1. What is uropathogenic E. coli
   1. UPEC causes disease and is an excellent model organism for the study of opportunistic pathogens
   2. What is a UTI and how is it defined?
      1. Symptoms of UTI?
      2. Incidence of UTI
      3. What is recurrent UTI?
      4. What bacteria are able to invade bladders and what bacteria cause disease
      5. Asymptomatic bacteria are also capable of existing in the bladder
   3. How is UPEC cleared from the host
      1. Natural clearance
      2. Antibiotic therapy
2. What is the population structure of UPEC
   1. Population structure of E. coli and UPEC
      1. Describe four major clades of E. coli
         1. How are the four clades separated?
         2. Sub-categorization of intra-clade diversity is capable
            1. At least 9 groups of strains with clade B2{LeGall:2007bq}
      2. Where are E. coli found? Where are UPEC found?
         1. Majority of urine isolates of E. coli (69%) are from clade B2 {Zhang:2002wo}
      3. What is the function of E. coli and UPEC in their habitats?
         1. Act as commensals. Eat stuff in the gut.
   2. Transmission of UPEC
      1. Fecal-oral route of transmission
      2. Sex-partner
      3. Recurrence occurs by the same strain approximately 50% of the time (LOOK UP CITATION)
      4. Perineum washing with antimicrobials did not prevent recurrence, indicating that another mechanism aside from anal to urogenital transmission is occurring{Cass:1985uc}
   3. Within host population structure of UPEC
      1. During UTI population structure in the bladder
         1. Figure
         2. IBCs
            1. IBCs are important for avoiding host defenses{Anderson:2003kb}
            2. IBCs are clonal{Schwartz:2011cy}
         3. QIRs
         4. Chronic
      2. Gut UPEC
         1. Strains containing urovirulence factors exist in the gut{Wold:1992tg}
         2. Few strains dominate in the gut during UTI {Moreno:2006ji, Moreno:2008eg}
         3. Other papers
3. Distribution of virulence factors in UPEC
   1. Introduction of pan-genome
      1. A number of UPEC strains have been sequenced
         1. 536 {Brzuszkiewicz:2006cu}
         2. CFT0079{Welch:2002bj}
         3. UTI89{Chen:2006wz}
      2. E. coli differ markedly in their accessory genome
         1. Comparison of whole genomes shows differences between UPEC strains{Brzuszkiewicz:2006cu}
   2. What virulence factors exist in UPEC and their abundance
      1. Move through a list of factors
      2. Abundance of virulence factors
         1. Indicates that there are many ways to be pathogenic in E. coli
      3. Virulence factors may be clade specific.
         1. Virulence factors specific to pathogenic isolates are common in isolates from clades B2 and D and rare in other clades, indicating that they are ancestral to those clades (B2 and D){Boyd:1998ub}.
         2. Analysis of virulence factors has shown that many virulence factors co-occur and display low levels of intra-group diversity, indicating that a structured and frequent horizontal gene transfer of virulence genes.{Johnson:2001cl}
         3. Genomic hybridization shows a correlation between the presence and absence of specific genomic content and the phylogenetic history of the core-genome of B2 isolates, indicating the co-evolution of the accessory and core genomes{LeGall:2007bq} through a process of “fine-tuning” {EscobarParamo:2004to}
4. Swords or plowshares?
   1. A greater number of virulence factors are present in “persistent” recurrent UTI strains compared to strains from secondary invasions{Luo:2012bm}.
   2. In healthy women, dominant E. coli clones had higher urovirulence scores than non-dominant clones{Moreno:2009jc}
   3. Deletion of PAIs in CFT0073 reduces rate of intestinal colonization{Diard:2010fr}
   4. Presence of PAIs in CFT0073 is linked to reduced growth rate in urine{Diard:2010fr}
   5. Common extra-intestinal virulence genes have been found to affect the fitness of strains within the gut environment{LeGall:2007bq}

1. Questions that remain in the host
   1. Why don’t more people have UTIs?
   2. What factors shape the abundance and distribution of virulence factors?
   3. Previous research into urovirulence genes in the bladder have focused mainly on the presence or absence of the genes, rather than sequencing particular alleles.
   4. Mounting evidence suggests that there are multiple ways to skin a cat, so single gene investigations may not capture a complete picture of UPEC pathogenicity.
2. What studies of these questions will reveal
   1. Treatment plan – if you know that gut E. coli are part of the problem, then it must be addressed.
   2. Enable better understanding of how E. coli inhabit multiple environments
   3. Better understanding of how E. coli have evolved, and why they are pathogenic in the first place.
      1. Accidental pathogen model (by-product hypothesis)
      2. Opportunistic pathogen model
      3. Source-sink model
3. Experimental plan

Hypothesis: Identify virulence genes that play a part in

Sub-aim 1: Assess population structure of UPEC in the host – determine population complexity using MLST – describe strain richness

Sub-aim 2: Sequence representative genomes of MLST subtypes – allow for description of synteny and provide information on the genomic organization of strains

Sub-aim 3: Identify abundance and allelic distribution of virulence factors in patients suffering recurrent UTI – describe strain evenness

Questions I am trying to answer:

1. Are recurrent UTIs caused by the same strain (i.e., the same gene network?)
2. Wholesale shifts occur in the gut and the bladder at the same time. A minor member rising to prominence, or is it a factor of a secondary invasion?
   1. Alternatively, look to see if there is a concordance in change in virulence genes. Are they linked, or are they changing independently.
3. Is there a core UPEC genome? How different is the core UPEC genome compared to the core genome of E. coli?
   1. How variable is the UPEC accessory genome?
   2. Do virulence genes co-occur?
4. What is the competitive advantage of virulence genes? Do they offer fitness advantage in the gut?
   1. In patients with a secondary invasion of UPEC, could do subtraction between the genomes to identify regions that are different and ask if they are these virulence genes are responsible for the competitive advantage between isolates.

Interesting Side notes:

* 1. How many colicin producing strains are there in the gut?

Models of virulence evolution