ASSESSING THE EVOLUTIONARY POTENTIAL OF NOVEL RESISTANCE ELEMENTS TO THE CANDIDATE ANTIBACTERIAL, NICLOSAMIDE

Hannah Lee-Harwood, Victoria University of Wellington
hannah.leeharwood@vuw.ac.nz
Dr Alistair Brown, Victoria University of Wellington
Dr Michelle Rich, Victoria University of Wellington
Prof David Ackerley, Victoria University of Wellington

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Antibacterial resistance is predicted to contribute to 10 million deaths annually by 2050. The FDA-approved, anthelmintic drug, niclosamide, has been shown to exert potent antibacterial effects in gram-positive pathogens and is currently in Phase II clinical trials for treating methicillin-resistant *Staphylococcus aureus*. Importantly, it has been reported that gram-positive bacteria do not develop detectable levels of spontaneous resistance for clinical usage. To assess possible resistance mechanisms more deeply, we have taken a functional metagenomics approach to screen small-insert soil libraries for primordial niclosamide resistance elements. A bespoke library generation method was developed that facilitates the precision-cloning of captured genes immediately downstream of a strong *E. coli* promoter, ribosome binding sequence and hexahistidine tag. Niclosamide selection recovered 88 unique resistance elements that varied in the strength of niclosamide resistance conferred. Forty-four resistance elements were annotated as flavoenzymes and were found to detoxify niclosamide by nitroreduction. A further ten resistance genes were annotated as methyltransferases, and a final resistance element, an alpha-beta hydrolase, was found to degrade niclosamide. To assess the evolutionary potential of these resistance elements, parallel directed evolution campaigns using error-prone PCR were conducted for increased niclosamide detoxification. Selected flavoenzymes were demonstrated to be easily evolvable, requiring only one to two mutations to develop a clinically relevant resistance phenotype. In contrast, the alpha-beta hydrolase was also shown to be evolvable but required the accrual of up to eight amino acid substitutions. No improved methyltransferases or putative drug-sequestering proteins were identified, suggesting that while these other mechanisms might confer low levels of resistance, their evolutionary potential to become mature resistance elements is low. The collateral resistance and sensitivity profiles of improved enzymes were shown to be altered, suggesting potential strategies for niclosamide stewardship in the clinic. This work presents a novel strategy for the surveillance of antibiotic resistance-associated enzymes.