

Detection and Characterisation of Bacterial Antimicrobial Resistance Genes in Mothers and their Offspring



Aseel Al-Araji^{1,2}, Fiona Collier², Anthony Chamings², Mimi Tang^{4,5,6}, Anne-Louise Ponsonby^{5,7}, Peter Vuillermin^{1,2,3,4}, Soren Alexandersen², Eugene Athan^{1,2,3} & the Barwon Infant Study Investigator Group

¹Centre for Innovation in Infectious Diseases and Immunology Research, Geelong, VIC 3220, Australia; ²Deakin University, School of Medicine, Geelong, VIC 3220, Australia; ³Barwon Health, University Hospital Geelong, Geelong, VIC 3220 Australia; ⁴The Murdoch Children's Research Institute, Parkville, Victoria, 3052, Australia; ⁵The University of Melbourne, Parkville, Victoria, 3052, Australia; ⁶The Royal Children's Hospital, Parkville, Victoria, 3052, Australia; ⁷The Florey Institute for Neuroscience and Mental Health, Parkville Victoria, 3052, Australia * Presenting Author: alaraji@deakin.edu.au

INTRODUCTION

- Antimicrobial resistance genes (AMR) confer microorganisms the ability to resist antimicrobial agents, which can result in frequent and previously curable infections becoming lethal (WHO, 2018).
- The gut microbial community is developed throughout infancy up until adulthood (Ferretti et al., 2018).
- Moore (2013) explains that if antibiotics are overprescribed in the first year of life, the subsequent disruption to the infant's gut and increase in AMR genes.
- Transmission of bacteria occurs from mother to child primarily during birth, and stabilization of the child's gut microbiome occurs in the first years of life (Asnicar et al., 2017).

This study aims to investigate the prevalence of AMR genes in mothers and their offspring at one year of age and to estimate the incidence of transfer of these genes and shared bacteria.

METHODS

Fecal samples from mothers (n=10) and their respective one-year old children's samples were obtained from the Barwon Infant Study (BIS), University Hospital Geelong, Australia in conjunction with data on the mode of delivery, antibiotic usage during pregnancy (mothers) and in the first year of life (children). Ethics approval was obtained from Barwon Health Human Research Ethics Committee (10/24). DNA was extracted (using the Power soil DNA Isolation Kit) and sequenced using next generation sequencing (NGS) (Ion Torrent S5x1 System (Thermo Fisher Scientific) and analysed by matching AMR sequences against multiple resistance gene databases: In-house (using AMR local database created from NCBI Gene Bank (Bioproject PRJNA313047) in July 2019), ResFinder, KmerResistance (Both of these web tools rely on the same reference database), AMRFinder plus (using database downloaded in October 2019) and CLC Genomics Workbench. Specific AMR genes were identified and the abundance per million nucleotides estimated (AMR gene coverage and depth metrics were calculated using the Torrent Suite Coverage Analysis v5.6.0.1 plugin).

RESULTS

- Overall, there was resistance to up to 10 antibiotic classes (with 50 resistance genes in total). AMR genes were more abundant in the samples from mothers than in the samples from children. The most abundant AMR genes often conferred resistance to the three most prescribed antibiotics in Australia: tetracycline; Beta-lactam; and macrolide, lincosamide, and streptogramin B (MLSB) (Figure 1).
- The In-house method, two web-based methods (KmerResistance and ResFinder), and AMRFinder plus gave relatively comparable results with variations due to different reference databases. The CLC method found extra AMR genes (i.e., multidrug efflux pumps) due to its more extensive AMR genes databases (Table 1).
- Transfer of nine AMR genes from mother to child was identified in five mother-child paired samples irrespective of antibiotic exposure or mode of delivery. In the antibiotic-treated VD group, transfer of AMR genes was detected in one mother-child pair (with one gene only), while in the antibiotics-treated CS group, AMR genes were transferred in two pairs (with three genes). In contrast, in the non-antibiotic treated VD group, there was a transfer of a total of five genes in two mother-child pairs (Figure 2-A). The most abundant and widespread AMR genes are those responsible for Beta-lactam and trimethoprim resistance. (Figure 2-B).

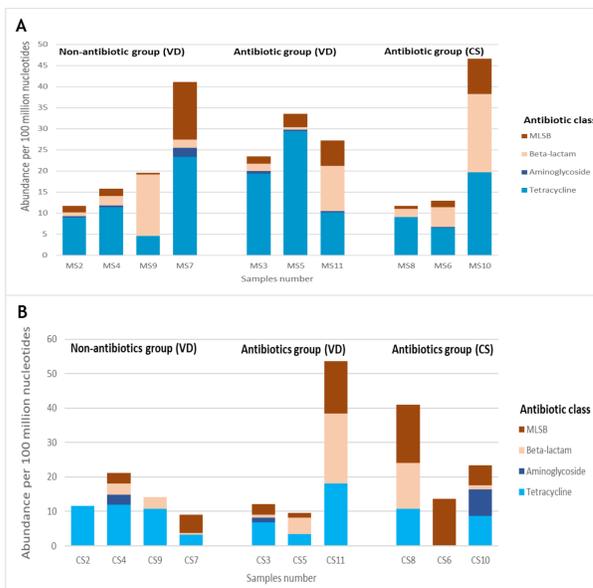


Figure 1: Summary of the abundance of AMR genes conferring resistance to the major antibiotic classes in the mothers' (A) and the children's samples (B). CS: caesarean section, VD: vaginal delivery.

	Non-antibiotic group (VD)				Antibiotic group (VD)			Antibiotic group (CS)		
Barcode number (mother)	MS2	MS4	MS9	MS7	MS3	MS5	MS11	MS8	MS6	MS10
AMR genes	15	15	12	7	19	22	15	8	11	6
Antibiotics classes	8	6	4	3	7	10	5	4	6	4
Efflux pump resistance genes	0	2	1	1	2	11	3	12	43	0

	Non-antibiotic group (VD)				Antibiotic group (VD)			Antibiotic group (CS)		
Barcode number (Child)	CS2	CS4	CS9	CS7	CS3	CS5	CS11	CS8	CS6	CS10
AMR genes	5	9	3	4	4	7	7	8	2	11
Antibiotics classes	2	7	3	3	4	5	3	5	2	6
Efflux pump resistance genes	1	0	0	0	0	34	5	3	0	0

Table 1: Number of AMR genes, antibiotic classes, and efflux pump resistance genes identified in the mothers' and children's samples. The data for the mothers is positioned directly above their children. CS: caesarean section, VD: vaginal delivery.

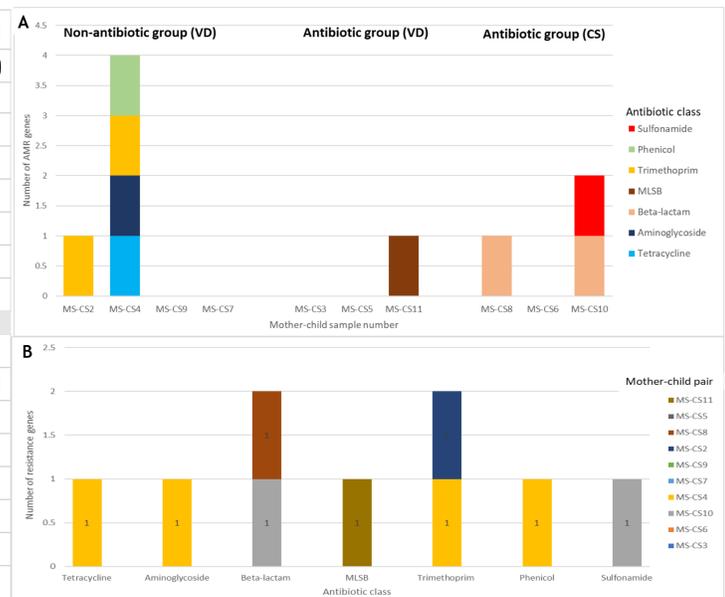


Figure 2: (A) Overview of the antibiotic resistance classes of identical AMR genes found in mother-child pairs (B) The main antibiotic classes detected in the mother-child pairs. CS: caesarean section, VD: vaginal delivery.

CONCLUSIONS

This study provides results that show the ubiquity of bacterial AMR genes in fecal samples from mothers and their one-year-old offspring. A higher diversity of AMR genes was identified in mothers when compared to their respective children's samples. The study illustrates the importance of using several analysis methods to provide a comprehensive picture of the AMR genes in children and their mothers and to utilize this information to develop a long-term plan to test for and analyse AMR gene data. CLC Genomics workbench was a time efficient analysis method with an added advantage of being able to detect more efflux pump genes than the other methods. Substantial carriage of AMR genes were found in pregnant mothers and evidence of transfer of AMR genes between mothers and infants. This work has shown that AMR genes are present from early life in infants, and further work is ongoing to understand the transfer of genes between mothers and babies and the impact of antibiotic use on the development of resistance early in life. This would include investigating infants born by vaginal delivery and either exposed to antibiotics or not in the first year, and more detailed investigation of specific antibiotic usage.

ACKNOWLEDGEMENTS



References

- WHO – UN, 2018, 'Monitoring global progress on addressing antimicrobial resistance', Geneva < <http://apps.who.int/iris/bitstream/handle/10665/273128/9789241514422-eng.pdf?ua=1> >
- FERRETTI, P., PASOLI, E., TETT, A., ASNICAR, F., GORFER, V., FEDI, S., ARMANINI, F., TRUONG, D. T., MANARA, S., ZOLFO, M., BEGHINI, F., BERTORELLI, R., DE SANCTIS, V., BARILETTI, I., CANTO, R., CLEMENTI, R., COLOGNA, M., CRIFO, T., CUSUMANO, G., GOTTARDI, S., INNAMORATI, C., MASÉ, C., POSTAI, D., SAVOI, D., DURANTI, S., LUGLI, G. A., MANCABELLI, L., TURRONI, F., FERRARIO, C., MILANI, C., MANGIFESTA, M., ANZALONE, R., VIAPPANI, A., YASSOUR, M., VLAMAKIS, H., XAVIER, R., COLLADO, C. M., KOREN, O., TATEO, S., SOFFIATI, M., PEDROTTI, A., VENTURA, M., HUTTENHOWER, C., BORK, P. & SEGATA, N. 2018. Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. *Cell Host & Microbe*, 24, 133-145.e5.
- ASNICAR, F., MANARA, S., ZOLFO, M., TRUONG, D. T., SCHOLZ, M., ARMANINI, F., FERRETTI, P., GORFER, V., PEDROTTI, A., TETT, A. & SEGATA, N. 2017. Studying Vertical Microbiome Transmission from Mothers to Infants by Strain-Level Metagenomic Profiling. *mSystems*, 2.
- MOORE, A. M., AHMADI, S., PATEL, S., GIBSON, M. K., WANG, B. & NDAO, M. I. 2015. Gut resistome development in healthy twin pairs in the first year of life. *Microbiome*, 3.