;55(8):2400-5.