ABSTRACT

Urinary tract infections (UTIs) are most commonly caused by uropathogenic *Escherichia coli* (UPEC). Around 50 % of all women will experience a UTI during their lifetime. UTIs have been considered acute, self-limiting infections. 25 % of young women with cystitis will however experience a recurrence within 6 months of the initial infection.

During cystitis, UPEC bind to bladder epithelial cells (BECs), invade superficial BECs and form intracellular bacterial communities (IBCs). Inside the IBCs, UPEC multiply in numbers and transition through different developmental stages. The pathogenicity cascade of the acute infection cycle ends with exfoliation of the superficial BECs, and UPEC bursting out of the host BECs as long filaments. A second round of infection can proceed in the remaining superficial BECs. UPEC is also able to invade the underlying layer of less differentiated BECs, which have become exposed as a consequence of exfoliation of infected superficial BECs. Here, UPEC form quiescent intracellular reservoirs (QIRs) where they are viable but unable to divide. QIRs can persist for months, and it is speculated that they are involved in the recurrence of UTIs because of the high prevalence of repeat infections by the same strain.

UPEC utilize multiple virulence- and fitness-associated genes to aid adhesion and invasion of BECs as well as form IBCs and QIRs. The identification of these genes, as well as virulence genes in other bacterial species, has been achieved by several different laboratory methods. Promoter trap library analysis by use of reporter genes has been utilized numerous times, and advances in technology both with emergence of fluorescence activated cell sorting (FACS) and next-generation sequencing (NGS) have furthered *in vivo* expression technology. Transposon mutagenesis is another method used during virulence gene identification and combining it with NGS has greatly aided in achieving high-throughput gene analysis. Methods not manipulating the bacteria prior to analysis are also available such as microarray and latest dual RNA-seq. These methods enable analysis of the transcriptome of both pathogen and host simultaneously.

The work presented in manuscript I describes the development of a method for virulence- and fitness-associated gene identification by combining promoter trap library analysis with both FACS and NGS. This was done to develop a method for genome-wide gene expression analysis that was independent of RNA purification. The development of this method was also conducted in order to aid in the further understanding of UPEC pathogenesis. In the manuscript, it was identified that amino acid biosynthesis genes were upregulated in human urine and many mutants missing these genes had a diminished ability to adhere to and invade or proliferate inside cultured BECs. A gene encoding an unknown protein was identified to be upregulated inside cultured BECs, and when deleted, the mutant had an enhanced ability

to adhere to and invade or proliferate inside cultured BECs. The improved method was termed DFI-seq and is a promising method by which potential fitness and virulence genes can be identified during *in vivo* infection.

The second manuscript describes the development of a flow-chamber based infection model. This *in vitro* model emulated the pathogenicity cascade of UPEC previously only observed in mouse models. It can be used to evaluate for example null mutants of identified virulence genes.