

Evidence for dysfunctional antibiotic resistance genes in commensal populations of *Escherichia coli*

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Abstract. Bacterial genes found on chromosomes and plasmids undergo random, but infrequent mutations during DNA replication. In environments where a gene product is needed, these mutations are quickly purged from the population. Conversely, in the absence of selection we should see accumulation of “dysfunctional” genes at the population level. The goal of this project was to determine if mutations accumulate in antibiotic resistance genes that are harbored by *E. coli* and *Salmonella enterica*. We predict that non-synonymous mutations will accumulate in populations of “commensal” *E. coli* whereas the prevalence of these mutations will be much lower in pathogenic bacteria. These latter populations are expected to experience more intensive antibiotic selection pressure. As a preliminary study we examined the correlation between antibiotic resistance genotypes and phenotypes for 52 strains of commensal *E. coli* from cattle. Antibiotic resistance phenotypes were determined by Kirby-Bauer disc diffusion tests. We deployed a newly developed antibiotic resistance gene microarray to determine the antibiotic resistance genotype of the bacteria. The process involved extracting genomic DNA, nick translating this material in the presence of biotinylated nucleotides, and hybridizing the labeled DNA to the microarray where enzymatic amplification was used to detect hybridized products. Out of 52 strains tested, 9 showed evidence of dysfunctional antibiotic genes whereby there was clear microarray evidence for the presence of antibiotic resistance genes, but the strains were susceptible to the corresponding antibiotics. Interestingly, we also encountered 7 strains that were antibiotic resistant, but negative for recognized antibiotic resistance genes included on the microarray. PCR confirmations are underway for the former group and sequencing will be used to determine if missing phenotypes are due to non-synonymous mutations in the coding regions of these genes. This study will be expanded to determine if pathogenic strains of *E. coli* and *Salmonella enterica* have a lower prevalence of putatively dysfunctional genes.