



The Urobiome Discovery, Replication, Exploration & Transitions

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A Tale of Two Researchers AND MANY MORE



Disclosures

AJW: Membership on scientific advisory boards of
Astek
Cerillo
Pathnostics
Urobiome Therapeutics

LB: Full time Deputy Editor, JAMA

It Never Made Sense

Clinical paradigm and test strategies

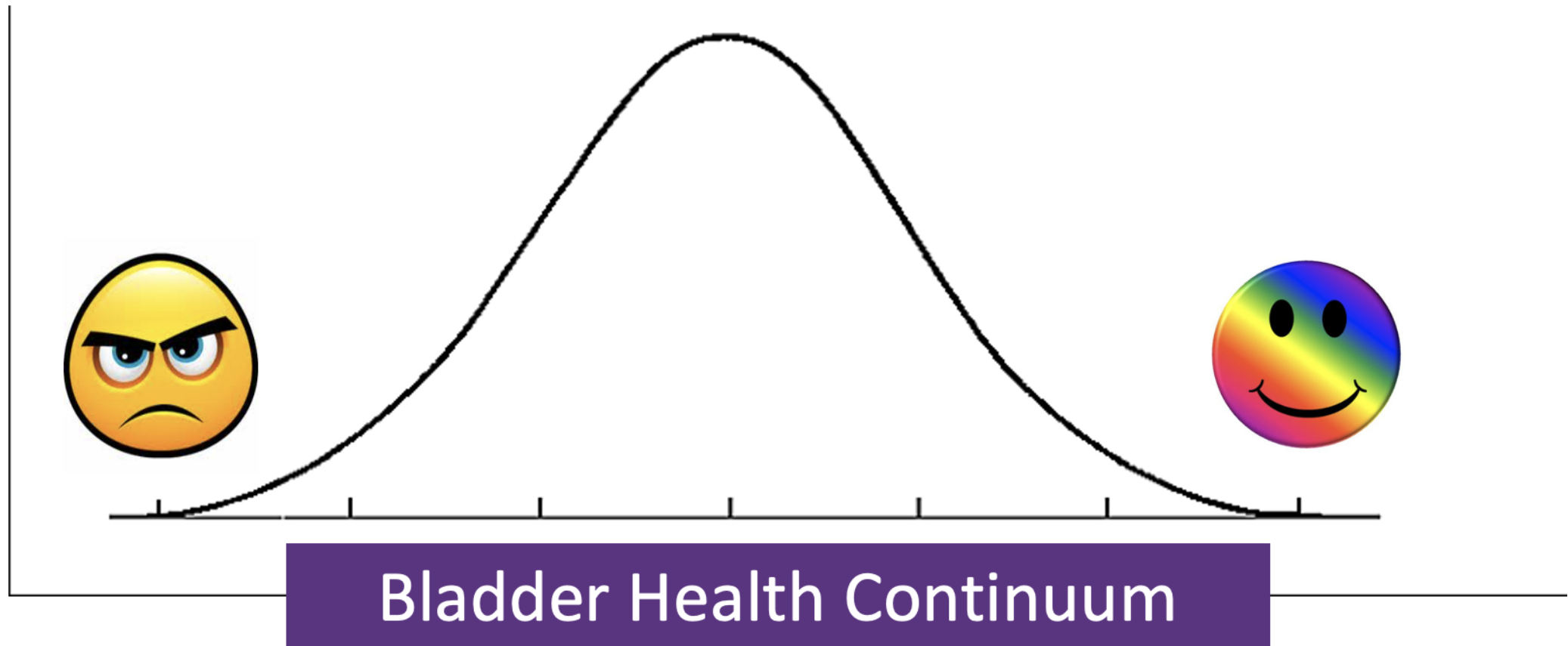
Pragmatic clinical care (with blinders on)

So many relevant clinical conditions +/- symptoms

So many problematic clinical practices

Too many dichotomies

Moving beyond the dichotomy



Urinary Tract Infection – Was, Is, but Doesn't Need to Continue to Be – A Problem

Defining UTI (digression – breast cancer)

Context matters (pregnant? surgery?)

Symptom assessment (new, worsening?)

Testing (none?, bad?, terrible?)

Test interpretation (rarely done correctly)

Defining resolution

Urinary Tract Infection

- “Uncomplicated” vs. “Complicated” – says who?
 - Is the **patient** complicated or are there **complicated aspects of infection**?
- “Uncomplicated” vs. “Complicated” dichotomy drives treatment
- Self-care rampant (without strong evidence base), globally!!
- Lack of clarity for clinician treatment goal
 - Symptoms relief?
 - Microbiologic cure (culture based?)
 - Lack of relapse?
 - Interval until next event?

Post-Episode Assessment and Care

- Lay people increasingly take pre/probiotic after antibiotic to restore their gut microbiome
- No effort to restore the urinary microbiome, even though we know there are missing microbes after UTI antibiotic therapy
- Can we predict and PREVENT occurrence of recurrent UTI if we attend to restoration of a health urobiome? How?

Gap from Laboratory Science to Clinical Care

Elegant laboratory work hasn't effectively improved clinical care

WHY?

