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| **UROBIOME RESEARCH INTEREST GROUP** | | | | | | *5* | *7* | *3* | *5* | *4* | *3* | *3* | *8* |  |  |  |
| 1. **Knowledge gaps and research needs according to the categories created in Google Doc after everyone added their thoughts** | **Whose idea?** | **Signed on** | **Signed on** | **Signed on** | **Signed on** | **Microbemicrobe intxns** | **Factors influencing colonization dynamics** | **Methods** | **Host-microbiota intxns** | **Functional characterization** | **Geography** | **Models** | **Clinical** |  |  |  |
| Molecular triggers for biofilm formation in the urinary tract | Aaron |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |
| Metabolic cross-feeding between members of the urinary microbiota | Nicole D |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |
| Cross-feeding between microbiota and potential pathogens | Chelsie |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |
| Direct cell interactions and metabolite dependent | Tanya |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |
| 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. calcium oxalate crystal formation) | Aaron |  |  |  |  | x |  |  |  |  |  |  |  |  |  |  |
| Are any of the associations between urobiome composition and urologic diseases a consequence of an altered environment? (the chicken-and-egg question) | Nicole G | Lisa |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| Hormonal regulation of urinary microbiome colonization dynamics in women (especially associated with menopause) | Nicole D | Lisa |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| Bacterial flows through the urinary tract: Direction, timing & succession of microbes migrating from reservoirs (skin and periurethra, gut, vagina) to bladder/kidney | Vanessa | Nicole G | Emily |  |  |  | x |  |  |  |  |  |  |  |  |  |
| Microbial colonization and persistence in the urinary tract | Emily | Nicole G |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| 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 | Aaron | Vanessa | Nicole G |  |  |  | x |  |  |  |  |  |  |  |  |  |
| 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? | Vanessa |  |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| 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) | Chelsie |  |  |  |  |  | x |  |  |  |  |  |  |  |  |  |
| Standardizing methodology and best practices, ongoing refinement of low biomass data handling, contaminant IDs, bioinformatic training | Emily | Lisa | Vanessa |  |  |  |  | x |  |  |  |  |  |  |  |  |
| Resources and databases to underlie computational models | Lisa |  |  |  |  |  |  | x |  |  |  |  |  |  |  |  |
| Methods for examining virus/fungi/parasite nucleic acids in addition to bacteria |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |  |
| Which urobiome bacteria are directly responsible for disease features? | Nicole G, Chelsie | Lisa | Emily | Tanya | Tanya |  |  |  |  | x |  |  |  |  |  |  |
| Which bacteria are selected by urinary tract environment based on their stress resistance, interactions, and metabolism | Tanya | Nicole G |  |  |  |  |  |  |  | x |  |  |  |  |  |  |
| Metabolic requirements of urogenital lactobacilli within the urinary and vaginal niches | Nicole D | Tanya | Lisa |  |  |  |  |  |  | x |  |  |  |  |  |  |
| 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) | Nicole G |  |  |  |  |  |  |  |  | x |  |  |  |  |  |  |
| Does composition change between urethra, different regions of the bladder, ureters, different regions of the kidneys? |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |  |
| How representative is urine of the complete urinary tract composition? |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |  |
| Which microbes tend to be exclusively extracellular vs forming intracellular communities? |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |  |
| 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? | Vanessa |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |
| Is there a consensus on approaches / limitations / or what advances are needed to study the urobiome in animal models? | Nicole G |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |
| Can we develop a dual oxic/anoxic urine-tolerant human organoid model for studying microbiota-host and microbe-microbe interactions? | Chelsie |  |  |  |  |  |  |  |  |  |  | x |  |  |  |  |
| If and how can we modify the urobiome, using diet, probiotics, novel biotherapeutics like FMT/BMT, etc | Tanya | Emily | Vanessa | Nicole D | Lisa |  |  |  |  |  |  |  | x |  |  |  |
| 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…) | Nicole D | Lisa |  |  |  |  |  |  |  |  |  |  | x |  |  |  |
| Identifying the “actional component’ of the urobiome | Lisa | Nicole G |  |  |  |  |  |  |  |  |  |  | x |  |  |  |
| 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. | Chelsie |  |  |  |  |  |  |  |  |  |  |  | x |  |  |  |
| Suggest to re-Define/break UTI by some markers/types of damage and pathogen from available data | Tanya |  |  |  |  |  |  |  |  |  |  |  | x |  |  |  |
| Does the urobiome help in refining phenotypic subtypes of lower urinary tract disorders? | Lisa |  |  |  |  |  |  |  |  |  |  |  | x |  |  |  |
| Point of care diagnostics that can predict SYMPTOMATIC UTI now that we know urine is not sterile - using host inflammatory markers AND microbial markers | Nicole D |  |  |  |  |  |  |  |  |  |  |  | x |  |  |  |
| Diagnostic/feasible approaches of sampling for basic science vs clinical disease presentation |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |  |
| How does the urinary microbiota contribute to urothelial barrier function? | Nicole D | Nicole G |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Does urinary microbiota modulate host response? |  |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |
| 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.) | Aaron |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |
| How does the urinary microbiota influence bladder pain responses? (microbial metabolites interacting with DRGs, etc.) | Nicole D |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |
| How does disruption of urothelial barrier feedback to promote colonization? | Nicole D |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |
| How does the urinary microbiota influence urothelial repair and adaptive immunity following a UTI? | Nicole G |  |  |  |  |  |  |  | x |  |  |  |  |  |  |  |
| How can we determine which models are more/less "translatable" to human health, i.e., determining model value | Emily |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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|  | 1. **Knowledge gaps and research needs (numbered) in order of those we listed on the Google Doc first, i.e., following round robin introductions** | **Microbe microbe intxns** | **Factors influencing colonization dynamics** | **Methods** | **Host-microbiota interactions** | **Functional characterization** | **Geography** | | **Models** | **Clinical** |
| 1 | Mechanistic knowledge of uromicrobiota interactions: There is little knowledge of how the members of the urinary microbiota interact with each other as a community beyond associative studies | x |  |  |  |  |  | |  |  |
| 2 | Host factors modulating urinary microbiota composition: What accounts for the large differences in urinary microbiome composition between individuals and especially as women age? |  | x |  |  |  |  | |  |  |
| 3 | Many computational and bioinformatic approaches are dependent on underlying reference databases and tuning parameters for properties of the data. There is a lack of urobiome-specific resources (guidelines, protocols, workflows, reference databases |  |  | x |  |  |  | |  |  |
| 4 | There is obvious variability in the urobiome composition amongst individuals and within individuals over time. Factors that contribute to this variation are unknown/lacking |  | x |  |  |  |  | |  |  |
| 5 | Full urobiome composition and stability in different patient populations: how stable are not just the bacteria but also fungi/viruses/parasites over time in different patients, and do fluctuations correspond to clinical outcomes? |  | x |  |  |  |  | |  | x |
| 6 | Role of the urinary microbiota in modulating infection: do members of the urinary microbiota modulate virulence of pathogens? If so, can we use this information to better inform diagnostics and treatment? | x |  |  |  |  |  | |  |  |
| 7 | Does urinary microbiota modulate host response? |  |  |  | x |  |  | |  |  |
| 8 | Functional characterization of the urinary microbiota: are there key functions that contribute to urinary health vs disease, and are these functions shared across multiple genera? |  |  |  |  | x |  | |  |  |
| 9 | How and where are urobiome bacteria colonizing the urogenital tract? |  |  |  | x | x |  | |  |  |
| 10 | If not stable in the bladder, where are they colonizing and how are they transferring? |  |  |  |  | x | x | |  |  |
| 11 | Are there species and strain level differences in pathogenesis? |  |  |  |  | x |  | |  |  |
| 12 | ​​Which urobiome bacteria are directly responsible for disease features? |  |  |  |  | x |  | |  |  |
| 13 | Are any of the associations between urobiome composition and urologic diseases a consequence of an altered environment? (the chicken-and-egg question) |  | x |  |  |  |  | |  | x |
| 14 | Mechanisms underlying urobiome function and disease (improved functional insight and causal exploration; interactions between microbes/immune responses; bridging the gap to clinical application) |  |  |  |  | x |  | |  |  |
| 15 | Standardizing methodology and best practices within the field (improved ability to compare data cross studies, meta-analyses, standardized databases) |  |  | x |  |  |  | |  |  |
| 16 | Ongoing refinement of low biomass data handling, contaminant IDs, bioinformatic training |  |  | x |  |  |  | |  |  |
| 17 | Improved characterization of how variables are influencing urobiome composition (e.g., meds/diet) |  | x |  |  |  |  | |  |  |
| 18 | Microbial colonization and persistence in the urinary tract (if/how can we modify, probiotics, novel biotherapeutics like FMT/BMT) |  |  |  |  |  | x | |  |  |
| 19 | Combine *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 |  |  |  |  |  |  | | x |  |
| 20 | Is there a consensus on approaches / limitations / or what advances are needed to study the urobiome in animal models? (e.g. rodents? dogs?) |  |  |  |  |  |  | | x |  |
|  | - Develop evidence based guidelines and/or outline critical next steps for methodological studies to inform the field |  |  | x |  |  |  | |  |  |
| 21 | 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? |  | x |  |  |  |  | |  |  |
| 22 | Molecular triggers for biofilm formation in the urinary tract | x |  |  | x | x |  | |  |  |
|  | - Pathogen Dose ↔ Immune Response |  |  |  | x |  |  | |  |  |
|  | - Thresholds linking bacterial burden (CFU) to urothelial & systemic immunity remain undefined |  |  |  | x |  |  | |  |  |
|  | - Host metabolites, urine chemistry, shear stress & quorum signals that induce biofilm transitions are largely unknown |  |  |  | x |  |  | |  |  |
|  | - Use urinary organ‑on‑chip flow platforms + pathogen transcriptome/proteome screens under variable urine; chemists identify inductive metabolites, screen inhibitors |  |  |  |  |  |  | | x |  |
| 23 | Bacterial flows through the urinary tract |  |  |  |  | x |  | |  |  |
|  | - Direction, timing & succession of microbes migrating from reservoirs (skin and periurethra, gut) to bladder/kidney are poorly mapped |  |  |  |  |  | x | |  |  |
| 24 | Timespan development of urobiome |  | x |  |  |  |  | |  |  |
|  | - how this system is colonized and changed by disease/events |  |  |  | x |  | x | |  |  |
|  | - from childhood to aged |  | x |  | x |  | x | |  |  |
| 25 | Urobiome constituents characterization |  |  |  |  | x |  | |  |  |
| 26 | Separating specific UTIs into classes |  |  |  |  |  |  | |  | x |
|  |  |  |  |  |  |  |  | |  |  |
| 1. **RESEARCH CHALLENGES AND BARRIERS** | | | | | | | |
| 1. We don’t know what healthy is | | | | | | | |
| 2. We don’t know the molecular changes associated with different UTDs | | | | | | | |
| 3. Appropriate model systems – currently available mouse models do not recapitulate the human urogenital microenvironment | | | | | | | |
| 4. Clinician-scientist divide, insufficient interactions and knowledge exchange | | | | | | | |
| (a.) Lack of interest at urology meetings; (b.) lack of interest in basic science at urology journals | | | | | | | |
| 5. Disparate funding sources - no single centralized source so need to be aware and on top of multiple sources | | | | | | | |
| 6. Difficulties getting articles published (i.e., past reviewers) | | | | | | | |
| 7. Lack of longitudinal sample collection | | | | | | | |
| 8. Methods of collection, processing, sequencing, and cleaning data not standardized | | | | | | | |
| 9. Need for methods to cultivate more components of the urobiome | | | | | | | |
| 10. Lack of suitable in vitro models for co-culturing urobiome and more traditional pathogens, ideally incorporating host tissue | | | | | | | |
| 11. High cost of multi-omics approaches for low-biomass samples | | | | | | | |
| 12. Lack of multisite collection and better protocols for collection and analysis | | | | | | | |
| 13. Many uncharacterized microbes | | | | | | | |