Longer Life Foundation Final Report
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TITLE: The Trajectory of the Gut Microbiome in Patients with MDRO UTIs

LAY SUMMARY: The goal of this study is to determine the role of the gut microbiome in the development of urinary tract infections (UTI) due to multidrug-resistant organisms (MDRO). We recruited people who had an MDRO UTI, and collected stool and urine samples to study if the organisms found in the stool were linked to those in the gut. Using a combination of genomic sequencing and microbiologic culture, we found that the same clonal organism in the gut microbiome could also be found in causing a clinically significant UTI. We further found that prior to a recurrent UTI, there were higher concentrations of the clonal MDRO in the gut. This means that there is a direct link between the gut concentration of MDROs and clinically significant UTIs. This data can be used to create methods to detect, and potentially prevent, MDRO UTIs. These results were published in mBio (PMID 31455657).

DISCUSSION: Infections due to MDROS are increasingly common, and with the CDC estimating that nearly 3 million people across the US will face an MDRO infection, and over 35,000 people will die as a result. To combat antibiotic resistance, the CDC has declared that we must stop relying only on new antibiotics, but we need to adopt aggressive strategies to prevent the development of antibiotic resistance, and infections due to AROs. If we do nothing, the WHO predicts that antibiotic resistance will kill 10 million people per year globally by 2050, outpacing the rate of cancer deaths. A major deficit in our knowledge is understanding the role of the gut microbiome as reservoir for MDROs that cause clinically significant UTIs. Currently, the rate of new antimicrobial development is greatly outpaced by the rate of antimicrobial resistance, thus novel methods to understand the role of the gut microbiome in MDRO infections is necessary. A better understanding of the transmission dynamics between the intestinal and urinary tracts, combined with phenotypic characterization of the uropathogen populations in both habitats, could inform prudent therapies designed to overcome the rising resistance of uropathogens. Here, we integrate genomic surveillance with clinical microbiology to show that drug-resistant clones persist within and are readily transmitted between the intestinal and urinary tracts of patients affected by recurrent and non-recurrent UTIs. Thus, our results advocate for understanding persistent intestinal uropathogen colonization as part of the

ABSTRACT: The gut microbiome of patients with urinary tract infections (UTI) due to multidrug-resistant organisms (MDRO) is poorly understood. Examining the gut microbiome trajectory in patients with MDRO UTIs wil allow us to identify bacterial colonies associated with recurrent MDRO UTIs. Here we propose a prospective cohort study, collecting 11 stool and urine specimens from patients with MDRO UTIs over a 6 month period. We will compare patients who had a recurrent MDRO UTI to those who do not. The proposed study will define the natural history of the gut microbiome in patients with MDRO UTI to better identify those at greatest risk for recurrent MDRO UTI and persistent MDRO colonization. This study will address a critical barrier to scientific progress in the field of recurrent MDRO UTI and gut MDRO colonization, and improve our scientific knowledge by delineating the microbial communities that are protective and/or permissive to MDRO colonization and recurrent UTI. This study will improve clinical practice and outcomes by providing a tool that can be used to identify patients at risk for recurrent UTI.

INTRODUCTION: UTIs affect 150 million people a year worldwide and account for $3.5 billion dollars in healthcare and societal costs in the US alone. UTIs of particular concern are those caused by MDROs, which consist of bacteria resistant to most available antibiotics and are often referred to as “superbugs.” The human gastrointestinal tract is a common source of MDRO UTIs, as the gut bacteria contaminate the outside of the urethra, then travel to the bladder to cause a UTI. In order to create methods to prevent MDRO infections and recurrence of infections, we propose a study to understand the gut microbiome in patients with MDRO UTIs.

Our study has 2 main goals. The first is to understand changes in the gut microbiome of patients that develop MDRO UTI. There are no studies of the gut microbiome in patients with MDRO UTI, therefore this is a necessary step. We hypothesize that at the time of the acute infection, the MDRO causing the UTI will be present at a high concentration in the gut microbiota. After appropriate treatment, we hypothesize that the MDRO concentration will decrease in the gut (as compared to the pre-treatment time-point), but will not be completely eliminated. Our second goal is to identify bacterial communities that are associated with recurrent

MDRO UTIs. We hypothesize there are microbial communities in the gut that are associated an increased risk for recurrent UTI, and identification of these communities can be used to predict patients who will develop recurrent UTI. Once we understand the types of microbial communities that present in the gut microbiota, we can then develop interventions to predict recurrent MDRO infections and future methods to prevent infection.

This study will address a critical barrier to progress in the field of MDRO infections, and improve our scientific knowledge by identifying the microbial communities associated with recurrent MDRO UTI. This study is significant to the insurance industry because we currently do not have methods to predict patients at risk for MDRO UTIs. If we are able to identify methods to predict recurrent UTIs, interventions can be taken to prevent fulminant infections and hospitalizations. With the increasing rates of morbidity and mortality associated with MDRO infections, this study will fill a knowledge gap that will ultimately improve patient care.

METHODS: This is a prospective cohort study to identify predictors of recurrent MDRO UTI, MDRO colonization and characterize the evolution of MDRO in response to antimicrobial exposures. Patients with Gram-negative MDRO UTIs were recruited and 11 stool and urine specimens from patients with MDRO UTIs over a 6 month period. Specimens were interrogated with semi-quantitative microbiologic culture for the MDRO of interest and whole genome shotgun sequencing (WGS) for clonal tracking. The primary analysis was based on patients with a recurrent UTI vs those without. Clonal tracking of the MDRO of interest in the stool and urine were analyzed in conjunction with clinical and culture data.

RESULTS: Leveraging funding from the LLF, we recruited 14 patients with MDRO UTI (7 with recurrences, 7 without). Our WGS and culture results determined that clonal MDROs including uropathogenic *Escherichia coli, Proteus mirabilis*, and *Klebsiella pneumoniae* isolates can be found in the intestinal and urinary tracts of patients affected by recurrent and non-recurrent UTIs. Clonal tracking of isolates in consecutively collected urine and gut specimens indicated repeated transmission of uropathogens between the urinary tract and their intestinal reservoir. Our results further implicate three independent routes of recurrence of UTIs: (i) following an intestinal bloom of uropathogenic bacteria and subsequent bladder colonization, (ii) reinfection of the urinary tract from an external source, and (iii) bacterial persistence within the urinary tract. Taken together, our observation of clonal persistence following UTIs and uropathogen transmission between the intestinal and urinary tracts warrants further investigations into the connection between the intestinal microbiome and recurrent UTIs. These results were published in mBio (PMID 31455657).

DISCUSSION: Infections due to MDROS are increasingly common, and with the CDC estimating that nearly 3 million people across the US will face an MDRO infection, and over 35,000 people will die as a result. To combat antibiotic resistance, the CDC has declared that we must stop relying only on new antibiotics, but we need to adopt aggressive strategies to prevent the development of antibiotic resistance, and infections due to AROs. If we do nothing, the WHO predicts that antibiotic resistance will kill 10 million people per year globally by 2050, outpacing the rate of cancer deaths. A major deficit in our knowledge is understanding the role of the gut microbiome as reservoir for MDROs that cause clinically significant UTIs. Currently, the rate of new antimicrobial development is greatly outpaced by the rate of antimicrobial resistance, thus novel methods to understand the role of the gut microbiome in MDRO infections is necessary. A better understanding of the transmission dynamics between the intestinal and urinary tracts, combined with phenotypic characterization of the uropathogen populations in both habitats, could inform prudent therapies designed to overcome the rising resistance of uropathogens. Here, we integrate genomic surveillance with clinical microbiology to show that drug-resistant clones persist within and are readily transmitted between the intestinal and urinary tracts of patients affected by recurrent and non-recurrent UTIs. Thus, our results advocate for understanding persistent intestinal uropathogen colonization as part of the pathophysiology of UTIs, particularly in patients affected by recurrent episodes of symptomatic disease.

FUTURE PLANS/GRANT SUBMISSIONS: The results of this pilot study were published in mBio, and currently we are building upon our growing cohort. The LLF was cited as a funder. Utilizing this pilot study, I was able to successfully obtain a NIH/NIAID K23 Career Development Award, and have garnered additional funding to support this study. We have recruited a total of 53 patients with MDRO UTIs for this study. In future plans, we will continue our microbiologic and genomic analyses, utilizing the framework developed by this pilot study. Completion of this project will advance our knowledge of the relationship between the gut and urine microbiome and recurrent MDRO UTI. The data generated by this project can be used to develop culture- and genomic-based diagnostics to identify those at greatest risk for recurrent UTI, and factors that are permissive

to MDRO colonization. The resulting unique clinical, dietary, microbiologic, and genomic data set can be used for future analyses of the impact of diet and medical comorbidities on the gut and urine microbiome. Additional future applications include metabolomic analyses to identify if gut microbiota derived urine metabolites may be protective or permissive to recurrent UTI and future studies focused on the impact of antimicrobials on the microbiome. Future grant applications utilizing the preliminary data from this study include a Doris Duke Clinical Scientist Development Award, and a NIH R01 award focused on understanding the mechanistic link between gut MDRO colonization and the development of clinically significant MDRO infections.