1. What is uropathogenic E. coli
   1. UPEC causes disease and is an excellent model organism for the study of opportunistic pathogens{Dobrindt:2010fe}.
   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. Population Structure of UPEC
   1. Description of Phenotypes of pathogenic E. coli.
   2. UPEC are predominantly found in clade B2
      1. ExPEC fall predominantly into clade B2, to a lesser extent into clade D, and are absent from other clades{EscobarParamo:2004to}
      2. Sub-categorization of intra-clade diversity is capable
         1. At least 9 groups of strains with clade B2{LeGall:2007bq}
   3. Clade B2 is overrepresented in *E. coli* strains isolated from gut and bladder
      1. Majority of urine isolates of E. coli are from clade B2 {Zhang:2002wo, Moreno:2008eg, Moreno:2009jc}
      2. Describe persistent and transient strains of E. coli
      3. Interestingly, clade B2 has been shown to persist at a greater rate in the gut environment of human infants{Nowrouzian:2005uu}.
      4. Act as commensals. Eat stuff in the gut
   4. UTIs are correlated with reduced strain richness in the gut and bladder
      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
            1. Found in the lamina propria of the bladder and may seed future recurrence{Mysorekar:2006ja}
         4. Chronic
      2. Gut UPEC during UTI
         1. During acute UTI, strains isolated from the urine are found to be the dominate strain in the rectal and fecal populations of *E. coli* {Gruneberg:1969wo, Yamamoto:1997wk}.
         2. Strains from clade B2 are associated with increased virulence
   5. Recurrence of UPEC
      1. Recurrence occurs by the same strain approximately 50% of the time (LOOK UP CITATION)
      2. Perineum washing with antimicrobials did not prevent recurrence, indicating that another mechanism aside from anal to urogenital transmission is occurring{Cass:1985uc}
3. 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. Virulence factors, phylogeny, and phenotype.
      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. Increased number of virulence factors is correlated with increased levels of extra-intestinal pathogenesis{Picard:1999uk}.
      5. The context of virulence factors is important in determining pathogenicity. The mere presence or absence of a virulence factor is not enough to predict pathogenicity{Picard:1999uk} and the genetic context of the virulence factors is important in determining pathogenicity within a strain{EscobarParamo:2004up, Nowrouzian:2005uu}. In many cases, non-pathogenic and pathogenic bacteria contain similar sets of virulence factors, as can be seen in commensal probiotic strain *E. coli* Nissle 1917 and the uropathogenic bacterium *E. coli* CFT073 {Grozdanov:2004bd}, however, despite their similar genomic architecture, these strains have widely different pathogenic potential.
   3. 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}. In addition, persistent strains were more likely than transient strains to present uropathogenic phenotypes{Wold:1992tg}.
      3. Persistent strains in the gut environment were statistically more likely to belong to the uropathogenic subgroup of clade B2, indicating a potential link between fitness in the gut and pathogenicity in the bladder{Nowrouzian:2006bu}. Additionally, in patients with UTI, strains with greater numbers of urovirulence factors were associated with reduced species richness in the gut habitat and were more likely to belong to clade B2 {Moreno:2008eg}. This suggests that urovirulence factors may facilitate the clonal expansion of UPEC strains in the gut at the cost of competing *E. coli* strains, which results in a reduction of species diversity.
      4. Deletion of PAIs in CFT0073 reduces rate of intestinal colonization{Diard:2010fr}
      5. Presence of PAIs in CFT0073 is linked to reduced growth rate in urine{Diard:2010fr}
      6. Common extra-intestinal virulence genes have been found to affect the fitness of strains within the gut environment{LeGall:2007bq}
      7. Virulence factors, such as hemolysin, type I fimbriae, and P fimbriae, are associated with persistence of E. coli in the gut{Nowrouzian:2003bs}.
4. Questions that remain in the host
   1. Why don’t more people have recurrent UTIs?
   2. 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{Picard:1999uk}
   3. What factors shape the abundance and distribution of virulence factors?
   4. Previous research into urovirulence genes in the gut have focused mainly on the presence or absence of the genes, rather than identification particular alleles.
   5. Something in addition to known virulence genes is enabling clade B2 strains to persist in the gut better than strains from other clades{Nowrouzian:2005uu}.
5. 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, what it means to succeed in the gut and how to make sure that success isn’t abused.
   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
6. 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