Models don't replicate humans well

Hypothesis testing limited

Complexity difficult to capture

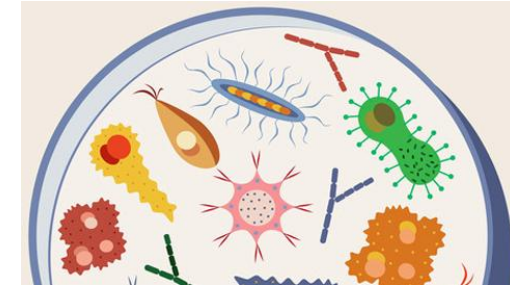
Clinicians often slow (very, very slow) to implement changes
especially when they sense that “all is well enough”

Progress Has Been Made Though (I don't mean more antibiotics...)

- Complexity Increasingly Recognized
 - (Although little change in front line clinical care)
- Urine Samples for Research – PLUS scientific contributions:
 - The RISE FOR HEALTH study: Methods for in-person assessment and biospecimen collection. Neurourol Urodyn. 2022 Dec 27. doi: 10.1002/nau.25108. PMID: 36573845; PMCID: PMC10290574.
 - Feasibility of Home Collection for Urogenital Microbiome Samples. Urogynecology 2024. 30(11), pp. 896-905. doi: 10.1097/SPV.0000000000001544. PMID: 38958286; PMCID: PMC11502285
 - Concordance of Urogenital Microbiome From Sequentially Self-collected Specimens. Urogynecology. November 21, 2024. doi: 10.1097/SPV.0000000000001607.

RISE FOR HEALTH Study

Urogenital Microbiome



	Above KatharoSeq Threshold	Not Above KatharoSeq Threshold	Sequenced
n	274	161	435
Age Group (yr; 4 Levels) (%)			
18-25	36 (13.1)	12 (7.5)	48 (11.0)
26-44	85 (31.0)	39 (24.2)	124 (28.5)
45-64	95 (34.7)	73 (45.3)	168 (38.6)
65+	56 (20.4)	37 (23.0)	93 (21.4)
Missing	2 (0.7)	0 (0.0)	2 (0.5)

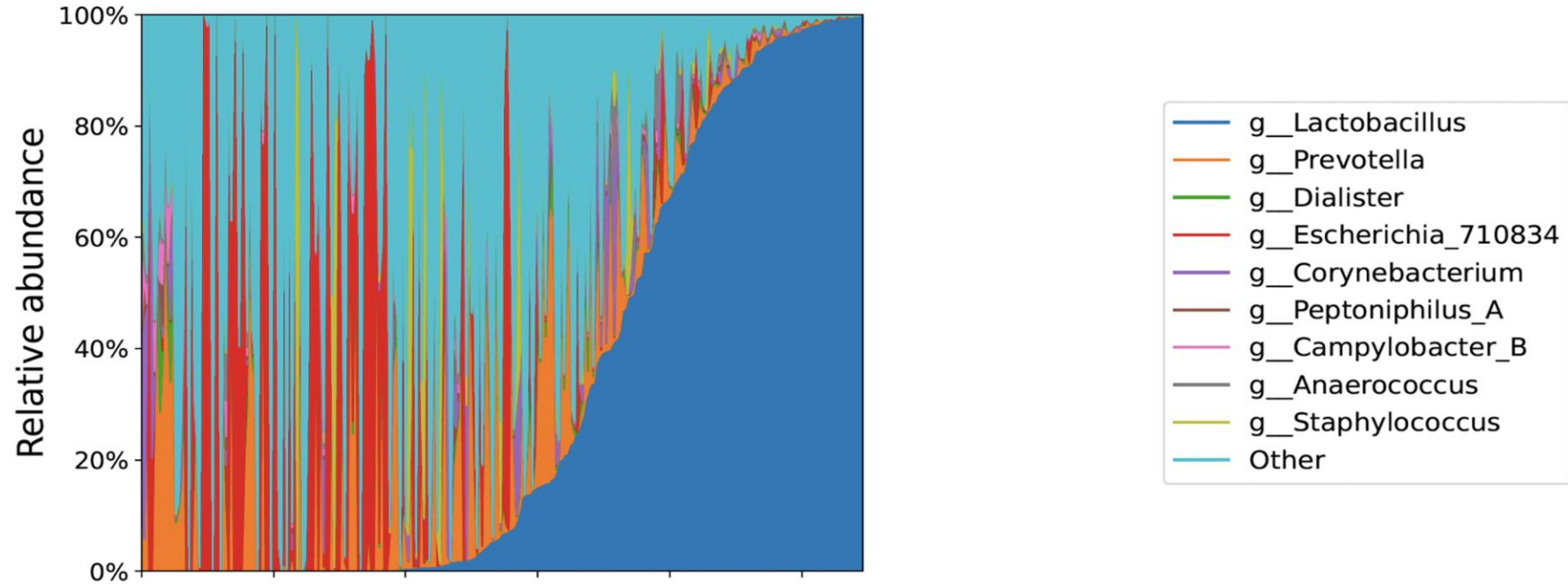
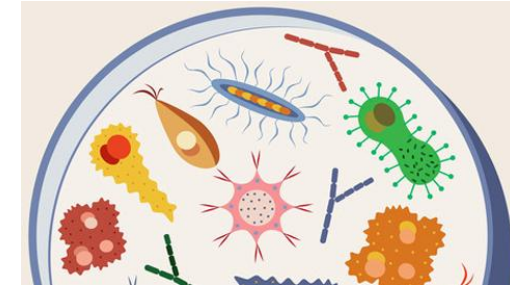
Participants with samples below threshold
tended to be older and a larger percentage were white.

