DAY 1

IDENTIFY RESEARCH GAPS & BARRIERS

KNOWLEDGE GAPS & RESEARCH NEEDS

chanistic knowledge of uromicrobiota interactions: There is little knowledge
ow the members of the urinary microbiota interact with each other as a
mmunity beyond associative studies (A - MICROBIAL INTERACTIONS)
t factors modulating urinary microbiota composition: What accounts for the
e differences in urinary microbiome composition between individuals and
ecially as women age? (B - FACTORS INFLUENCING DYNAMICS)
y computational and bioinformatic approaches are dependent on
erlying reference databases and tuning parameters for properties of the
. There is a lack of urobiome-specific resources (guidelines, protocols,
kflows, reference databases (C - METHODOLOGIES)
re is obvious variability in the urobiome composition amongst individuals
within individuals over time. Factors that contribute to this variation are
nown/lacking. (B - FACTORS INFLUENCING DYNAMICS)
urobiome composition and stability in different patient populations: how
le are not just the bacteria but also fungi/viruses/parasites over time in
erent patients, and do fluctuations correspond to clinical outcomes? (B -
CTORS INFLUENCING DYNAMICS; CLINICAL)
e of the urinary microbiota in modulating infection: do members of the
ary microbiota modulate virulence of pathogens? If so, can we use this
rmation to better inform diagnostics and treatment? (A - MICROBIAL
ERACTIONS)
s urinary microbiota modulate host response? (D - HOST MICROBIOTA
ERACTIONS)
ctional characterization of the urinary microbiota: are there key functions
contribute to urinary health vs disease, and are these functions shared
oss multiple genera? (E - FUNCTIONAL CHARACTERIZATION)
v and where are urobiome bacteria colonizing the urogenital tract? (D -
ST MICROBIOTA INTERACTIONS; E - FUNCTIONAL
ARACTERIZATION)
ot stable in the bladder, where are they colonizing and how are they
sferring? (F - GEOGRAPHY/ COLONIZATION DYNAMICS)
there species and strain level differences in pathogenesis? (E -
NCTIONAL CHARACTERIZATION)
ch urobiome bacteria are directly responsible for disease features? (E -
NCTIONAL CHARACTERIZATION)
any of the associations between urobiome composition and urologic
ases a consequence of an altered environment? (the chicken-and-egg
stion) (B - FACTORS INFLUENCING DYNAMICS; D - HOST MICROBIOTA
ERACTIONS)
chanisms underlying urobiome function and disease (improved functional
ght and causal exploration; interactions between microbes/immune
onses; bridging the gap to clinical application) (E - FUNCTIONAL
ARACTERIZATION)
ndardizing methodology and best practices within the field (improved ability
ompare data cross studies, meta-analyses, standardized databases) (C -
THODOLOGY)
going refinement of low biomass data handling, contaminant IDs,
nformatic training (C - METHODOLOGY)
roved characterization of how variables are influencing urobiome
position (e.g., meds/diet) (B - FACTORS INFLUENCING DYNAMICS)

BARRIERS & CHALLENGES

1. We don't know what healthy is
2. We don't know the molecular changes associated wi
   UTDs
3. Appropriate Model Systems: Currently available mou
   do not recapitulate the human urogenital microenviro
4. Clinician-scientist divide, insufficient interactions and
   exchange
   a. Lack of interest at urology meetings
   b. Lack of interest in urology publications
5. Disparate funding sources - no single centralized sou
   need to be aware and on top of multiple sources
6. Difficulties publishing
7. Need for longitudinal sample collection
8. Need for standardized methods of collection, process
   sequencing, and cleaning data
9. Need for methods to cultivate more components of th
10. Need for better in vitro models for co-culturing urobio
    more traditional pathogens, ideally incorporating hos
11. Cost of multi-omics approaches for low-biomass sam
12. multisite collection and better protocols for collection
    analysis
13. Many uncharacterized

RESEARCH QUESTIONS

1. Hormonal regulation of urinary microbiome colonizati
   dynamics in women (especially associated with men
   (Lisa)
   SA1: Determine the urobiome shifts through
   menopause and identify factors that are ass
   with urobiome composition.

