Writing QE

Rationale:

Why are we and what will we get?

Hypothesis:

Experiment:

Expected Results:

Anticipated challenges and alternative methods:

OVERALL HYPOTHESIS

There is a bloom of uropathogenic *E. coli* in the gut that coincides with the onset of a UTI.

1. Compare the community structure of the gut microbiota during UTI and after recovery.
   1. Rationale
      1. Gut, skin, and vaginal microbiome is generally stable generally stable through time {Consortium:2012iz, Schloissnig:2012hx}.
      2. Changes in the gut microbiota have been observed in pregnant women{Koren:2012ji}, a population of women who are prone to UTIs{Law:2012jr}.
      3. Changes in the periurethral prevalence of *E. coli* indicate that changes in human microbiota accompany the onset of UTI.
   2. Hypothesis
      1. There is a greater representation of *E. coli* in the gut microbiota of a patient at the onset of a UTI than at times when the patient is healthy.
   3. Experimental methods
      1. Genomic DNA from the fecal samples collected from cohort will be extracted following the protocols of Methe *et al.* (2012). These extracts will be used as template in the amplification of the V3-V5 regions of the 16S rRNA gene using 454 pyrosequencing. Sequence data from this reaction will be then used to estimate the community structure of the gut microbiota. This method has been used previously to analyze the human microbiomes of several body habitats{Consortium:2012iz}.
      2. Binning of the amplicons into phylotypes using the QIIME software package will allow for the representation of those phylotypes in the gut microbiota to be measured{Caporaso:2010bi}. The stringency of the binning can be set to cluster 16S sequences into families, such as Enterobacteriaceae, of which *E. coli* is a member.
      3. Statistical analysis will include application of the Shannon diversity index to identify intra- and inter-host differences in community structure, Mann-Whitney non-parametric test of means to identify changes in the representation of Enterobacteriaceae during the UTI, and prinicipal component analysis to measure the tendency of the samples to cluster to their treatment groups (before treatment, after treatment, and after recovery).
      4. Antibiotic treatment has been shown to dramatically effect the composition of the gut microbiota, and some genera may take months to reappear {Trinidad:2012ja, Ubeda:2010kw, Hill:2010he, Croswell:2009hc, Sekirov:2008ec, Dethlefsen:2008kn}. This will reduce the overall species richness of the gut microbiota, and may artificially reduce the divergence between populations calculated by the Shannon diversity index and PCA. As an additional control against this error, data on the gut community structure of healthy adults available from the Human Microbiome Project will be included in these analyses in order to identify differences between healthy microbiomes and microbiomes that may be altered after antibiotic treatment. The addition of the HMP data as a control has been used successfully in other investigations into changes in the gut community structure{Koren:2012ji}.
      5. These methods will enable discrimination between the sample groups (during UTI, after antibiotic treatment, and after recovery) and include data obtained from analysis of gut microbiomes from healthy adults. The statistical analyses will allow measure the abundances
   4. Anticipated challenges:
      1. Recurrence of a UTI episode within 6-12 months after an initial UTI caused by UPEC occurs in approximately 25-30% of cases. The chance of recurrence is greatest within the first 3 months following the initial UTI, and is often caused by the same UPEC strain as the one that caused the initial UTI. The chance of another UTI episode caused by the same strain drops after 6 months. This indicates that there may be a short-lived reservoir of UPEC in the patient that survives antibiotic treatment and immune system clearance. This reservoir may be located in the gut, which would complicate analysis of the gut carriage of UPEC during an acute UTI episode. As a result, any patients who self-reported another UTI episode within the 6 months following entry into the cohort will be excluded from the final analyses.
      2. It is possible that there are members of the Enterobacteriaceae that could be have increased representation in the gut at the onset of a UTI episode due to host factors, such as immunodeficiency. If there are large discrepancies between the estimated abundance of Enterobacteriaceae and *E. coli* specific markers identified in sub-aim2, then the relative representation of *E. coli* in the gut microbiome will be re-estimated using qPCR targeting *E. coli* specific housekeeping genes and comparing those results to a control region conserved in the family Enterobacteriaceae. This will identify the proportion of *E. coli* in the fecal sample relative to the abudance of the Enterobacteriaceae family
2. Compare relative abundance of urovirulence genes in the gut microbiome during UTI, after treatment, and after recovery.
   1. Rationale
      1. While studies have identified urovirulence genes that often co-occur (CITE NEEDED), no single set of urovirulence genes necessary and sufficient for pathogenesis have been defined{Picard:1999uk}. As a result, many urovirulence genes must be assayed to measure the prevalence and abundance of UPEC.
      2. Analysis of the *E. coli* population structure in the gut has shown that dominance in the gut is highly correlated with the presence of urovirulence genes during a UTI episode{Moreno:2008eg, Moreno:2006ji}; however, the relative representation of urovirulence genes in the gut microbiome during UTI and in a healthy state within the same patient has yet to be compared.
   2. Hypothesis
      1. There is a greater relative abundance of known urovirulence genes in the gut microbiome of a patient at the onset of a UTI than when the person is healthy.
   3. Experimental methods
      1. Genomic DNA extracted in sub-aim 1 will be used as template for quantitative polymerase chain reactions (qPCRs) targeting known urovirulence genes from gene clusters listed in Table 1. Additional qPCRs will be performed that target a conserved region of the 16S rRNA gene common to all bacteria as well as a region of the single copy housekeeping gene, *rpoB*, which is divergent in *E. coli*.
      2. Comparison of the qPCR results from the urovirulence genes to the qPCR results from the 16S rRNA and *rpoB* genes can be used to estimate the relative abundance of the urovirulence genes to the total gut microbiota population and the *E. coli* sub-population, respectively. Mann-Whitney non-parametric t-tests will be used to identify statistically significant differences between sample groups in uroviruelnce gene abundance relative to the total microbiota and to *E. coli* specifically.

What the hell am I writing? Below this line

There is a chance that there is an increase in the representation of UPEC strains in the gut microbiota that are not the same as the strain causing the UTI. This could possibly occur through environmental and behavioral shifts, such as changes in frequency and type of sexual intercourse, which has been found to affect the risk ratio for developing a UTI