PLUS Main Findings from Urobiome Substudy

Bladder health and the urogenital microbiome in community-dwelling adult females. mSystems. 2025 Nov 18;10(11):e0055825. doi: 10.1128/mSystems.00558-25. PMID: 41099505; PMCID: PMC12625691.

RISE FOR HEALTH Study

Urogenital Microbiome



Lactobacillus predominance - although the majority of samples contained various proportions of mixed microbes.

Taxonomic distribution of genus, showing
9 most abundant genera across 274 individuals

PLUS PLUS – Metabolomics

L Burnett PhD MD

<https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=fcc5b768696342bc8d552ffbd517b85d>

Zhao HN et al. A resource to empirically establish drug exposure records directly from untargeted metabolomics data. *Nature Communications*. 2025;16(1):10600. doi:10.1038/s41467-025-65993-5. PMID: 41366203.

Xing S et al. Navigating the conjugated metabolome. *bioRxiv* [Preprint]. 2026 Feb 9. doi:10.64898/2026.02.06.704496. PMID: 41727094. Under review at *Nature*.

Patan A et al. Charting the Undiscovered Metabolome with Synthetic Multiplexing. *bioRxiv* [Preprint]. 2025 Nov 19. doi:10.1101/2025.11.18.689170. PMID: 41332743.

Mannochio-Russo H et al. Pan-Metabolomics Repository Mapping of the Carnitine Landscape. *bioRxiv* [Preprint]. 2026 Mar 31. doi:10.64898/2026.03.27.714844. PMID: 41959195.

El Abiead Y et al. Structure-centric searching enables global mapping of the public metabolome. *Nature Biotechnology*. 2026 Apr 15. doi:10.1038/s41587-026-03082-8. PMID: 41986697.

Beyond UTI

Bladder Health – Associated Conditions

- Obtaining urine samples from other large cohorts
 - Diabetes, Weight management, Cardiovascular Disease, Renal Disease
- Assessing other urinary conditions (urgency incontinence, urinary stones, etc.) with known urobiome changes
- Aiming for PREVENTION and IMPROVEMENT of human health

Transitions

Challenging times for science

Clinical need remains – patients suffering, in need!

Microbial resistance an urgent global threat!!

High scientific standards needed NOW more than ever!!!

It is UP TO YOU now.

Transitions

We should retire the following concepts/terms!!!

Asymptomatic bacteriuria

What does this mean if everyone (at least all adult females) is bacteriuric?

Significant bacteriuria

Thresholds don't work

Transitions

We should retire the following concepts/terms!!!

***E. coli* causes 70-90% of UTIs**

Percentages based on the standard urine culture (SUC)
Biased for *E. coli* and other fast growing facultative anaerobes

Streetlight Effect



Transitions

We should retire the following concepts/terms!!!

A specific pathogen is the cause of symptoms

Based on the 19th century concept

Identify the pathogen and kill it

But no one does Koch's postulates

At best, it's the suspected pathogen

But is it?

Transitions

We should retire the following concepts/terms!!!

Uropathogenic *E. coli* (UPEC)

Could someone please define UPEC?

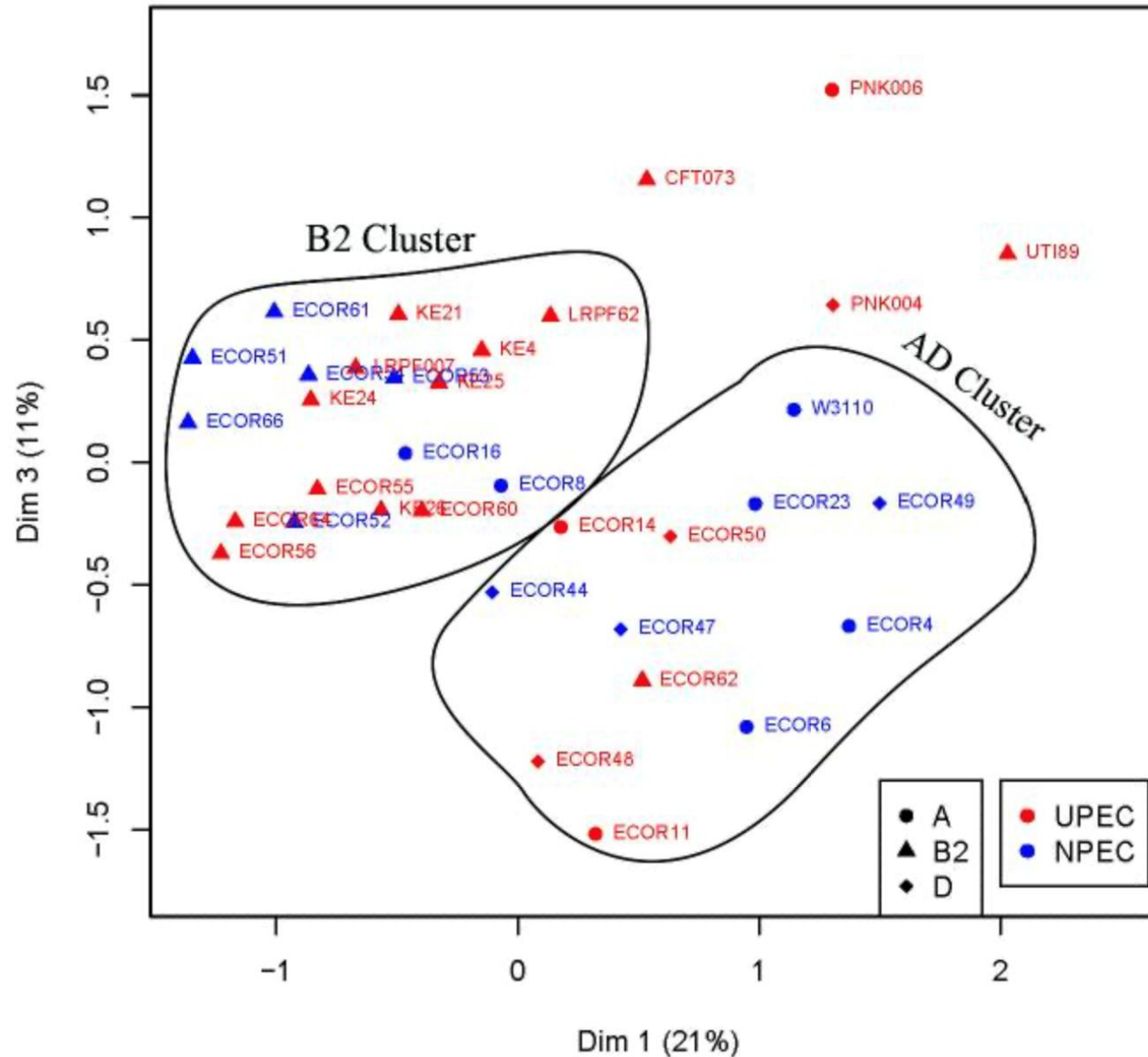
E. coli isolated from a patient diagnosed with UTI