   SA2: Identify the response of the urobiome t
   systemic and (b) vaginal estrogen treatment
   SA3: Identify microbiome characteristics ass
   with symptom severity (and improvement?)
2. Metabolic requirements of urogenital lactobacilli withi
   urinary and vaginal niches (Nicole). SA1) Define me
   requirements for exogenous FAs and membrane
   composition among Lactobacillaceae. SA2) Exa
   impact of FA auxotrophy and exogenous LCFAs
   Lactobacillus protective functions. SA3) Determi
   Lactobacillus host lipid utilization and the impact
   estrogen

3. Do fluctuations in bacteria/fungi/virus/ correspond to
   outcome risk or can this be - used to strategify patie

robial colonization and persistence in the urinary tract (if/how can we
lify, probiotics, novel biotherapeutics like FMT/BMT) (F - GEOGRAPHY/
LONIZATION DYNAMICS)
ctionality of the urobiome:

a. Microbe/microbe and microbe/host interactions in health/disease
b. Would be ideal to a combination of *in silco* modeling to predict microbe/host/bladder health outcomes iterating with high throughput *in vitro* experiments to refine the models which would, in turn, (ideally) allow us to narrow the hypothesis space for further *in vitro / in vivo* testing. (G - MODELS)

roaches: Animal models (G - MODELS)

- Is there a consensus on approaches / limitations / or what advances are needed to study the urobiome in animal models? (e.g. rodents? Dogs?)
- Develop evidence based guidelines and/or outline critical next steps for methodological studies to inform the field.

osome in the bladder: (B - FACTORS INFLUENCING DYNAMICS)

a. Many exposures get processed through the bladder - Diet, xenobiotics, pollutants. How are these layering on with immune cells/microbes/host cells to influence bladder health?

ecular triggers for biofilm formation in the urinary tract (A - MICROBIAL
ERACTIONS, D - HOST INTERACTIONS, E - FUNCTIONAL
ARACTERIZATION)

a. Pathogen Dose ↔ Immune Response
b. Thresholds linking bacterial burden (CFU) to urothelial & systemic immunity remain undefined.
c. Quantify load with digital PCR/imaging + multiplex immune profiling in organoid & murine UTI models; immunology-microbiology-data teams derive dose-response curves.
d. Host metabolites, urine chemistry, shear stress & quorum signals that induce biofilm transitions are largely unknown.
e. Use urinary organ-on-chip flow platforms + pathogen transcriptome/proteome screens under variable urine; chemists identify inductive metabolites, screen inhibitors.

terial flows through the urinary tract (F - COLONIZATION/ GEOGRAPHY)

a. Direction, timing & succession of microbes migrating from reservoirs (skin and periurethra, gut) to bladder/kidney are poorly mapped.
b. Longitudinal multi-site sampling with engineered fluorescence; collaboration between genetic engineers, germ-free mouse models, clinically relevant isolates.

espan development of urobiome (B - FACTORS INFLUENCING
NAMICS)

a. how this system is colonized and changed by disease/events
b. from childhood to aged

biome constituents characterization (E - FUNCTIONAL
ARACTERIZATION)

a. many microbes are functionally uncharacterized
b. access to strains and protocols
c. Sharing data amongst the community and public

arating specific UTIs into classes (CLINICAL
PLICATIONS/TREATMENTS)

ke other niches – UTI is defined as a single disease
k on identifying already 'known UTI' and others
ew and working with clinical colleagues

(Chelsie)

