WHAT MAKES AN ORGANISM PATHOGENIC?

**Uropathogenic Escherichia coli – a facultative pathogen and model system**

Approximately 50% of women will suffer a urinary tract infection (UTI) at some point in their life, and 20-30% of these women will suffer a recurrent UTI within three to four months. The rates of UTI increase in the immunosuppressed, including the elderly and children, and may result in significant complications, including renal scarring, septicemia, and pyelonephritis.

Nearly 80% of community acquired UTIs are caused by uropathogenic *E. coli* (UPEC), a Gram-negative gammaproteobacteria. UTIs caused by UPEC are responsible for 150 million cases of uncomplicated cystitis each year [JOHNSON AND RUSSO 2003]. Despite the rate of UPEC morbidity, UPEC is considered a facultative pathogen, as are many other types of *E. coli*{LeGall:2007bq, Denamur:2010ug}. Facultative pathogens, such as the deadly O157:H7 strain of *E. coli*, live commensally in one habitat, such as cattle gastrointestinal tracts, but are capable of causing disease in alternative habitats, such as the human gastrointestinal tract. This pattern differs from obligate pathogens, such as the *Shigella* species, which are unable to exist in a host without causing disease{Denamur:2010ug}.

In addition to being clinically important, UPEC is also an excellent model system to study virulence in facultative pathogens. . UPEC offer a number of unique advantages as a model system, including the range of laboratory tools available specific to *E. coli*, the tractability of genetic modification, and the wealth of genomic data available for the pathogen. UPEC have been used to study biofilm formation, pili expression, epithelial cell invasion, toxin production, and population bottlenecks, in addition to its obvious use as a model for uropathogenicity. The evolution of virulence in this facultative pathogen has also been studied, which has resulted in a number of competing theories, which will be discussed below. Finally, although there has been attention paid to the global phylogenetic structure of UPEC, relatively few investigations have sought to describe the within-host distribution of the UPEC populations or elucidate the changes in population structure that occur within patients with recurrent UTIs. However, new technologies and bioinformatic tools now enable high-resolution descriptions of bacterial population structures using genomic analyses, enabling research into these unexplored areas. These analyses into population structure will facilitate a better understanding of how virulence has evolved in *E. coli* by describing the selection pressures faced by UPEC in their host habitats.

**The population structure of UPEC – a tale of two homes**

*Classification of Escherichia coli isolates*

*Escherichia coli* are associated with a number clinical conditions, each caused by different strains of *E. coli* harboring different repertoires of gene sets and virulence factors, and, as such, have been categorized according to their pathology and genomic content. *E. coli* that cause disease in the gastrointestinal tract are grouped together into a supergroup labeled intestinal pathogenic *E. coli* (IPEC){Nataro:1998uo, DarfeuilleMichaud:2002dx, Kaper:2004bm: Kohler:2011cn}. A separate group consists of extra-intestinal pathogenic *E. coli* (ExPEC) and includes strains of uropathogenic *E. coli* (UPEC) capable of causing urinary tract infections (UTIs) {Russo:2000vr}. In addition to phenotype, *E. coli* can also be categorized according to their phylogenetic history. Currently, four main clades of *E. coli* have been described, A, B1, B2, and D, along with two smaller clades, C and E{EscobarParamo:2004to, EscobarParamo:2004up, Touchon:2009kw}. ExPEC fall predominately into clade B2, and to a lesser extent D and are generally absent from other clades{EscobarParamo:2004to} and the majority of urine isolates of E. coli are from clade B2 {Zhang:2002wo, Moreno:2008eg, Moreno:2009jc}.

Although there appears to be a connection between phylogeny and virulence, this correlation is not 100%, and ExPEC strains have been isolated from clades A, B1, B2, and B4 {Picard:1999uk, EscobarParamo:2004to}. Additionally, genomic content is not an absolute predictor of pathotype. Delineation of ExPEC, and consequently UPEC, strains from non-pathogenic strains of *E. coli* is difficult given the presence of ExPEC virulence factors in non-pathogenic strains {Kohler:2011cn, Grozdanov:2004bd}.

Currently, the most widely used method of grouping strains into a phylogenetic structure relies on Multi-Locus Sequence Typing (MLST).

*UPEC population structure in the bladder*

The population dynamics of UPEC during the course of a UTI are complex and consist of a number of bottleneck events that occur both outside and within the host epithelium{Hannan:2012jh} which result in a drift to clonality in UPEC in the bladder{Schwartz:2011cy, Walters:2012hq}. These bottlenecks occur recur many times during the course of the cyclical progression of UTI (Figure here). A stringent bottleneck occurs during the formation of intracellular bacterial colonies (IBCs), which is a critical step of UPEC pathogenesis that occurs during the acute phase of UTIs{Mulvey:1998wv, Anderson:2003kb, Justice:2004gx}. Although IBCs allow for significant clonal expansion of UPEC{Anderson:2003kb}, formation of the IBCs occurs at a very low rate, with only 50-700 IBCs persisting at 6h after inoculation of 107 UPEC bacteria{Schwartz:2011cy}. Formation of these IBCs requires known virulence factors, including the adhesin *fimH*{Wright:2007ha}. While the IBC bottleneck is important during the acute phase of UTI, the disappearance of IBCs at the end of the acute phase does not halt the continued loss in genetic diversity, suggesting a secondary bottleneck that occurs during the extracellular, chronic phase of UTI{Hannan:2012jh}. As with the IBC bottleneck, passage through the extracellular bottleneck may also be mediated by virulence factors. This hypothesis has been supported by inability of a mutant UTI89 lacking a pathogenicity associated island (PAI) containing known virulence factors, such as -hemolysin and P pili, to persist during chronic UTI{Hannan:2012jh}. These findings show that virulence factors have a significant effect on population structure of UPEC in the bladder, which, in turn, affects disease progression through acute and chronic phases of UTI.

*Escherichia coli population structure in the gut*

The gut populations of *E. coli* are surprisingly simple. The number of *E. coli* strains in the gut of women experiencing a UTI (~3) does not differ significantly from the number of *E. coli* strains in the guts of healthy women(2.5) as determined by PCR typing{Moreno:2008eg, Moreno:2009jc}. Interestingly, during acute UTI, The 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, Moreno:2006ji, Moreno:2008eg}. Further, dominance of a B2 strain in the gut is correlated with both increased number of urovirulence factors in the dominant strain and reduced species richness in the gut habitat{Moreno:2008eg, Moreno:2009jc}. This suggests that more urovirulent strains are able to outcompete less urovirulent strains in the gut habitat, which may result in local extinction of those less virulent strains. This pattern mirrors the population dynamics that occur in the bladder during UTI, indicating a shared functional effect on population structure by virulence factors in both habitats.

**UPEC virulence factors – multi-purpose tools**

*UPEC genotypes are varied, but structured*

*Virulence factors, phylogeny, and phenotype.*

*Urovirulence factors: swords or plowshares?*

**Future plans directions and unanswered questions**