Is there any significant difference
between any UPEC and its closest ABU relative

Not at the genome level

And...

Not at the transcriptome level



UPEC and **NPEC** cluster

by phylogroup
(sublineage)

NOT

by **UPEC** and **NPEC**

Transitions

We should retire the following concepts/terms!!!

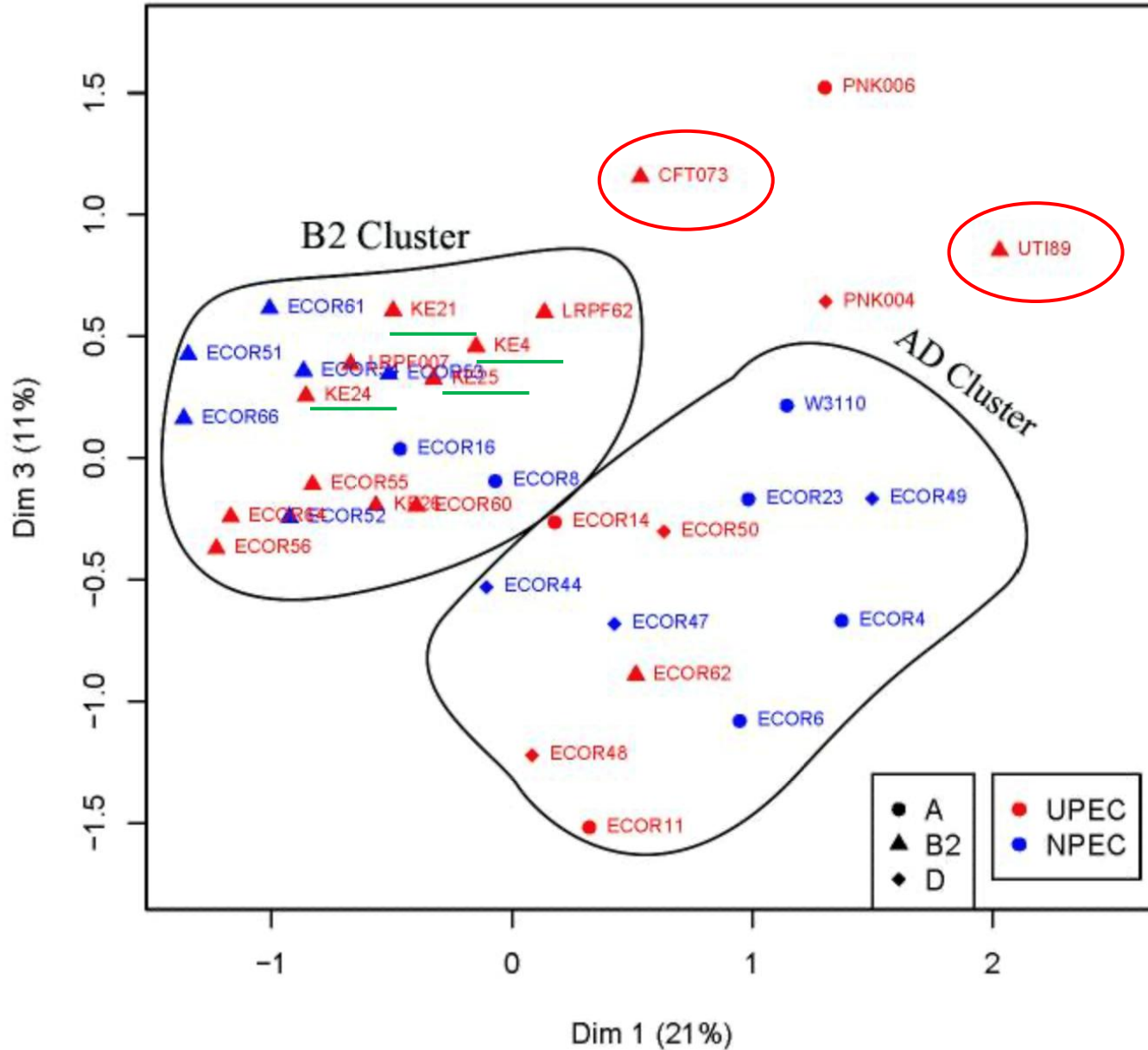
UTI89 and CFT073

Isolated decades ago

No longer represent *E. coli* currently circulating

Transcriptomes are outliers among other phylogroup B2 members

UTI89 and CFT073 are outliers



ECOR collection also isolated
decades ago

BUT KE strains are recent

Thus
ECOR collection
is more representative
of circulating strains
than
the canonical “UPEC” strains

Should we retire
“classic UPEC”
strains?

Anchor

Casadevall Pirofski 1999 PMID: 10417127
Casadevall Pirofski 2002 PMID: 12014430
Casadevall Pirofski 2003 PMID: 15040176

Transitions

We should retire the following concepts/terms!!!

Uropathogen

What's a pathogen?

Pathogenesis is easy to define

Pathogen not so much

Pathogenesis requires a host

Thus, other factors are involved

Bacterium of pathogenic potential?

Our hypothesis

- UTI/LUTS are ecological problems that involve
- the prospective, suspected “uropathogen(s)”
 - the rest of the urobiome
 - the host’s protective systems
(especially the immune response)

Just because a suspected “pathogen” is detected
DOES NOT MEAN

that this suspected “pathogen” is the cause of the symptoms
or that there is even pathogenesis/symptoms

An analogy:

A tale of two cities

Some cities are safe
Some are not

What's the difference?

Both have crime, but...

Some communities have
systems
to keep criminals under
control

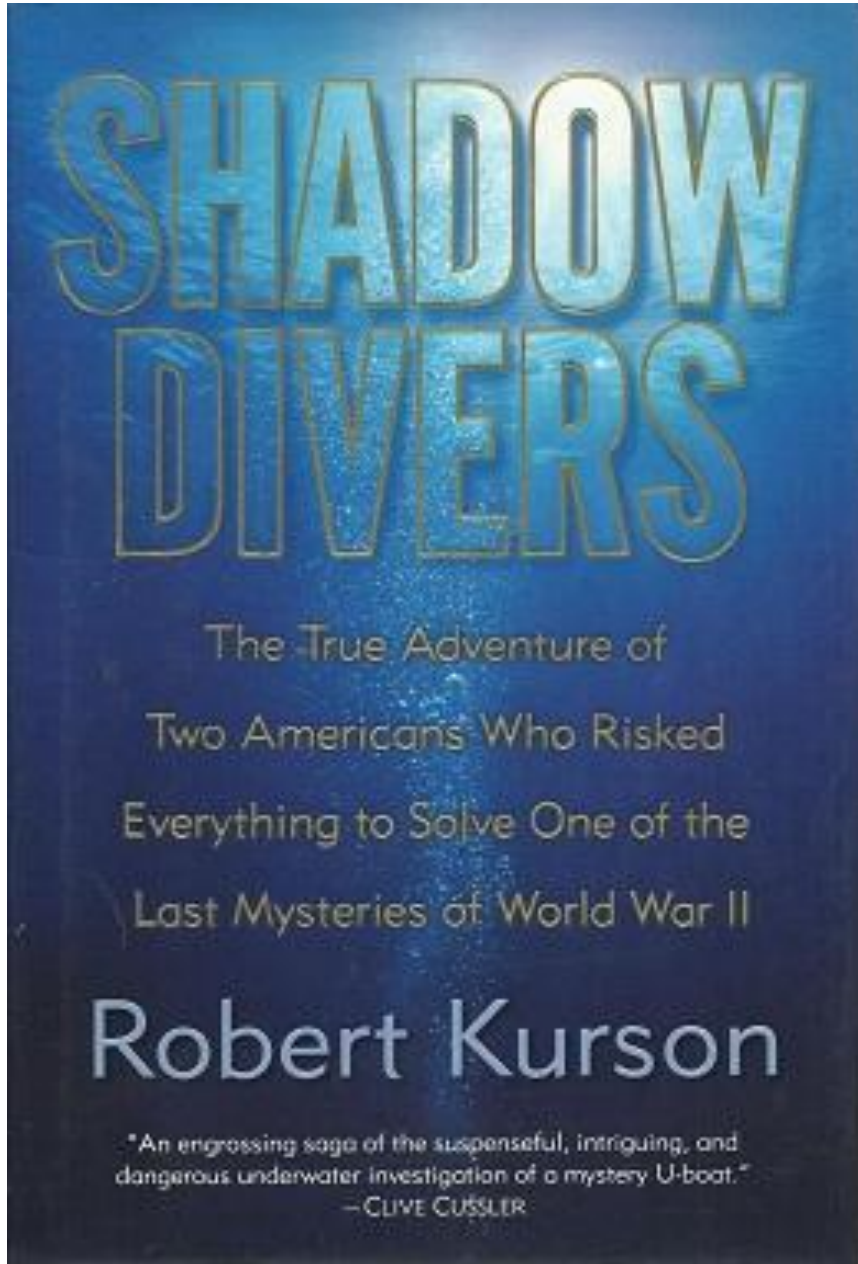
Others are run by the criminals



The urinary tract is no different

Some **communities** of microbes are “safe” = uroprotective
Some are not = uropathogenic

How can we tell them apart?



