Master of Science Thesis

The University of Western Ontario

Liam Brown

January 12, 2021

Contents

Chapter 1

Introduction

Antimicrobial resistance (AMR) is the natural phenomenon by which microorganisms acquire defences against harmful chemicals within their environment. All microorganisms have the potential to acquire AMR under various environments, including terrestrial and aquatic ecosystems, agri-food, hospitals, and the human body. In the context of an infection, resistant microorganisms limit available treatment options. Acquired antimicrobial resistance (AMR) is estimated to have caused 5,400 Canadian fatalities in 2015 — a number which is expected to climb to 13,700 deaths per year by 2050, while disproportionally affecting populations that are at a greater risk of acquiring infections (Council of Canadian Academies, 2019).

AMR is acquired in response to any chemical that imposes a selective pressure on the microorganism. AMR is a huge burden to global healthcare systems, economies, and societies. As Buongermino Pereira et al. (2020) has said...

Chapter 2

Methods

2.0.1 Class 1 integron gene cassettes

2.0.1.1 DNA isolation from soil microplots

Total genomic DNA was isolated from 2019 Day 30 post-application soil microplots using the DNeasy PowerSoil Kit (Qiagen) with an input of 250 mg of dry soil. The DNA was eluted in 100 uL of 10 mM Tris-HCl, and Nanodrop readings were taken to assess quality. The isolated DNA was stored at -20°C until it was used as template for gene cassette PCR.

2.0.1.2 Gene cassette PCR

Class 1 integron gene cassettes were amplified using primers and thermocycling conditions described by Stokes et al. (2001), except with 33 and 34 bp oligonucleotides ligated onto the 5' ends (Figure **XX**). The purpose of these oligonucleotides was to provide more distance between the 5' end of the PCR product (lost during library preparation) and the desired gene cassette sequence. DNA from each soil microplot was used as template for five technical replicates of 25 uL reactions (125 uL total). PCR product was analyzed by gel electrophoresis and visualized under UV light. Technical replicates were pooled together and PCR product was purified using the GenepHlow PCR Cleanup Kit (Geneaid). Cleaned PCR product was quantified using the Qubit dsDNA HS Assay Kit (Fisher Scientific). PCR and cleanup were repeated as described above using the same template DNA for a total of two technical replicates for each soil microplot.

2.0.1.3 Library preparation

The Nextera XT DNA Library Preparation Kit (Illumina) was used to prepare the library for sequencing by following the manufacturer's protocol. The indexed PCR product was quantified using the Qubit dsDNA HS Assay Kit and sized using the High Sensitivity DNA Kit on a Bioanalyzer 2100 (Agilent). Individual libraries were diluted to 10 nM, and 15 uL of each diluted library was pooled into a single library for 2 x 125 bp sequencing at Sick Kid's Hospital (Toronto, ON) on a HiSeq 2500 (Illumina).

2.0.1.4 Data processing

Adapters were trimmed using CUTADAPT (v2.10) and the sequence quality was assessed using FastQC (v0.11.9) and MultiQC (v1.8) (Martin, 2011; Andrews, 2010; Ewels et al., 2016). The adapter-trimmed reads were assembled into contigs using MEGAHIT (v1.2.9) with default options (D. Li et al., 2015). Contigs that didn't contain the terminal 9 bp of each primer sequence were removed using BBDuk with options `copyundefined=t rcomp=f mm=f k=9`. After filtering out contigs that didn't contain the terminal primer sequences, the metagenomic assemblies of each sample were merged into a single assembly, and the open reading frames (**ORFs**) of the remaining contigs were called using Prokka (Bushnell, 2020; Seemann, 2014). The ORFs were concatenated into a non-redundant protein database at 97% identity using CD-HIT (vX.X) and then reverse translated back to nucleotides (W. Li and Godzik, 2006; Fu et al., 2012). To obtain fold-coverages, the adapter-trimmed reads of each sample were mapped back onto the concatenated nucleotide database of

ORFs using BBMap (vX.X) (Bushnell, 2020). ORFs were then annotated using CARD-RGI with options `--low_quality --include_loose --clean -t protein` (vX.X) for ARGs and eggNOG-mapper (v2.0) for functional categories (Alcock et al., 2019; Huerta-Cepas, Forslund, et al., 2017; Huerta-Cepas, Szklarczyk, et al., 2019). CARD annotations with a percentage identity less than the mean percentage identity of all ARG hits, along with hits of less than 20% coverage to the subject ARG sequence were discarded.

2.0.1.5 Compositional data analysis and statistics

The fold-coverages of technical replicates were combined. A non-zero count of 0.1 was added to the resulting feature table prior to centred log-ratio (**CLR**) transformation, although we acknowledge that a non-zero count could lead to x, y, and z errors. The CLR transformation was performed using the base 2 logarithm, thereby producing relative abundances where the difference between two values is the fold-change between them. The Kruskal-Wallis test was used to determine if there were differences between the median relative abundances of class 1 integron gene cassettes ORFs between treatment groups (P <0.05), followed by Dunn's post-hoc test for multiple comparisons (P <0.05) with Bonferroni correction.

Chapter 3

Results

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus,

aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut

metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Chapter 4

Discussion

Lorem ipsum.

Chapter 5

Conclusion

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus, aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Etiam lobortis facilisis sem. Nullam nec mi et neque pharetra sollicitudin. Praesent imperdiet mi nec ante. Donec ullamcorper, felis non sodales commodo, lectus velit ultrices augue, a dignissim nibh lectus placerat pede. Vivamus nunc nunc, molestie ut, ultricies vel, semper in, velit. Ut porttitor. Praesent in sapien. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Duis fringilla tristique neque. Sed interdum libero ut metus. Pellentesque placerat. Nam rutrum augue a leo. Morbi sed elit sit amet ante lobortis sollicitudin. Praesent blandit blandit mauris. Praesent lectus tellus,

aliquet aliquam, luctus a, egestas a, turpis. Mauris lacinia lorem sit amet ipsum. Nunc quis urna dictum turpis accumsan semper.

Bibliography

Alcock, Brian P et al. (Oct. 29, 2019). "CARD 2020: Antibiotic Resistome Surveillance with the Comprehensive Antibiotic Resistance Database." In: *Nucleic Acids Research* 48.D1, pp. D517–D525. ISSN: 0305-1048, 1362-4962. DOI: 10.1093/nar/gkz935. URL: https://academic.oup.com/nar/advance-article/doi/10.1093/nar/gkz935/5608993 (visited on 01/24/2020).

Andrews, Simon (2010). *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Version 0.11.9. Babraham Bioinformatics. URL: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Buongermino Pereira, Mariana et al. (Dec. 2020). "A Comprehensive Survey of Integron-Associated Genes Present in Metagenomes." In: *BMC Genomics* 21.1, p. 495. ISSN: 1471-2164. DOI: 10.1186/s12864-020-06830-5. URL: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-020-06830-5 (visited on 07/27/2020).

Bushnell, Brian (Feb. 19, 2020). *BBMap*. Version 38.79. URL: https://sourceforge.net/projects/bbmap/.

Council of Canadian Academies (2019). *When Antibiotics Fail: The Expert Panel on the Potential Socio-Economic Impacts of Antimicrobial Resistance in Canada*. Ottawa, p. 268.

Ewels, Philip et al. (Oct. 1, 2016). "MultiQC: Summarize Analysis Results for Multiple Tools and Samples in a Single Report." In: *Bioinformatics* 32.19, pp. 3047–3048. ISSN: 1367-4803, 1460-2059. DOI: 10.1093/bioinformatics/btw354. URL: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btw354 (visited on 05/01/2020).

Fu, Limin et al. (Dec. 2012). "CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data." In: *Bioinformatics* 28.23, pp. 3150–3152. ISSN: 1367-4803, 1460-2059. DOI: 10.1093/bioinformatics/bts565. URL: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/bts565 (visited on 12/31/2020).

Huerta-Cepas, Jaime, Kristoffer Forslund, et al. (Aug. 1, 2017). "Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper." In: *Molecular Biology and Evolution* 34.8, pp. 2115–2122. ISSN: 0737-4038, 1537-1719. DOI: 10.1093/molbev/msx148. URL: https://academic.oup.com/mbe/article/34/8/2115/3782716 (visited on 12/31/2020).

Huerta-Cepas, Jaime, Damian Szklarczyk, et al. (Jan. 8, 2019). "eggNOG 5.0: A Hierarchical, Functionally and Phylogenetically Annotated Orthology Resource Based on 5090 Organisms and 2502 Viruses." In: *Nucleic Acids Research* 47.D1, pp. D309–D314. ISSN: 0305-1048, 1362-4962. DOI: 10.1093/nar/gky1085. URL: https://academic.oup.com/nar/article/47/D1/D309/5173662 (visited on 07/29/2020).

Li, Dinghua et al. (May 15, 2015). "MEGAHIT: An Ultra-Fast Single-Node Solution for Large and Complex Metagenomics Assembly via Succinct de Bruijn Graph." In: *Bioinformatics* 31.10, pp. 1674–1676. ISSN: 1460-2059, 1367-4803. DOI: 10.1093/bioinformatics/btv033. URL: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btv033 (visited on 05/02/2020).

Li, W. and A. Godzik (July 1, 2006). "CD-HIT: A Fast Program for Clustering and Comparing Large Sets of Protein or Nucleotide Sequences." In: *Bioinformatics* 22.13, pp. 1658–1659. ISSN: 1367-4803, 1460-2059. DOI: 10.1093/bioinformatics/btl158. URL: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btl158 (visited on 01/12/2021).

Martin, Marcel (2011). "Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads." In: *EMBnet. journal* 17, pp. 10–12.

Seemann, T. (July 15, 2014). "Prokka: Rapid Prokaryotic Genome Annotation." In: *Bioinformatics* 30.14, pp. 2068–2069. ISSN: 1367-4803, 1460-2059. DOI: 10.1093/bioinformatics/btu153. URL: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btu153 (visited on 12/31/2020).

Stokes, H. W. et al. (Nov. 1, 2001). "Gene Cassette PCR: Sequence-Independent Recovery of Entire Genes from Environmental DNA." In: *Applied and Environmental Microbiology* 67.11, pp. 5240–5246. ISSN: 0099-2240. DOI: 10.1128/AEM.67.11.5240-5246.2001. URL: http://aem.asm.org/cgi/doi/10.1128/AEM.67.11.5240-5246.2001 (visited on 07/03/2019).