Longer Life Foundation Final Report
Title: Discovery of Urinary Tract Infection Biomarkers to Predict Longevity in the Elderly
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Abstract
Urinary tract infections (UTIs) are a common problem among elderly patients. Here we combined multivariate mathematical analyses with traditional microbiological and biochemical techniques to identify virulence strategies among clinical *E.coli* isolates and individualistic metabolic contributors to antibacterial immunity in humans. Findings from these studies provide a basis for more sophisticated measures of disease risk and suggest new therapeutic strategies for at-risk patients.

Lay Summary
UTI is common among the elderly, where it can cause serious disability if it escapes to the bloodstream. Identifying those at elevated risk and preventing UTI has proven difficult because many of the risk factors remain unknown. In part, this reflects the fact that the process by which UTI develops is complex and affected by individualistic features of both humans and potential bacterial pathogens. To address this challenge, we incorporated mathematical approaches used in marketing, political strategy, and intelligence work to determine how UTI bacteria cause, and how the chemical composition of human urine can prevent, infections. The results confirm that important differences exist between different people or different bacteria of the same species but that these differences appear to be limited in number, such that useful new tests and therapies will be feasible.

Introduction
Effectively a longevity biomarker, urinary tract infections (UTIs) in the elderly are common. UTI in the elderly is associated with falls, mental status changes, and sepsis. Sepsis associated with urinary tract infections (common enough to have resulted in the shorthand term “urosepsis”) is highly prevalent among the elderly and is a common cause of hospital admissions. Although wide individual variation in susceptibility to UTI and its progression has long been appreciated, the biological bases for this remain incompletely understood.

Methods
Specimen collections. We examined genotypes from *E.coli* strains recovered from patients sufficiently ill to merit hospitalization at Barnes-Jewish Hospital in St. Louis, Missouri from August 1, 2009 to July 31, 2010. Urines were collected from asymptomatic male and female volunteers. This study was approved by the Institutional Review Board.

Urinary growth studies. We expressed and purified human siderocalin (SCN) from a laboratory *E.coli* strain for investigation. The effect of a fixed SCN concentration inhibit bacterial growth was assessed in both culture media and human urine using quantitative titering.

Urinary metabolome measurements. We used a liquid chromatograph-equipped mass spectrometer (LC-MS) to obtain quantitative profiles of urinary molecules using a protocol previously established in the Henderson Lab. Molecules of interest were identified through purification, high-resolution mass spectrometric analysis, and GC-MS with comparison to authentic standards, where possible.

Mathematical analysis. We used principal components analysis (PCA) to discern molecules associated with high SCN activity. To identify genetic networks in bacteria, we collaborated with the Mathematics group of Dr. Peter Mucha at the University of North Carolina, Chapel Hill), who used network community detection and statistical biclustering approaches.

Results

Our analyses revealed that 300 clinical urinary *E.coli* isolates can be sorted into four groups, each characterized by distinctive disease-associated gene combinations. Some of these groups were associated with antibiotic resistance and others with patient sex. Analysis of a diverse human population revealed that individual differences in urinary pH and metabolite composition determine whether an important immune protein is able to inhibit bacterial growth.

Discussion, including implications and potential long-term extensions

E.coli isolated from patients, despite being the same species, exhibit marked genetic diversity that have complicated efforts to understand how these bacteria cause disease. Nevertheless, it is likely that some gene combinations are more favorable than others, leading to their overrepresentation in a population. Mathematic network analysis is well-suited to identifying patterns such as this. The four genetic groups found here may represent different strategies used by urinary bacteria to cause infections. In this case, the genetic group associated with male patients may be best suited to defeat distinctive male barriers to urinary tract infections. Men colonized with these bacteria may thus be at elevated risk for UTI.

It has long been appreciated by physicians that there are profound individual differences in susceptibility to UTI, even among otherwise normal individuals. A serendipitous observation in the lab revealed that an antibacterial human protein called siderocalin functioned far better in the urine of some individuals. We biochemically traced this difference to a combination of urine pH and levels of specific urinary metabolites. These results suggest a way to identify those at elevated infection risk and also new strategies to prevent or treat UTI.

Future plans including planned grant submissions

Future studies will seek a more complete understanding of urinary metabolites that prevent bacterial growth so that interventional strategies may be devised and tested. The bacterial virulence gene data will be used as an organizing principal for devising improved risk models and antivirulence interventions.