Clinicians make decisions
with limited information

Dipstick
SUC
Experience

Typical clinical approach is dipstick with reflex to SUC

**But solution is not urine dipstick or SUC
Both are deeply flawed**

**Dipstick depends on detection
of nitrate reductase activity and white blood cells
Many species don't have nitrate reductase
And leukocytes are fragile (time)**

**SUC is biased
misses many of the most common bacterial species
with uropathogenic potential**

Moreland 2025 PMID: 39641639
Brubaker 2023 PMID: 40778073

Solution is not urine dipstick or SUC
Both are deeply flawed

Haley et al., (2024)
Res Rep Urol. **16**:19-29

ORGANISM	GRAM STAIN	OXYGEN TOLERANCE	SUC	EQUC	TIER	PERCENT OCCURENCE	NITRATE REDUCTASE
Escherichia coli	Negative	Facultative anaerobe	Yes	All Conditions	1	41 (336/823)	Yes
Aerococcus urinae	Positive	Facultative anaerobe	Variable	BAP CO ₂	1	27 (222/823)	No
Actinotignum schaalii	Positive	Anaerobe	Not Detected	CNA Agar CO ₂	1	22 (224/823)	No
Enterococcus faecalis	Positive	Facultative anaerobe	Variable	CNA Agar CO ₂	1	22 (177/823)	No
Viridians Group Streptococcus					1	19 (160/823)	
Streptococcus anginosus	Positive	Facultative anaerobe	Not Detected	CNA Agar CO ₂	1	-	No
Streptococcus oralis	Positive	Facultative anaerobe			1	-	Variable
Streptococcus pasteurianus	Positive	Facultative anaerobe			1	-	No
Klebsiella pneumoniae	Negative	Facultative anaerobe	Yes	All Conditions	2	13 (97/823)	Yes

% occurrence in patients diagnosed with UTI

Identification by M-PCR

For example,

VGS =
S. anginosus,
S. oralis, S. pasteurianus

M-PCR detected 19%

SUC <1%

Our hypothesis

- It's an ecological problem that involves
- the prospective, suspected “uropathogen(s)”
 - the rest of the urobiome
 - the host's protective systems