4. How does disruption of urothelial barrier feedback to colonization?**(Nicole G)** SA1) Identify pathways t differentially expressed in the uroepithelium of g compared to conventional mice. SA2) Assess fu impact of the microbiome on urothelial barrier re Compare susceptibility to urinary tract infection b germ free and conventionally-raised mice
5. **Colonization dynamics- What are the most co routes of colonization? How does colonizatio proximity impact urologic symptoms? (Aaron**
6. **How do multi-omic urobiome clusters predic symptoms and chronic inflammatory disease co-morbidities? (Aaron)**
7. **How to define healthy and disease urobiome**
8. How can we best combine of *in silco* modeling to pre microbe/host/bladder health outcomes iterating with h throughput
   in vitro experiments to refine the models which would (ideally) allow us to narrow the hypothesis space (for and metabolites) for further *in vitro / in vivo* testing **(V**
9. **How can we strategically modify the urobiom through diet or FMT? (Emily)**

CATEGORIZE RESEARCH GAPS & BARRIERS

SPECIFIC RESEARCH IDEAS AND QUESTIONS

A) Microbial interactions
   a) Molecular triggers for biofilm formation in the urinary tract
   b) Nicole De Nisco: Metabolic cross-feeding between members of the urinary microbiota
      i) Chelsie Armbruster: and cross-feeding between microbiota and potential pathogens
      ii) Tanya: direct cell interactions and metabolite dependent
   c) Aaron W. Miller: Titrate pathogen burden in gnotobiotic mice and single-cell-profile to the urothelium to generate quantitative dose–response models linking load to innate and adaptive outputs along with urologic phenotypes (i.e. CaOx crystal formation).

B) Factors influencing colonization dynamics
   a) Vanessa Hale: Bacterial flows through the urinary tract: Direction, timing & succession of microbes migrating from reservoirs (skin and periurethra, gut, vagina) to bladder/kidney. - Nicole G., Emily
   b) Vanessa Hale: Exposome in the bladder: Many exposures get processed through the bladder - Diet, xenobiotics, pollutants. How are these layering on with immune cells/microbes/host cells to influence bladder health?
   c) Emily: Microbial colonization and persistence in the urinary tract - Nicole G.
   d) Nicole Gilbert: Are any of the associations between urobiome composition and urologic diseases a consequence of an altered environment? (the chicken-and-egg question) - Lisa
   e) Nicole De Nisco: Hormonal regulation of urinary microbiome colonization dynamics in women (especially associated with menopause) - **Lisa, Vanessa** (Hormonal metabolism / production by microbes)
   f) Aaron W. Miller: In germ-free mice, using genetically engineered green and red fluorescent proteins in bacteria - track colonization through blood (i.e. gut origin) and urethral routes (i.e. skin origin) (i.e. same bacterial species - blood bacteria are red; urethral bacteria are green). Distinguish bottom up vs. top down colonization routes and where bacteria end up. (SO COOL! I'm not doing this but would love to - Vanessa Hale; DITTO, Nicole G.)
   g) Chelsie Armbruster: impact of catheterization (transient vs long-term) on microbiome composition, urothelium regeneration, and susceptibility to pathogen colonization. How long-lasting are the impacts of short-term catheterization? Can this be mitigated by different types of catheters (silicone vs latex, anti-fouling, etc)

C) Methodologies
   a) Emily: Standardizing methodology and best practices, ongoing refinement of low biomass data handling, contaminant IDs, bioinformatic training - Lisa, Vanessa
   b) Lisa - Resources and databases to underlie computational models
   c) methods for examining virus/fungi/parasite nucleic acids in addition to bacteria

D) Host-microbiota interaction
   a) Does urinary microbiota modulate host response?
   b) Aaron W. Miller: Mouse, bioreactor, biochemical, and cell culture models of bacteria and bacterial metabolite impacts on urologic-specific outcomes (biofilm formation, crystal formation, cellular invasion, inflammation, etc.)
   c) Nicole De Nisco: How does the urinary microbiota influence bladder pain responses? (microbial metabolites interacting with DRGs, etc.)
   d) Nicole De Nisco: How does the urinary microbiota contribute to urothelial barrier function? - Nicole G. and how does disruption of urothelial barrier feedback to promote colonization?
   e) Nicole Gilbert: How does the urinary microbiota influence urothelial repair and adaptive immunity following a UTI?