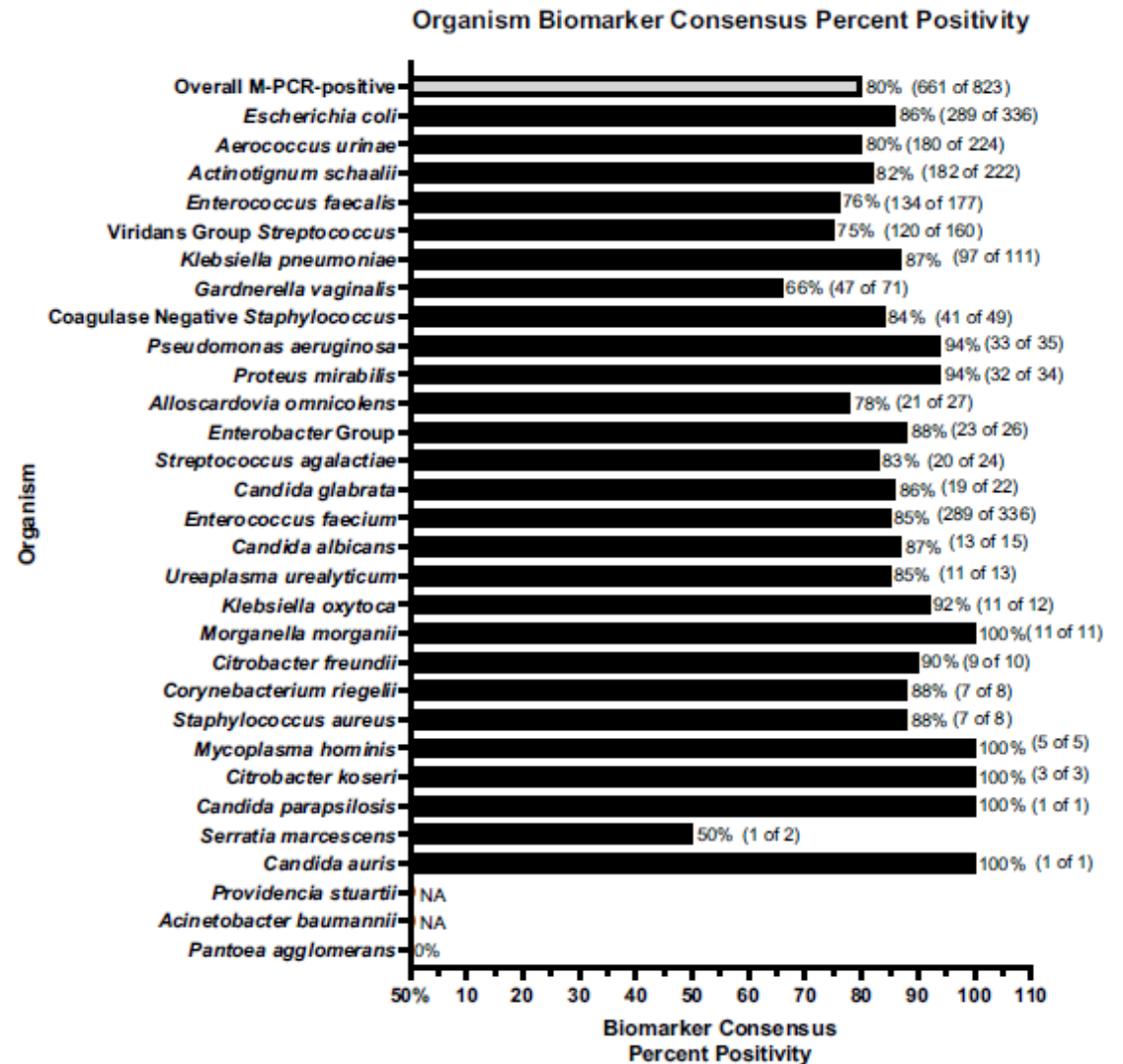
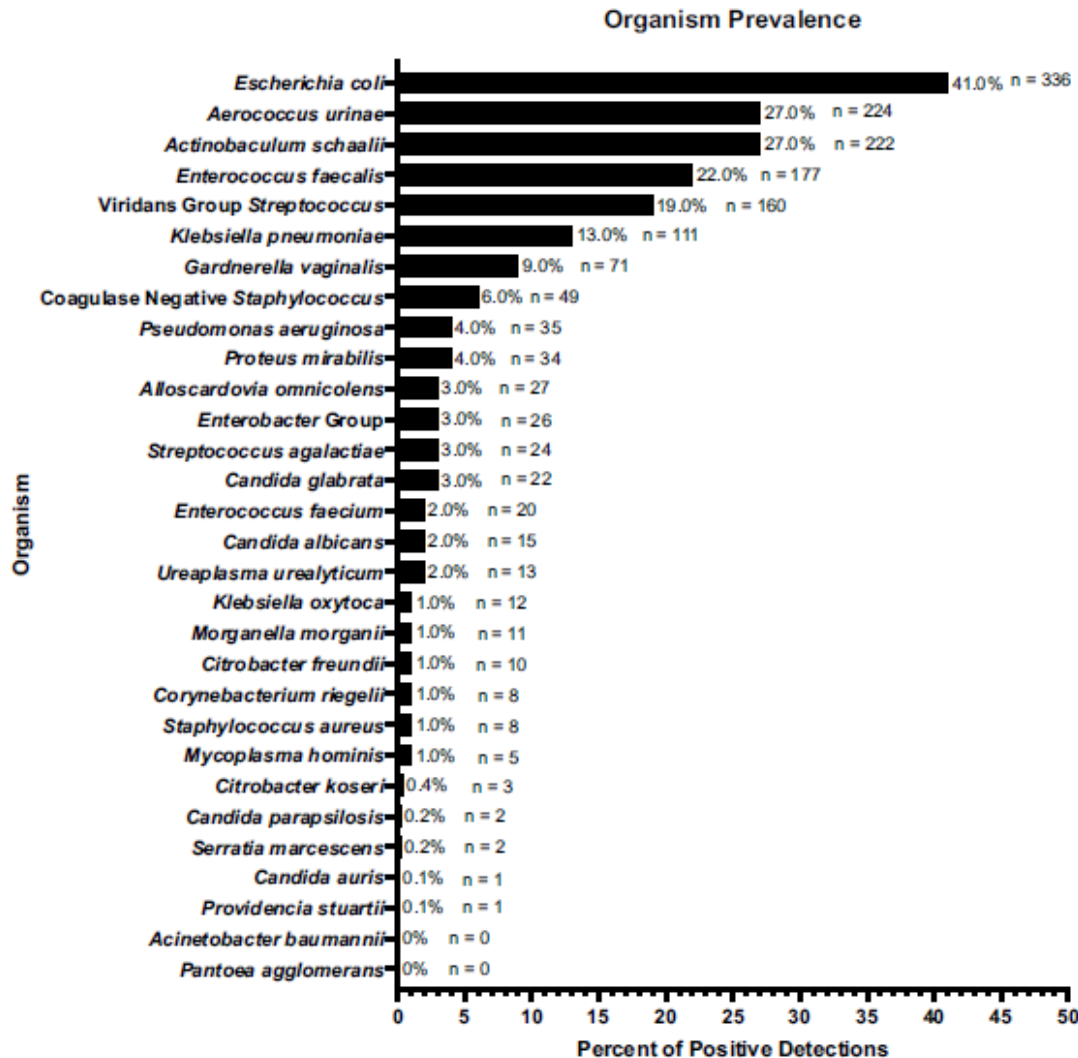
*Can we distinguish
healthy from unhealthy communities?*

Now let's look at the host response

A solution is more comprehensive detection method plus more predictive immune biomarkers

ORGANISM	GRAM STAIN	OXYGEN TOLERANCE	SUC	EQUC	TIER	PERCENT OCCURENCE	NITRATE REDUCTASE	NGAL (% ₁ , n)	IL-8 (% ₁ , n)	IL-1b (% ₁ , n)
Escherichia coli	Negative	Facultative anaerobe	Yes	All Conditions	1	41 (336/823)	Yes	81 (670/823)	86 (709/823)	64 (539/823)
Aerococcus urinae	Positive	Facultative anaerobe	Variable	BAP CO ₂	1	27 (222/823)	No	86 (192/224)	86 (193/224)	63 (142/224)
Actinotignum schaalii	Positive	Anaerobe	Not Detected	CNA Agar CO ₂	1	22 (224/823)	No	82 (182/222)	88 (196/222)	63 (140/222)
Enterococcus faecalis	Positive	Facultative anaerobe	Variable	CNA Agar CO ₂	1	22 (177/823)	No	76 (134/177)	86 (153/177)	58 (103/177)
Viridians Group Streptococcus					1	19 (160/823)		78 (125/160)	81 (130/180)	56 (90/180)
Streptococcus anginosus	Positive	Facultative anaerobe	Not Detected	CNA Agar CO ₂	1	-	No	-	-	-
Streptococcus oralis	Positive	Facultative anaerobe			1	-	Variable	-	-	-
Streptococcus pasteurianus	Positive	Facultative anaerobe			1	-	No	-	-	-
Klebsiella pneumoniae	Negative	Facultative anaerobe	Yes	All Conditions	2	13 (97/823)	Yes	86 (96/111)	91 (101/111)	68 (76/111)

M-PCR of Symptomatic UTI Patients Correlates with Urinary Biomarkers of Infection



Our hypothesis

UTI/LUTS are ecological problems that involve

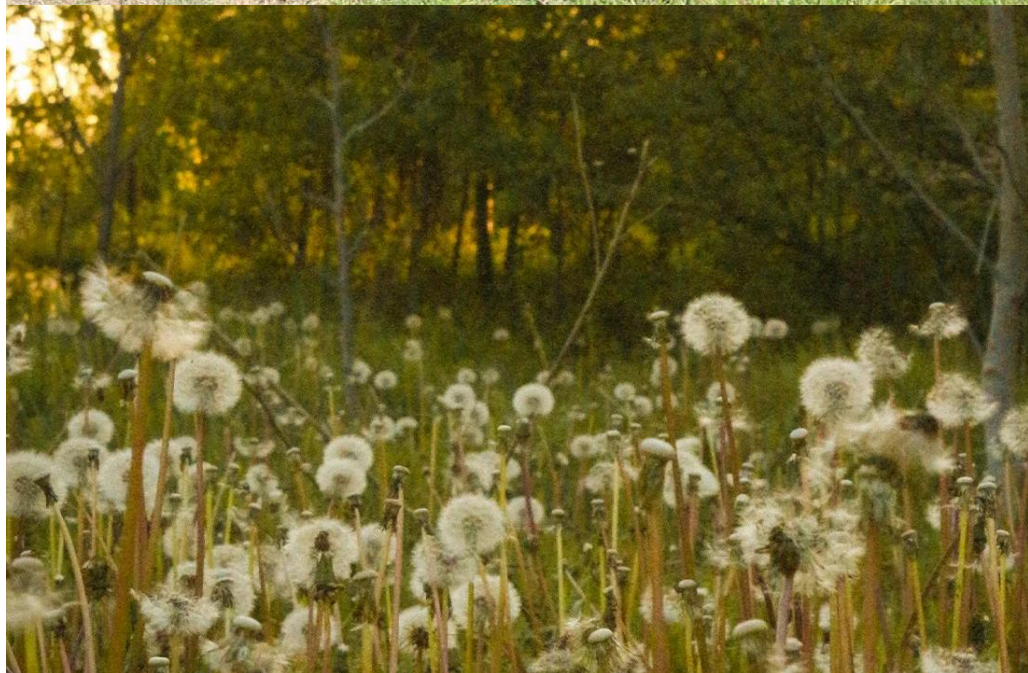
- the prospective, suspected “uropathogen(s)”
 - the rest of the urobiome
- the host’s protective systems

Can we restore balance?

*Push unhealthy (dysbiotic) communities
towards healthy (eubiotic) communities*

Another analogy

Prairie restoration



Regenerative farming

a way of growing food that heals the land,
focusing on **soil health**, **biodiversity**, and **water retention**.



A current attempt to restore the ecosystem



► [Top Spinal Cord Inj Rehabil.](#) 2023 Aug 16;29(3):44–57. doi: [10.46292/sci23-00004](#) [↗](#)

Intravesical *Lactobacillus rhamnosus* GG Alters Urobiome Composition and Diversity Among People With Neurogenic Lower Urinary Tract Dysfunction

[Suzanne L Groah](#)^{1,2,✉}, [Amanda K Rounds](#)^{1,3}, [Marcos Pérez-Losada](#)⁴

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PMCID: PMC10644857 PMID: [38076286](#)

Next Goal

Distinguish between
healthy (eubiotic) & unhealthy (dysbiotic) communities
Identify and sort ecosystems

Analogy
breast cancer

Luminal A, Luminal B, HER2-positive, and Triple-negative

Ultimate Goal

Embrace the new knowledge
to improve patient outcomes

Thanks to

Attendings, Fellows, Residents, Nurses

Wolfe lab members

Collaborators

Funders

&

Participants