E) Functional characterization
   a) **Nicole De Nisco**: Metabolic requirements of urogenital lactobacilli within the urinary and vaginal niches - add me:)) Tanya Sysoeva and Lisa (I tried to put a heart emoji) 🙂
   b) Nicole Gilbert and Chelsie Armbruster: Which urobiome bacteria are directly responsible for disease features? - Lisa, Emily, Tanya
   c) Tanya Sysoeva: which bacteria are selected by urinary tract environment based on their stress resistance, interactions, and metabolism - Nicole G.
   d) Nicole Gilbert: Changes in metabolism, virulence/colonization factor expression by bacteria as they move in and out of the urinary tract (e.g. gut or vagina to periurethral area to urethra to bladder)

F) Geography
   a) Does composition change between urethra, different regions of the bladder, ureters, different regions of the kidneys?
   b) How representative is urine of the complete urinary tract composition?
   c) Which microbes tend to be exclusively extracellular vs forming intracellular communities?

G) Models
   a) Vanessa Hale: How can we best combine of *in silco* modeling to predict microbe/host/bladder health outcomes iterating with high throughput *in vitro* experiments to refine the models which would, in turn, (ideally) allow us to narrow the hypothesis space (for microbes and metabolites) for further *in vitro / in vivo* testing?
   b) Nicole Gilbert: Is there a consensus on approaches / limitations / or what advances are needed to study the urobiome in animal models? \ - Emily (and how can we determine which are more/less “translatable” to human health. Ie., determining model value)
   c) Chelsie Armbruster: can we develop a dual oxic/anoxic urine-tolerant human organoid model for studying microbiota-host and microbe-microbe interactions?

H) Clinical applications and treatments
   a) Chelsie Armbruster: Do fluctuations in bacteria/fungi/viruses/parasites correspond to clinical outcome risk, or can this be used to stratify patients for monitoring/treatment strategies? Would require longitudinal monitoring at short intervals over prolonged follow-up times. Ideally multi-omic approach to better define clinical outcome of interest.
   b) Emily, Vanessa, (Nicole De Nisco and Lisa for humans), Tanya: If and how can we modify the urobiome, using diet, probiotics, novel biotherapeutics like FMT/BMT, etc
   c) Tanya: suggest to re-Define/break UTI by some markers/types of damage and pathogen from available data
   d) Lisa: Does the urobiome help in refining phenotypic subtypes of lower urinary tract disorders?
   e) Lisa: Identifying the “actional component’ of the urobiome - Nicole G.
   f) Nicole De Nisco: Point of care diagnostics that can predict SYMPTOMATIC UTI now that we know urine is not sterile - using host inflammatory markers AND microbial markers
   g) Nicole De Nisco: Improving estrogen hormone therapies for better modulation of urogenital microbiome during and after menopause for better disease outcomes (vaginal estrogen only works in about half of women right now…) - Lisa
   h) Diagnostic/feasible approaches of sampling for basic science vs clinical disease presentation

Urobiome modeling

- Linking Garnerella to LUTS - how do we connect what’s happening in mice/ in vitro to what’s happening in women?
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DAY 2

SHARE SPECIFIC AIMS AND/OR HYPOTHESES & PRIORITIZE

Research question 1

- Specific aims
- Hypothesis
- Collaborators

Research question 2

- Specific aims
- Hypothesis
- Collaborators

REFINE & ASSIGN

Project 1:

- Leaders
- Timeline

Project 2:

- Leaders
- Timeline

DEVELOP RESEARCH APPROACHES & STRATEGIES

Project 1:

Project 2:

CROSS FERTILIZE

Uro-Voiding & Dysfunction

Research question 1:

Research question 2:

Uro-Aging

Research question 1:

Research question 2: