



**Vasilios Kalas**



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# Urinary Tract Infection



Antibiotic-sparing Therapeutic Revolution

To Your Health

# The superbug that doctors have been dreading just reached the U.S.

By **Lena H. Sun** and **Brady Dennis** May 27 



CRE, a family of bacteria pictured here, is considered one of the deadliest superbugs. It causes infections that are often resistant to most antibiotics. (Centers for Disease Control and Prevention/Reuters)

For the first time, researchers have found a person in the United States carrying bacteria resistant to antibiotics of last resort, an alarming development that the top U.S. public health official says could mean “the end of the road” for antibiotics.

The antibiotic-resistant strain was found last month in the urine of a 49-year-old



Health » Diet + Fitness | Living Well | Parenting + Family

Live TV

## A dreaded superbug found for time in a U.S. woman



By **Jen Christensen** and **Debra Goldschmidt**, CNN

Updated 2:48 PM ET, Fri May 27, 2016

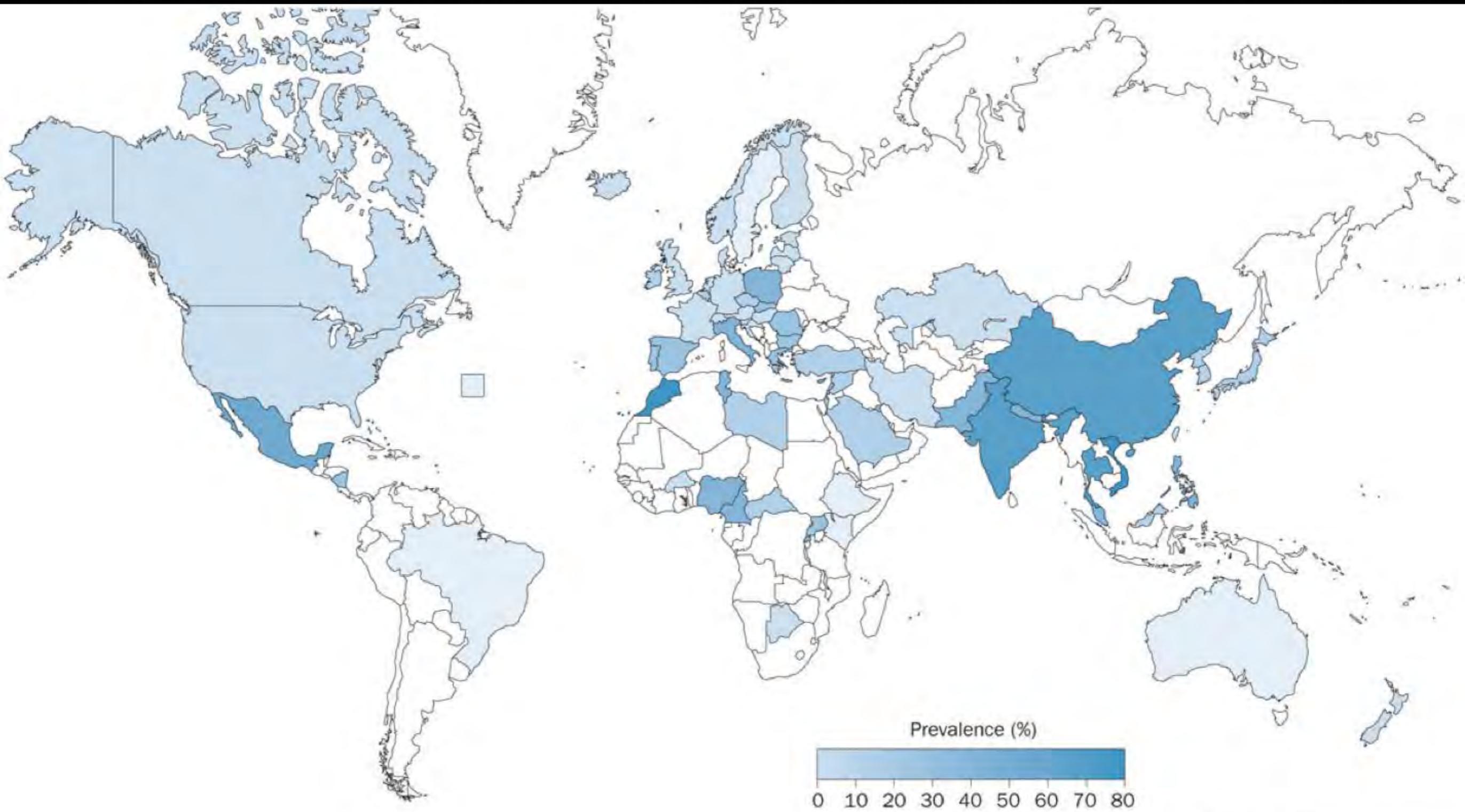


Navigation bar for video player with thumbnails and titles:

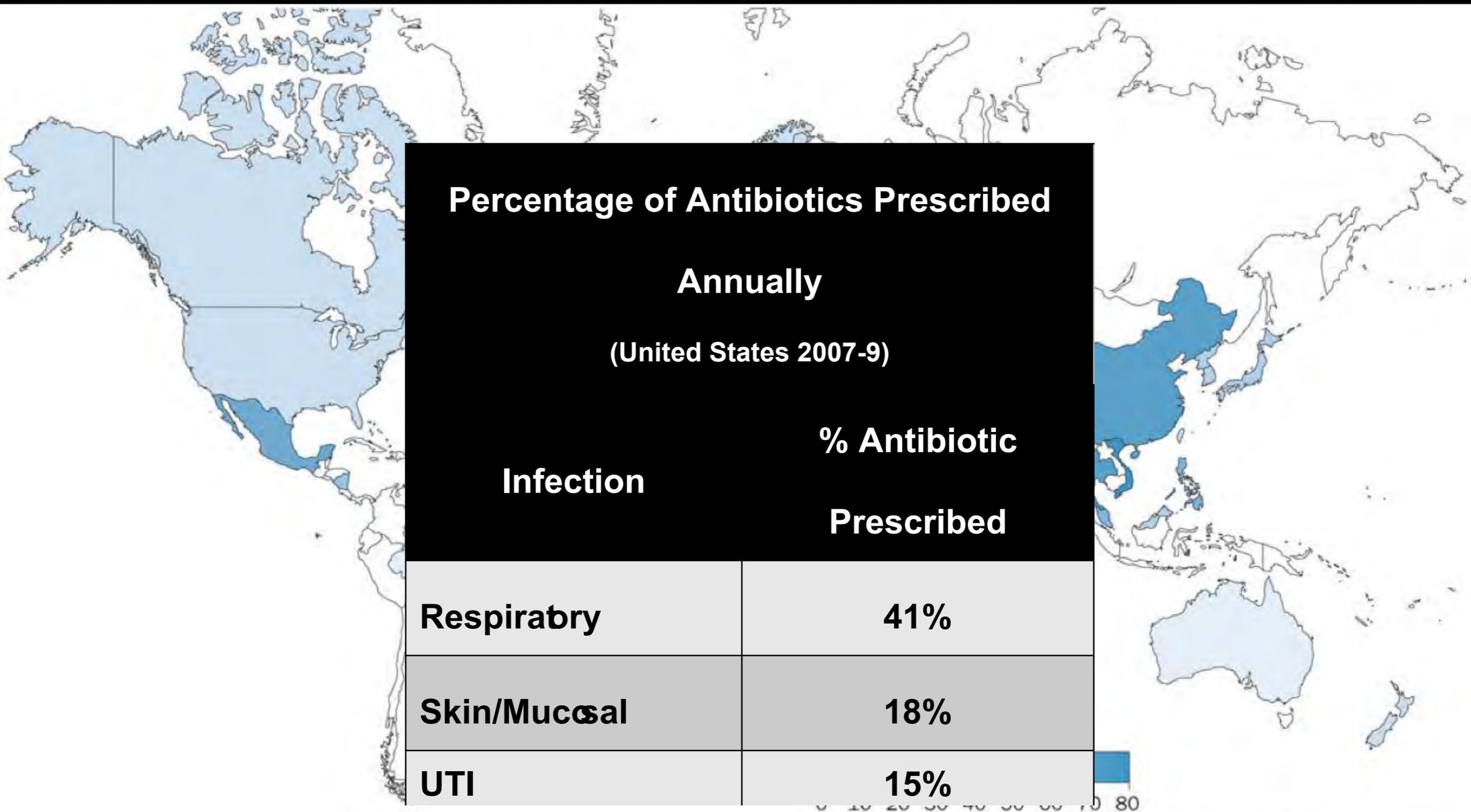
- Now Playing**: Superbug found in U.S. woman
- 00:55: How bugs become superbugs
- 02:07: CDC sees 'steady increase' in
- 01:49: These foods aren't as healthy as you think
- 02:40: Ways to treat heartburn

**Highlights an urgent need to develop a vaccine and new and better therapeutics**

# Global epidemiology of fluoroquinolone resistance in UPEC



# Global epidemiology of fluoroquinolone resistance in UPEC

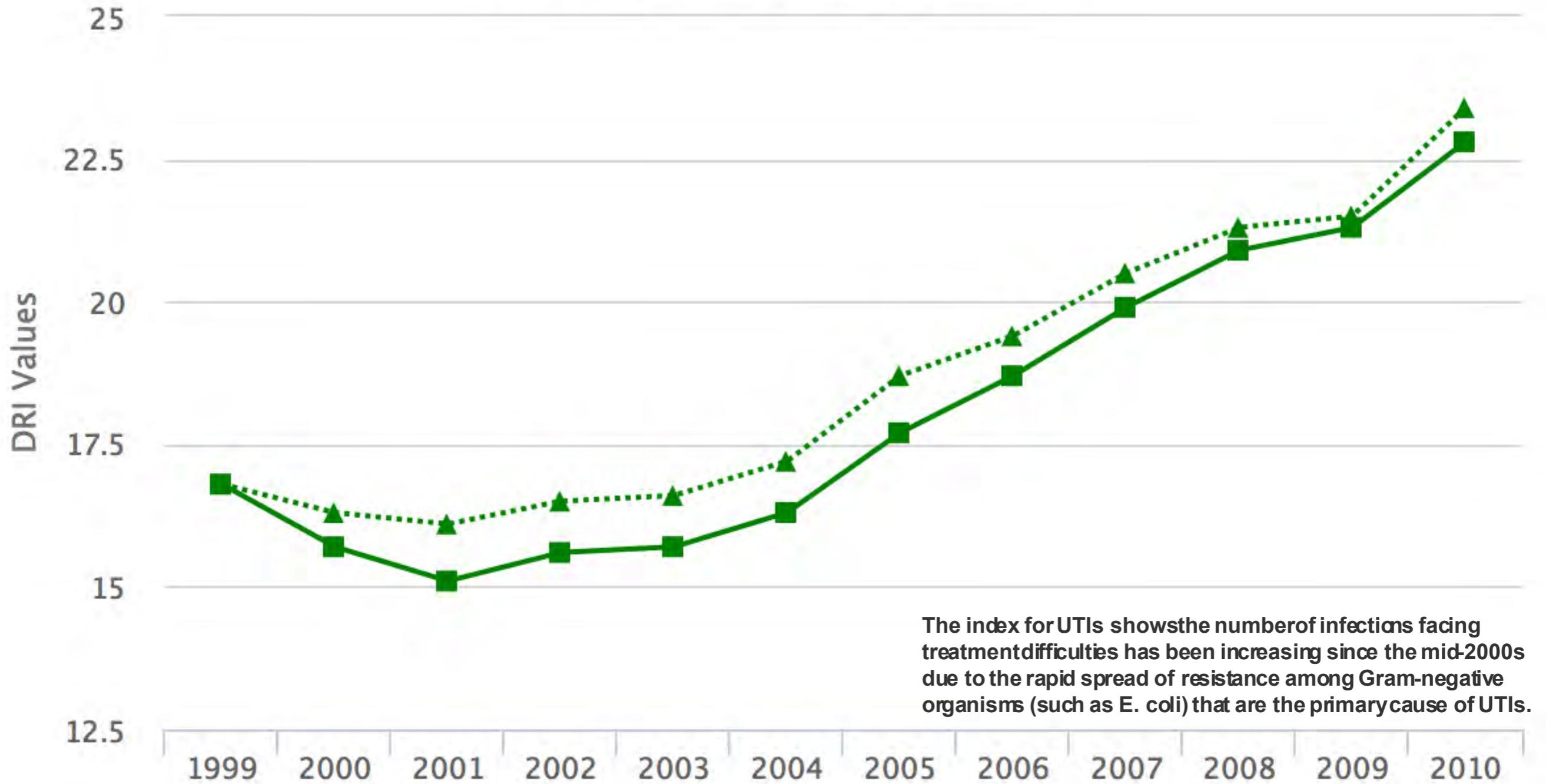


**Women's health is intricately intertwined with the spread of antibiotic**

# DRUG RESISTANCE INDEX

DRI provides an aggregate trend measure of the effectiveness of available drugs.

US region grouping based on census.



The index for UTIs shows the number of infections facing treatment difficulties has been increasing since the mid-2000s due to the rapid spread of resistance among Gram-negative organisms (such as E. coli) that are the primary cause of UTIs.

—■— Uropathogens DRI (Adaptive)

.....▲..... Uropathogens DRI (Fixed)

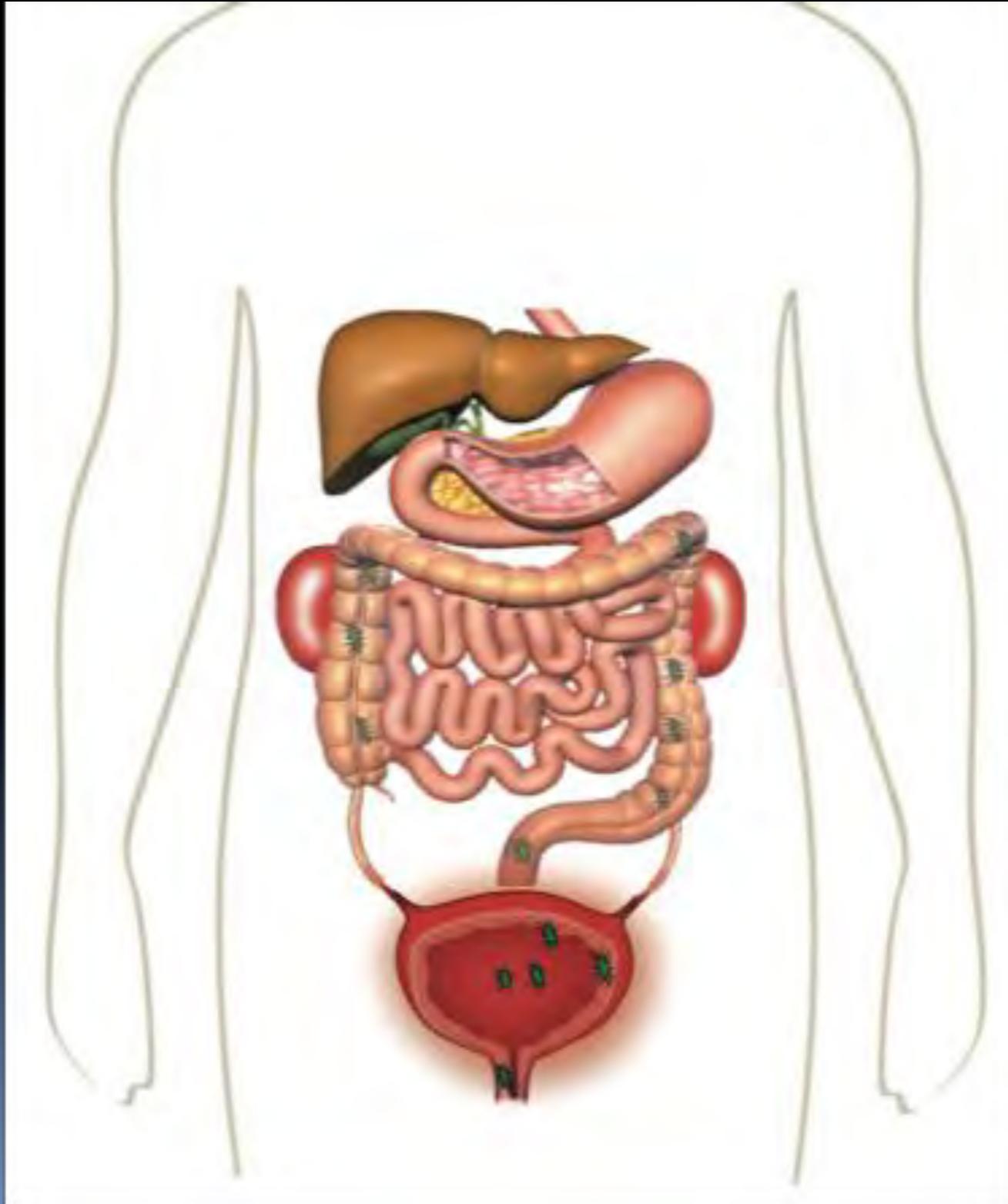
- Over 15M women suffer from UTIs per year with cost over \$2.5 billion
- Chronic/Recurrent
- Multi-drug resistant bacteria
- Lead to inadequate treatment options
- CA-UTI adds \$1 Billion to US healthcare costs
- Abx resistance is intricately intertwined with women's health

# UTI risk: matching urovirulence phenotypes with dynamic host susceptibility determinants

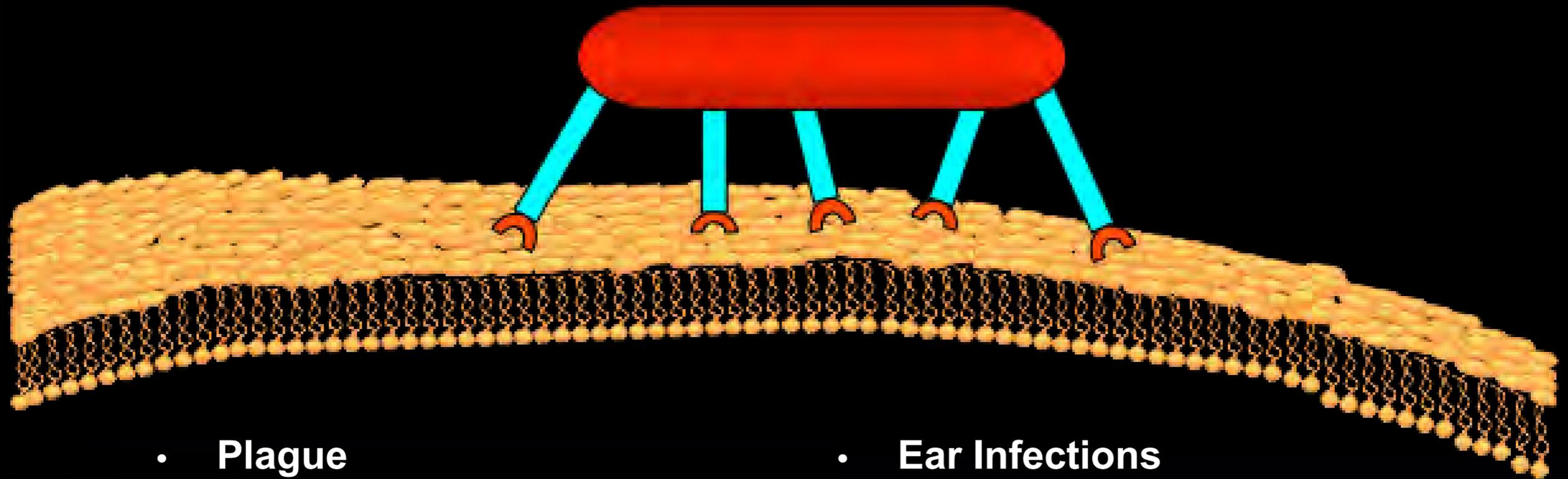


- **UPEC occupies diverse habitats (gut, bladder, kidney, etc.) and each has unique sets of colonization requirements (“Locks”)**
- **UPEC strains contain variable sets of fitness factors (“Keys”) enabling colonization depending on the host**
- **Colonization and persistence occurs when a “Lock” is opened by the matching “Key.”**
- **The shape of Locks can change based on history, genetics, and behavior.**
- **UTI Complexity Results from Diversity at the Bacterial-Host Interface**

# Bacterial Attachment



# Pili allow bacteria to stick around

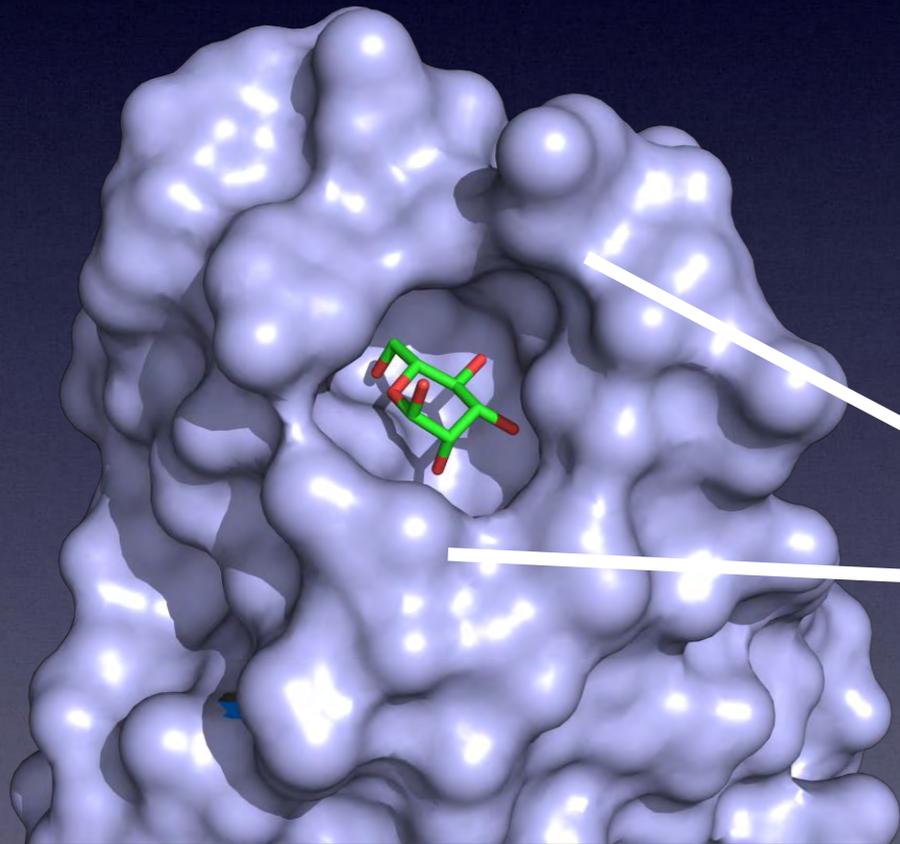
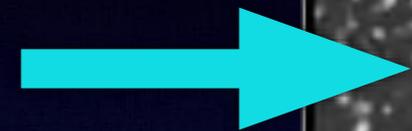


- Plague
- Pneumonia
- Cystic Fibrosis
- Biofilms - Wound Infections
- Food-Borne Illness
- Ear Infections
- Whooping Cough
- Urinary Tract Infection
- Catheter Infections
- Heart Infections (Endocarditis)

**Pili used by diverse human pathogens**

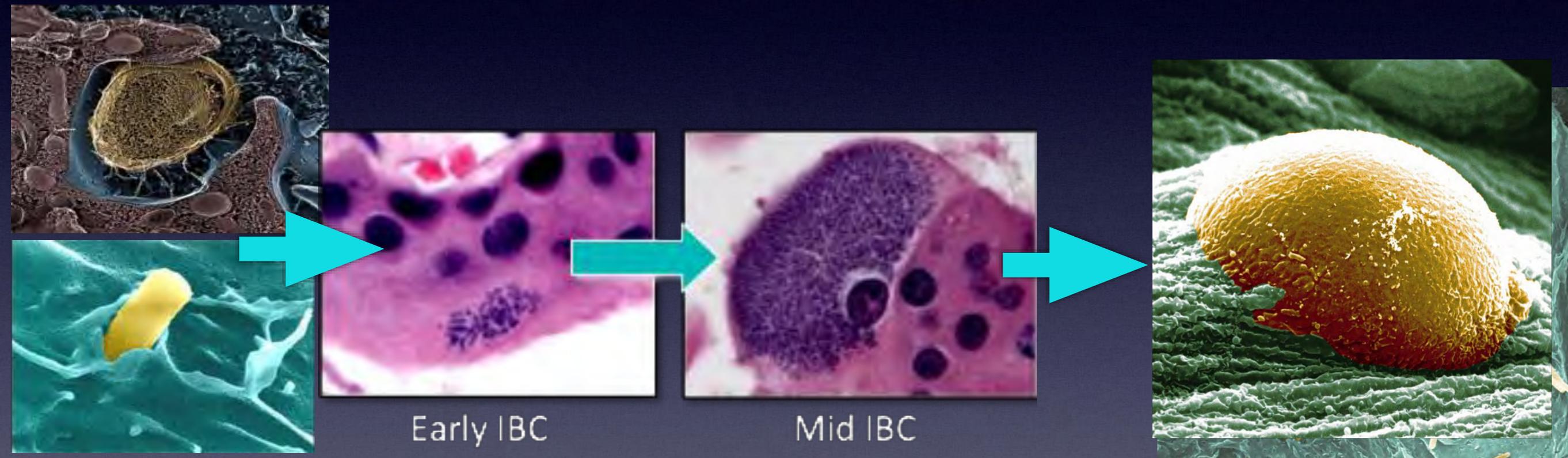
# FimH-Mediated binding of *E. coli* to bladder

Type 1 pili are tipped FimH



FimH-mannose

# UPEC form Intracellular Bacterial Communities (IBC)



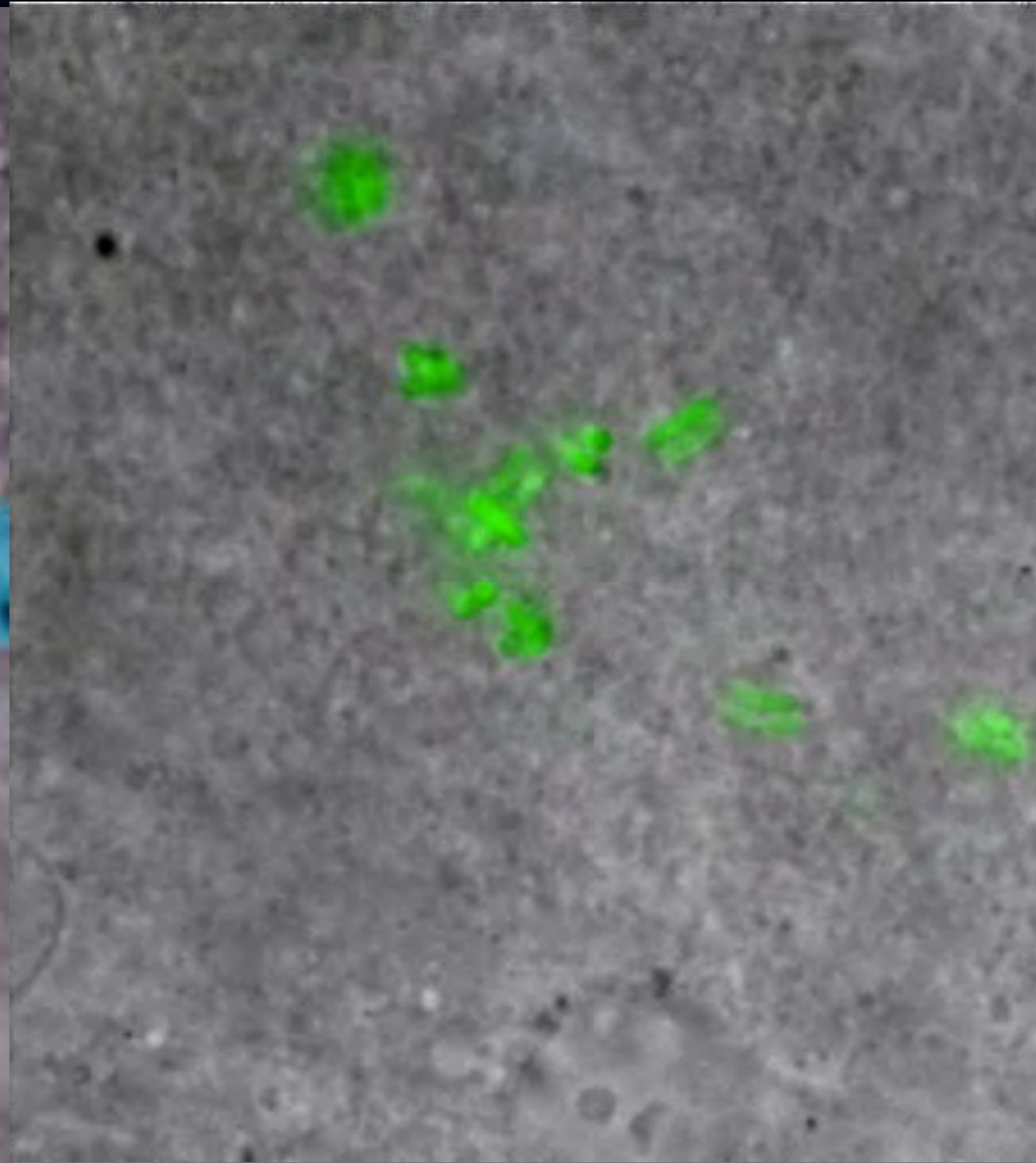
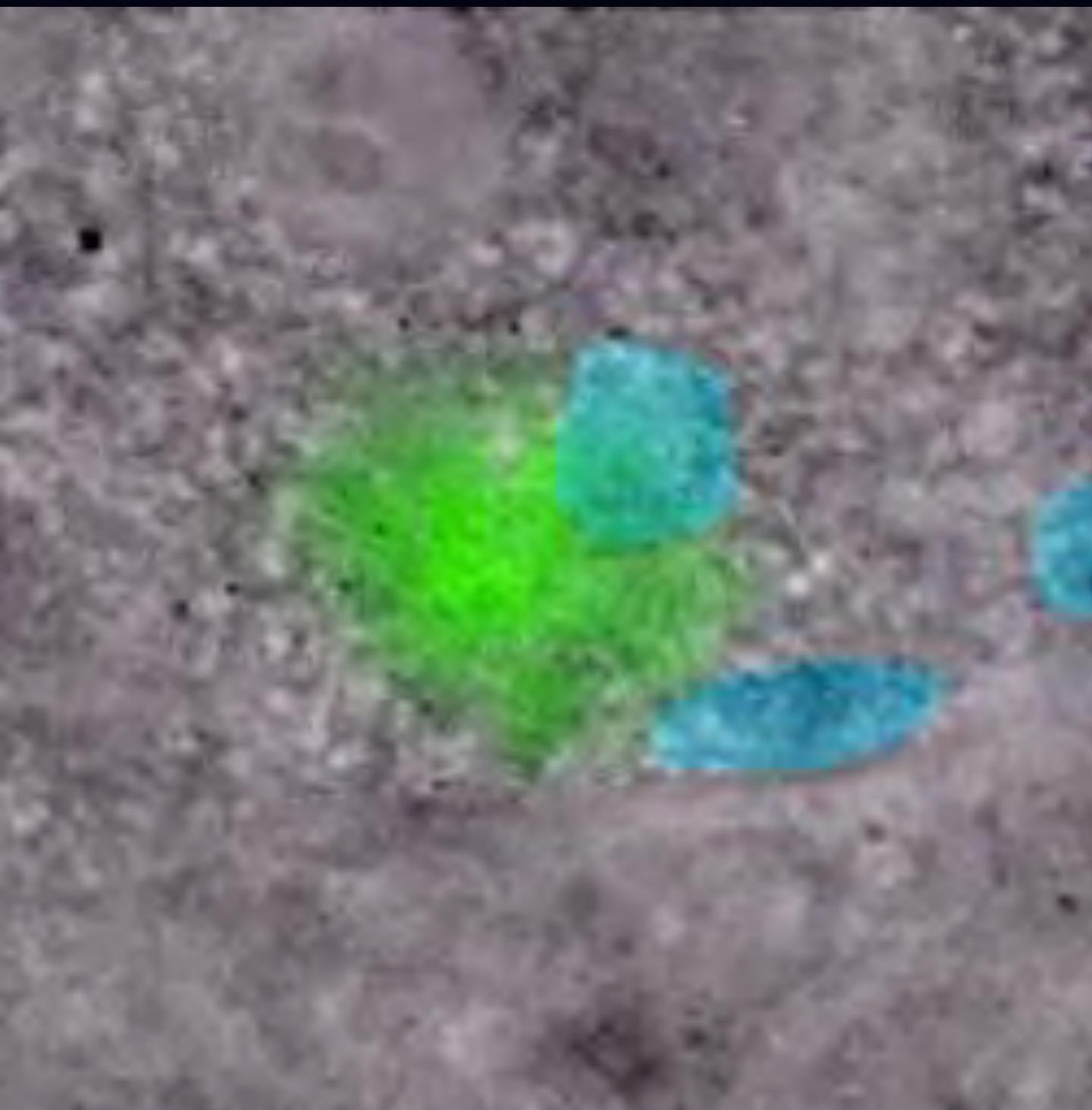
Early IBC

Mid IBC

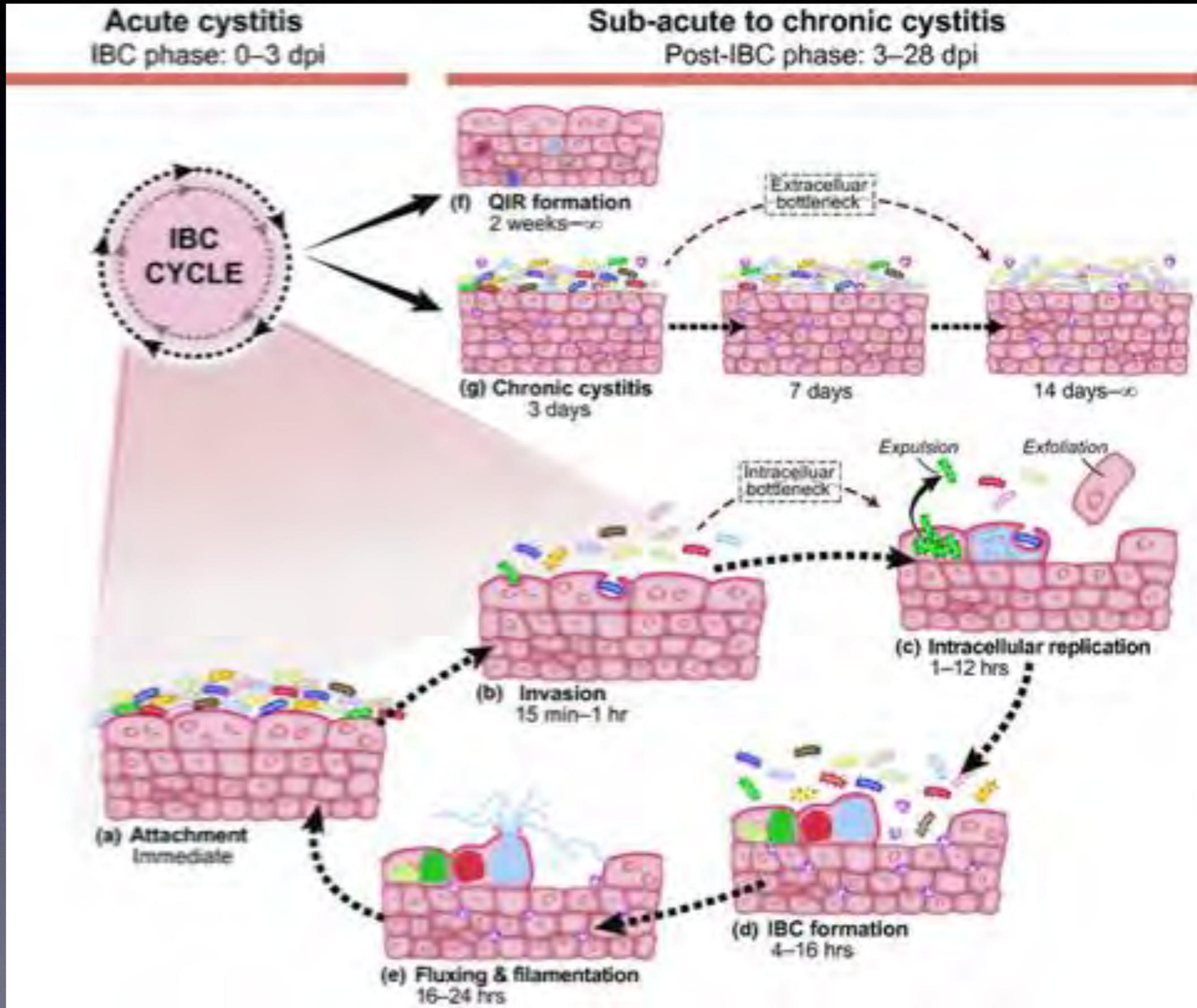
MOLECULAR VELCRO



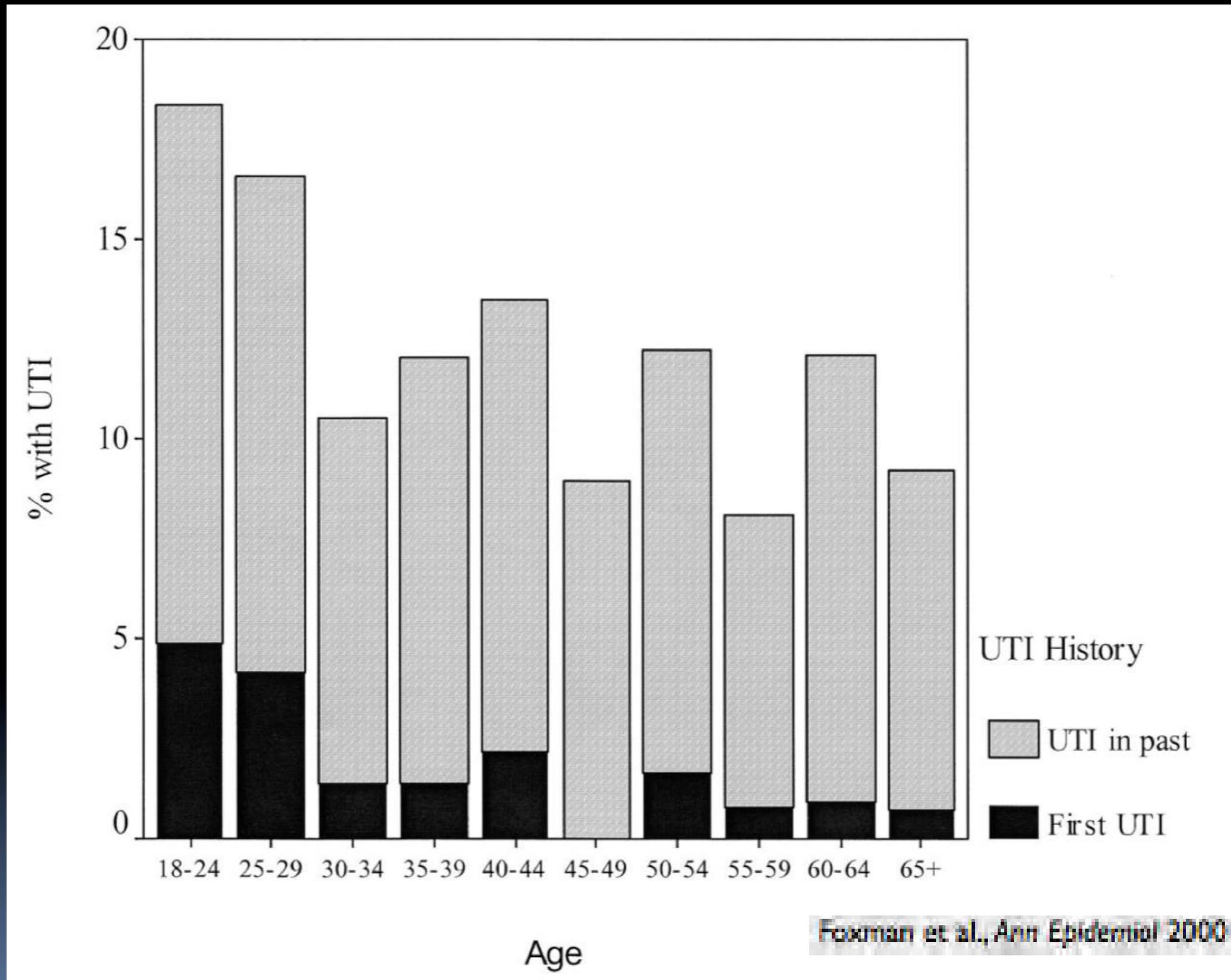
# Bacterial Communities Escape Attack by Immune Cells



# Uropathogenic *E. coli* (UPEC) infection of the urinary bladder has distinct acute and chronic phases

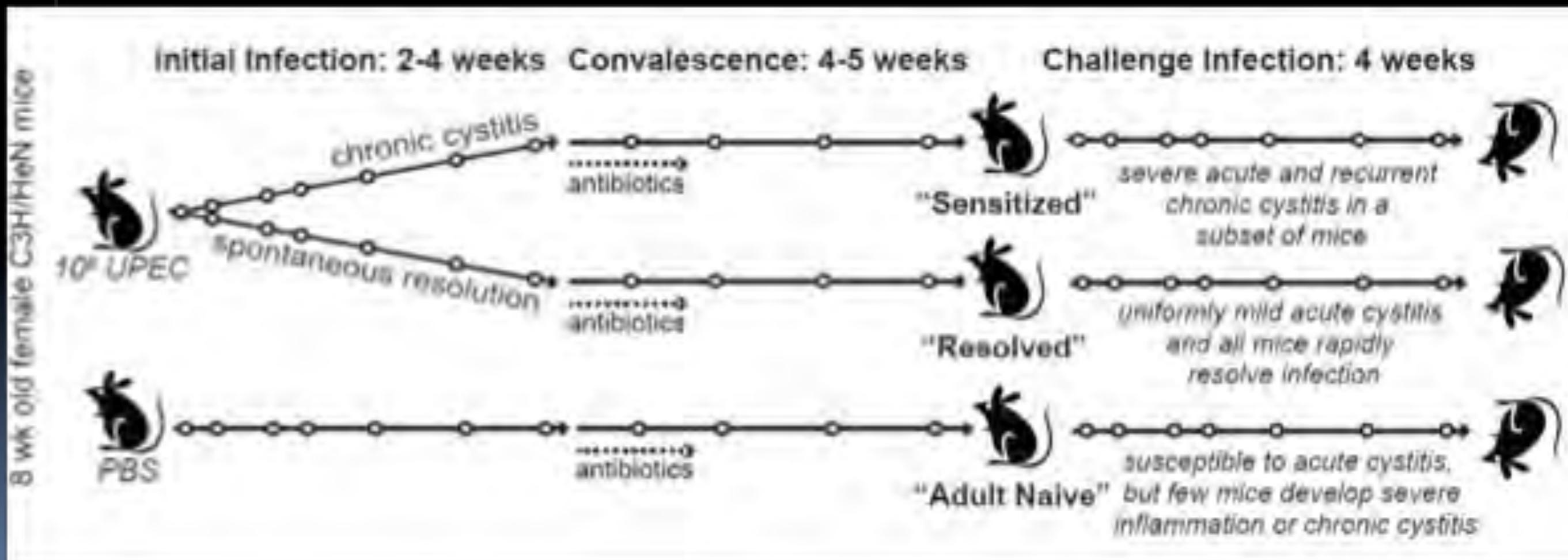


# History of UTI is among the most significant risk factors

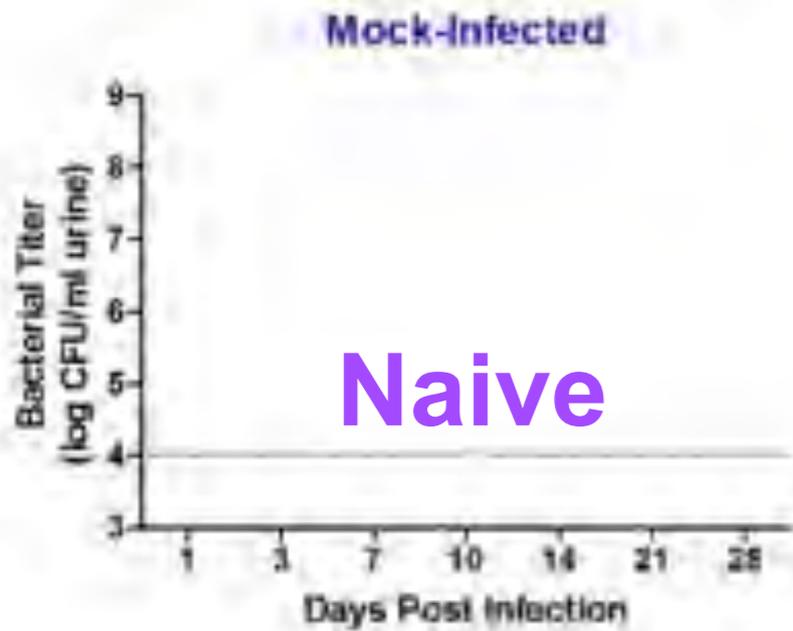


UTI pathogenesis has exclusively been studied in naive mice, but may not reflect rUTI pathogenesis.

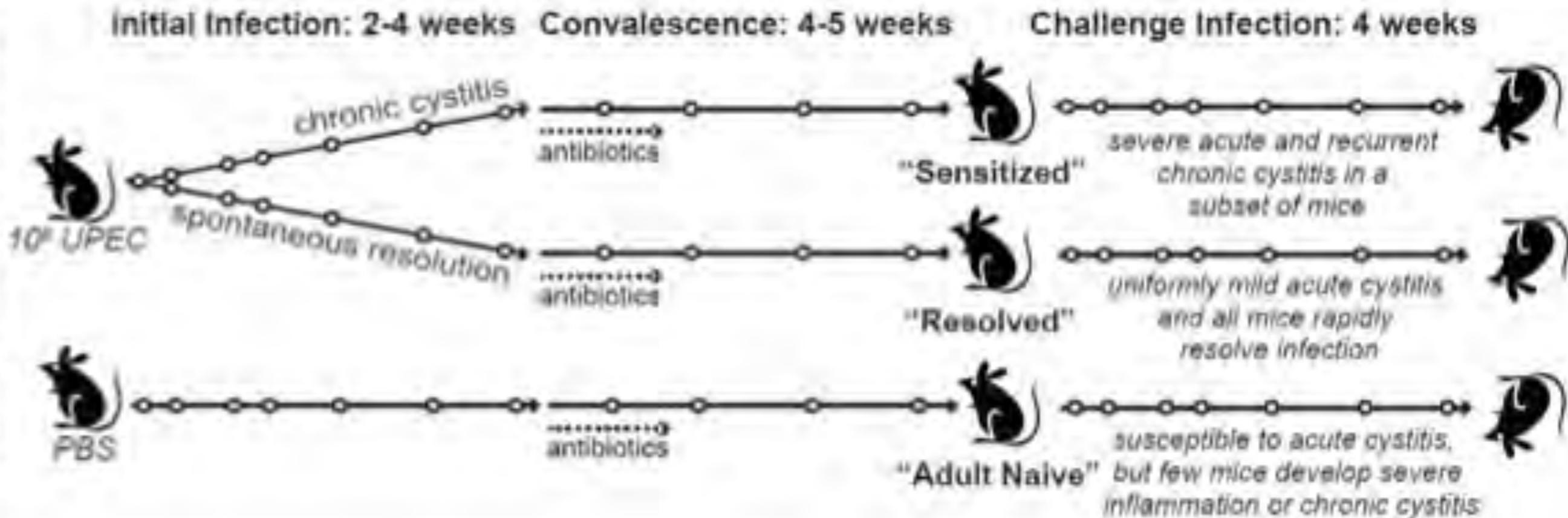
# Investigate how prior history of UTI impacts the pathogenesis of rUTI



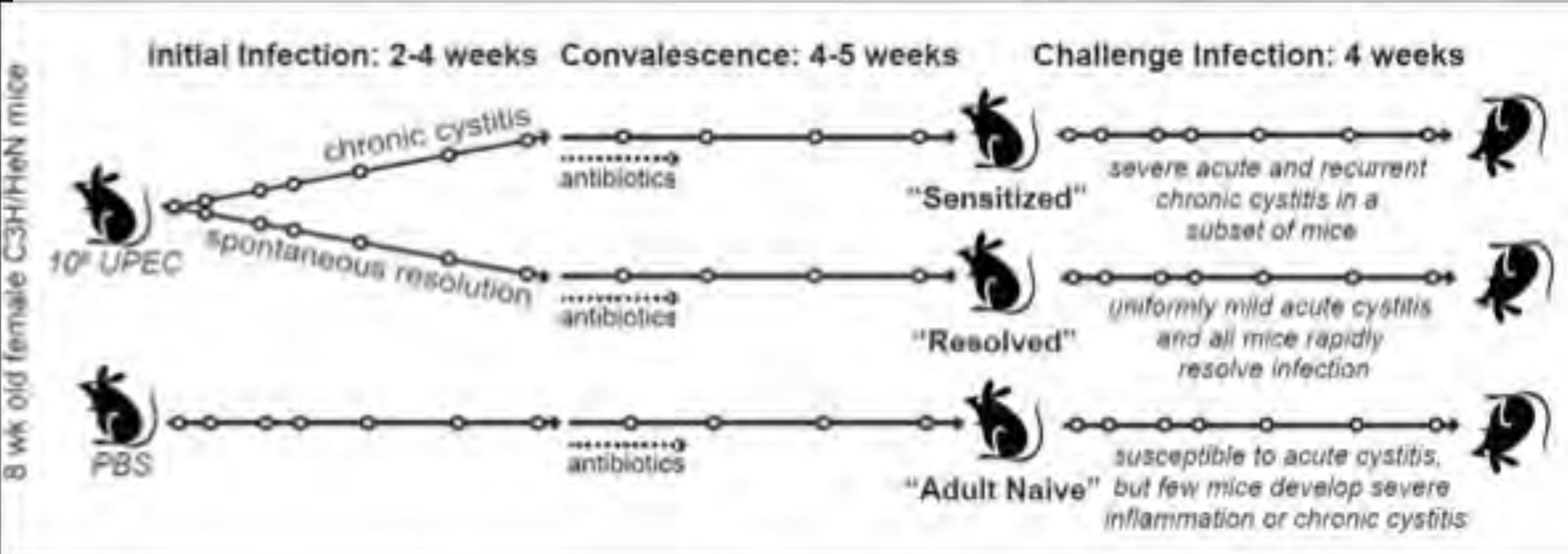
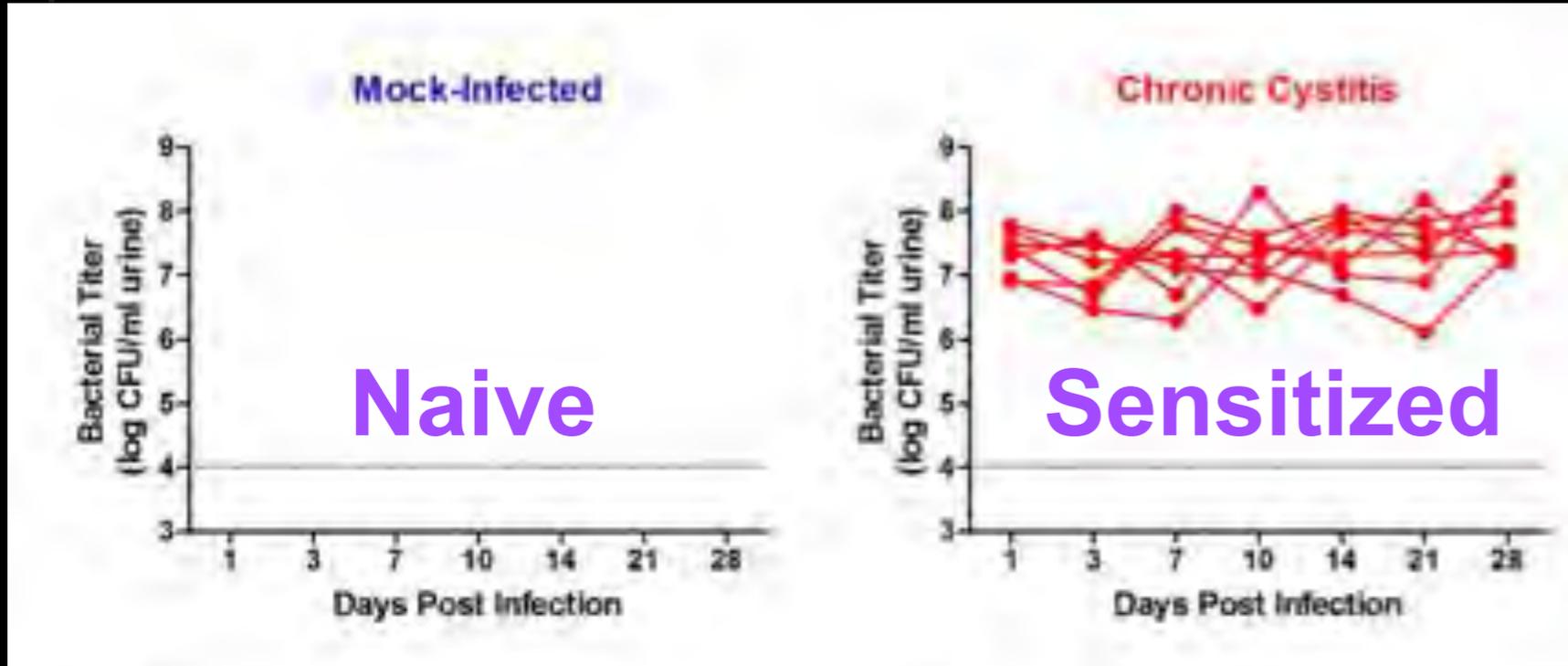
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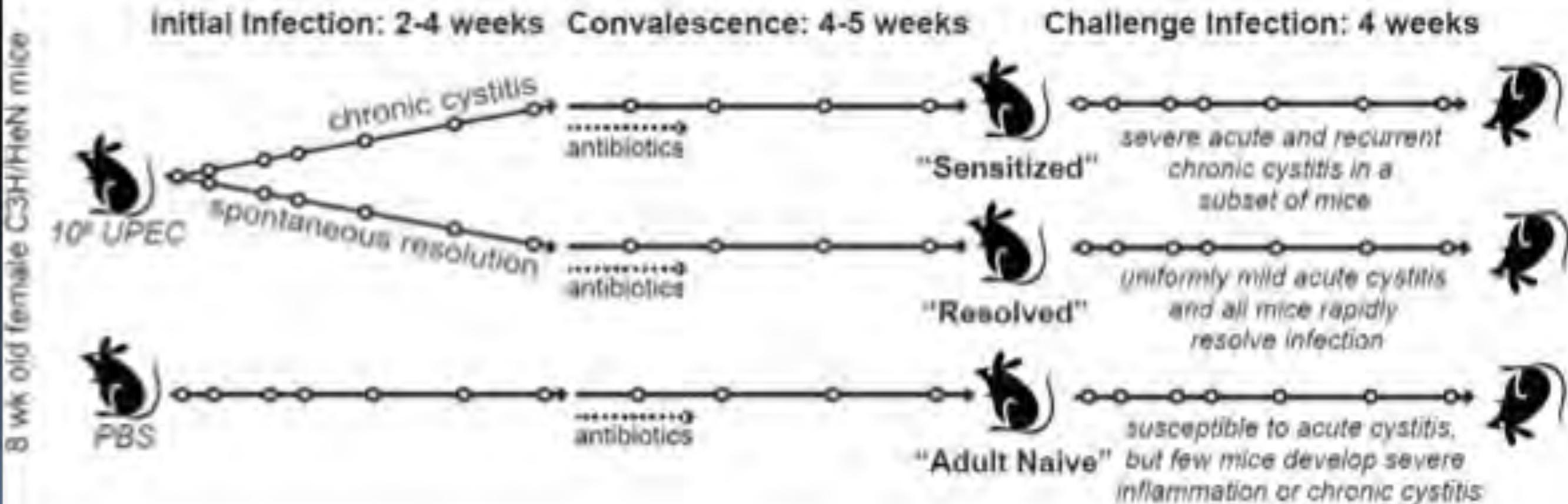
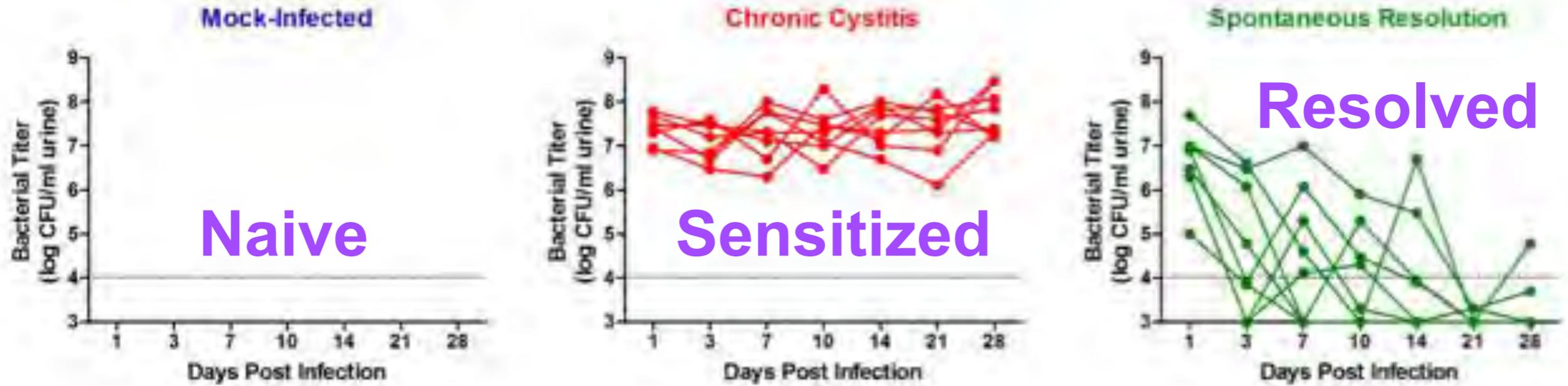
8 wk old female C3H/HeN mice



# Investigate how prior history of UTI impacts the pathogenesis of rUTI

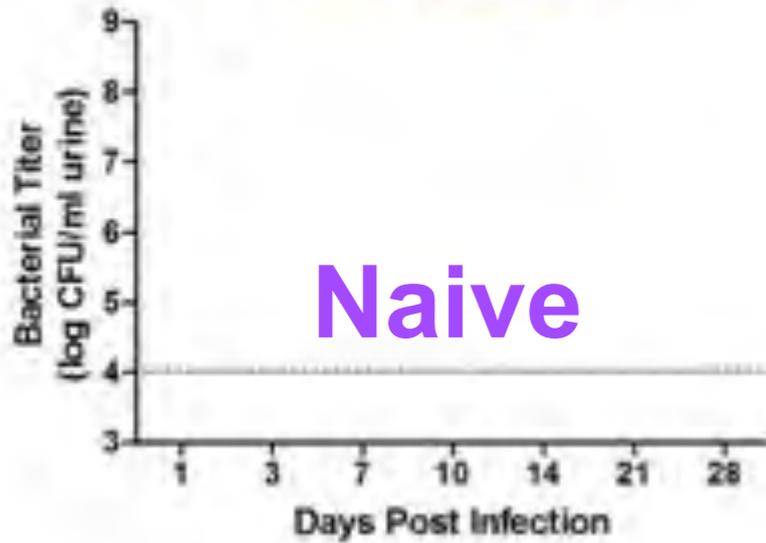


# Investigate how prior history of UTI impacts the pathogenesis of rUTI



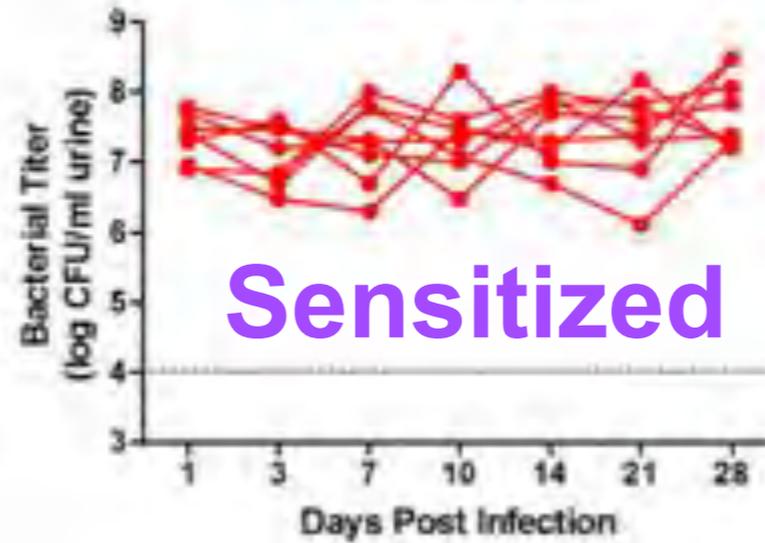
# History of infection sensitizes recurrent UTI

Mock-Infected



Naive

Chronic Cystitis



Sensitized

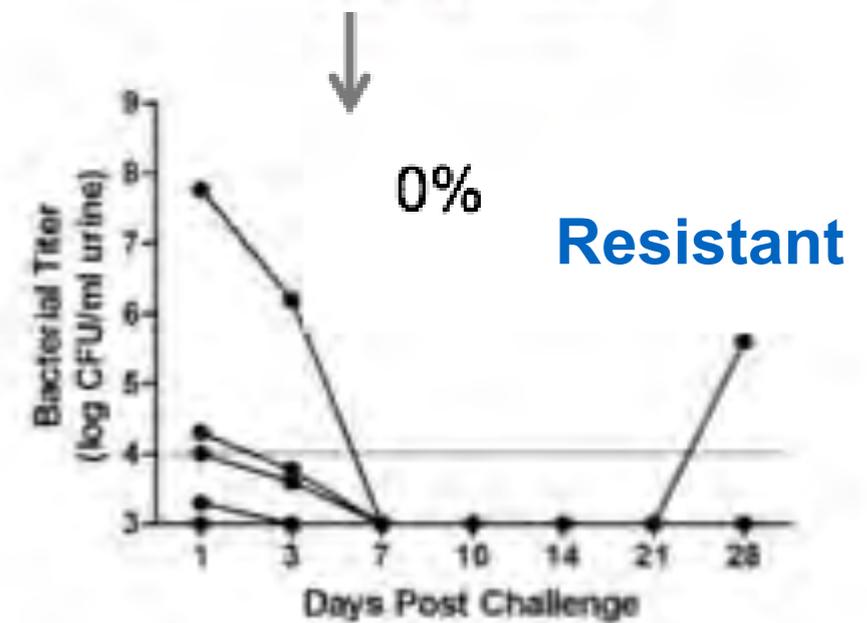
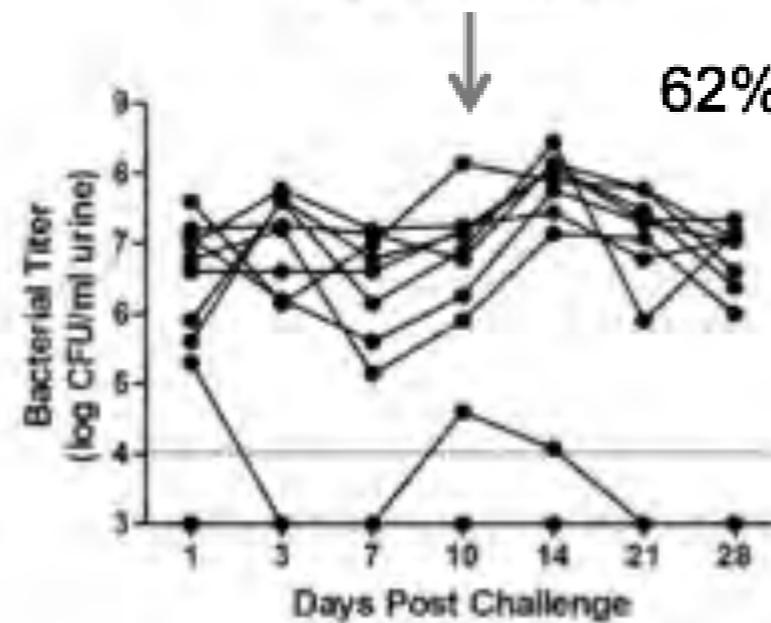
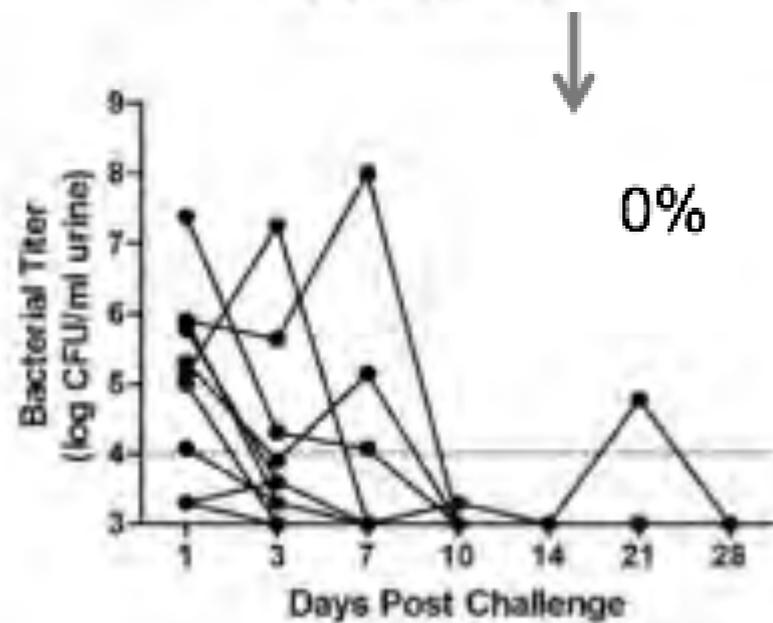
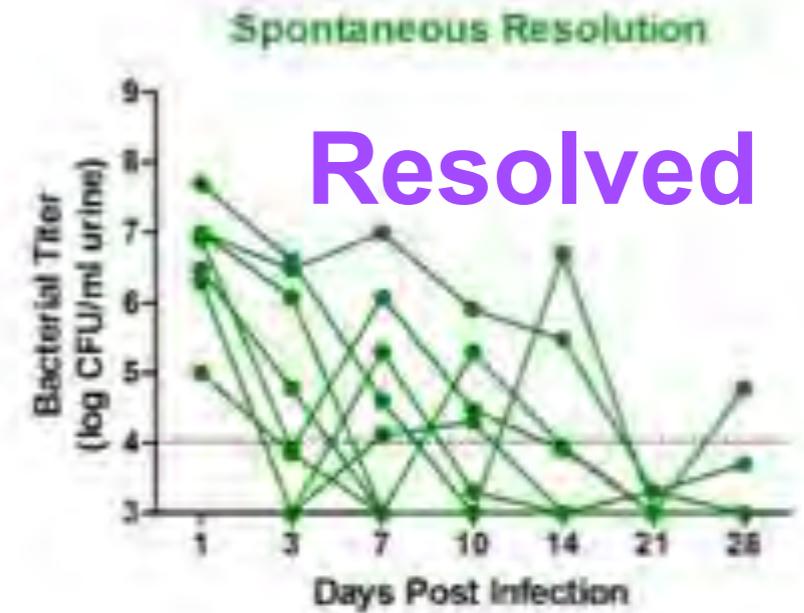
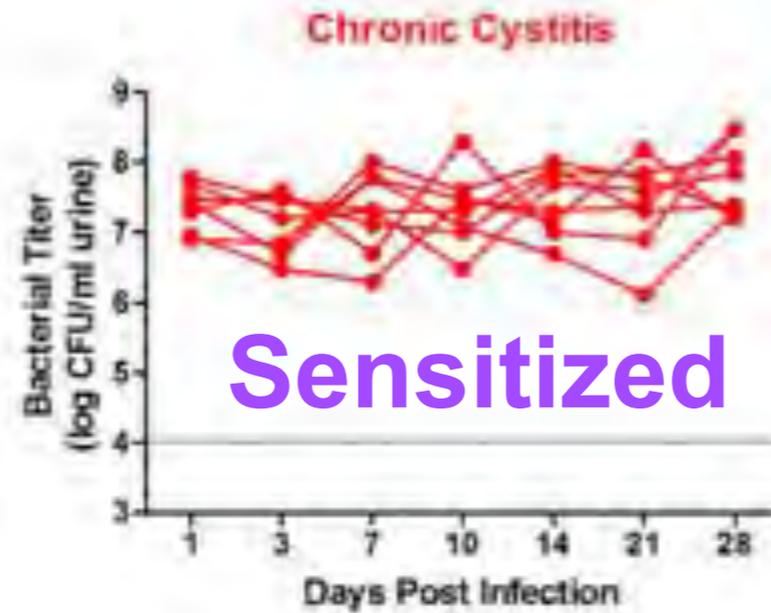
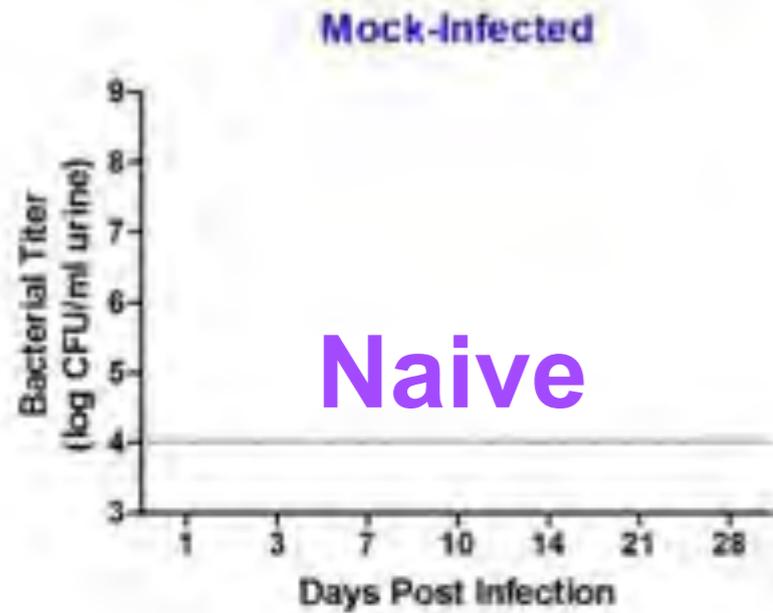
Spontaneous Resolution



Resolved



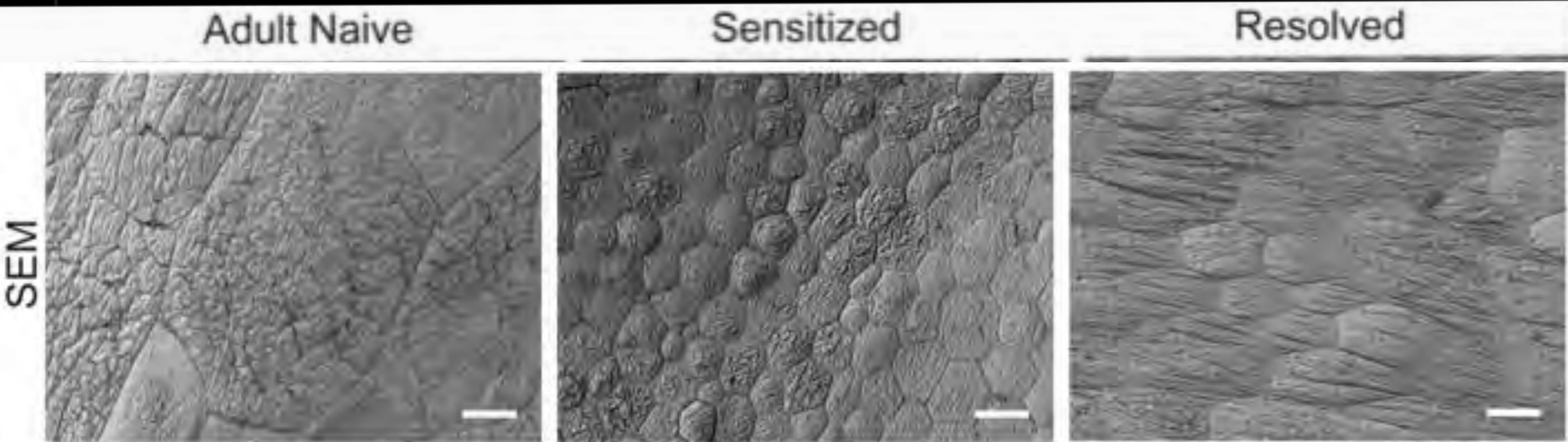
# History of infection sensitizes recurrent UTI



Bladders are remodeled through sensitization

**Valerie O'Brien, Tom Hannan**

# Defect in Terminal Differentiation



Bladders are remodeled through sensitization

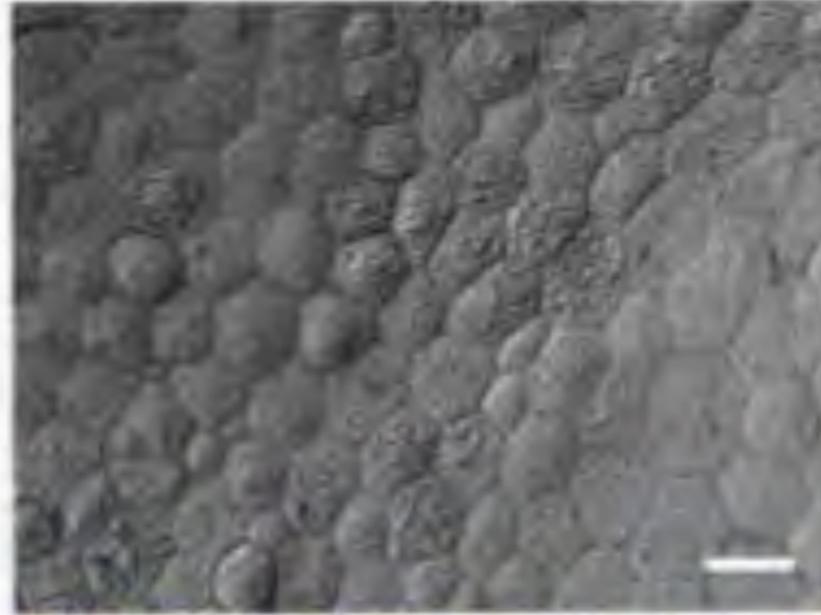
# Defect in Terminal Differentiation

Adult Naive

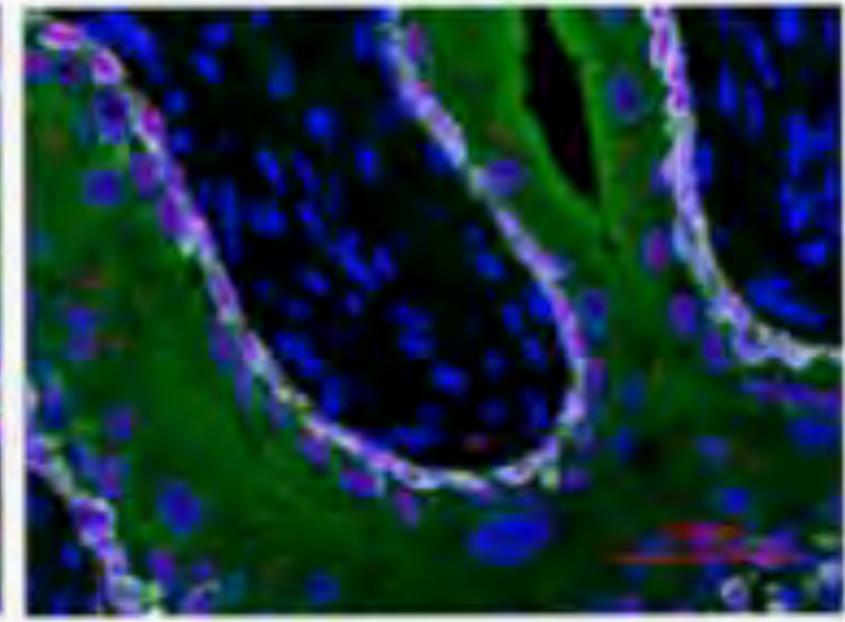
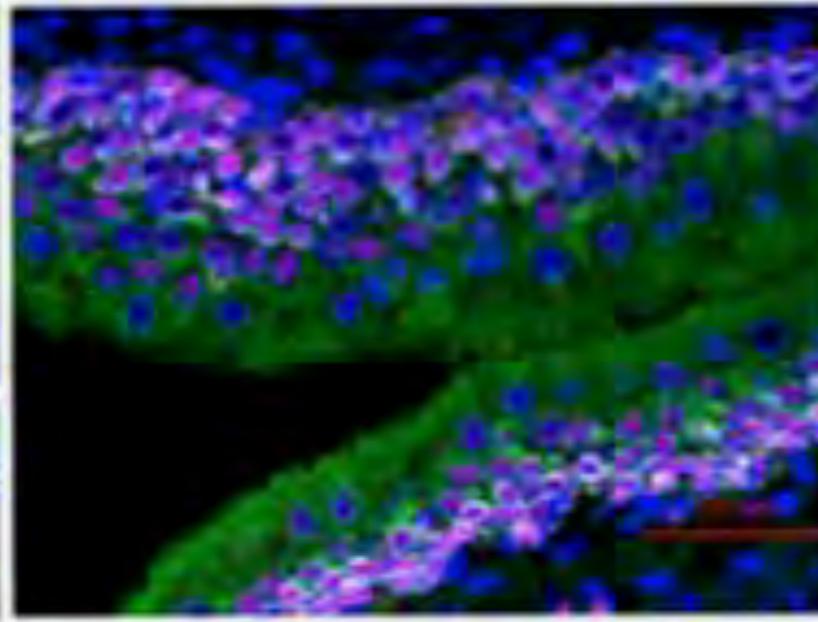
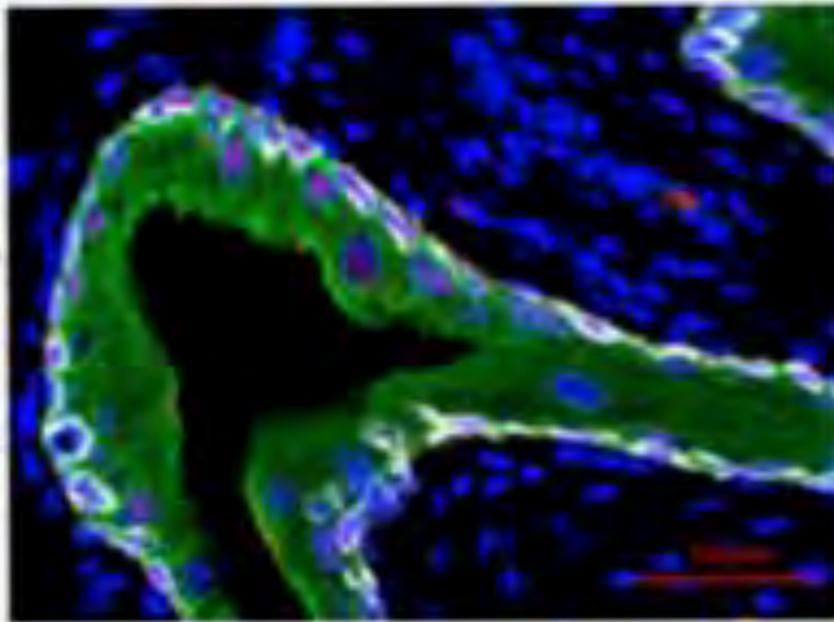
Sensitized

Resolved

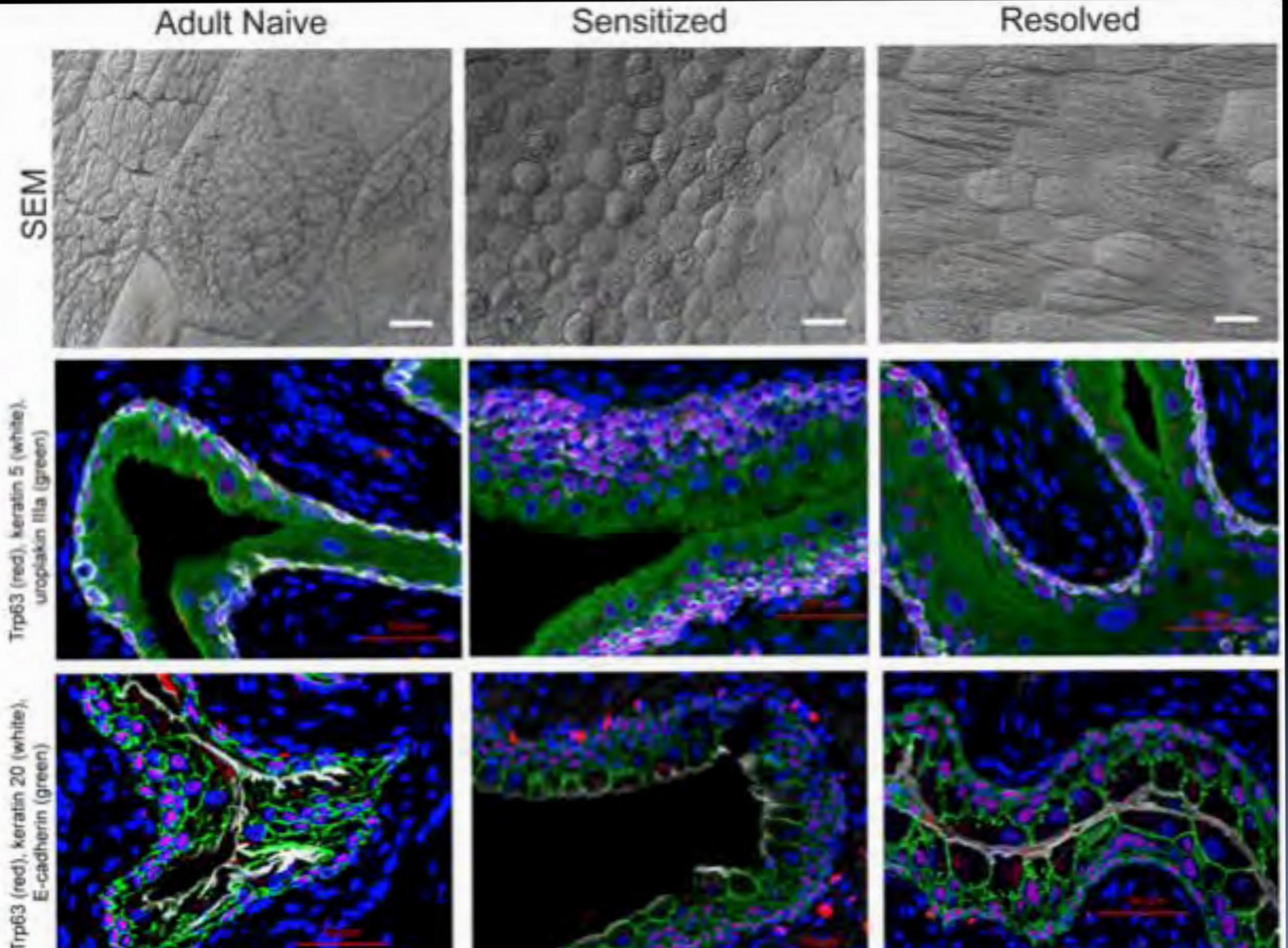
SEM



Tp63 (red), keratin 5 (white),  
uropilakin IIIa (green)



# Defect in Terminal Differentiation

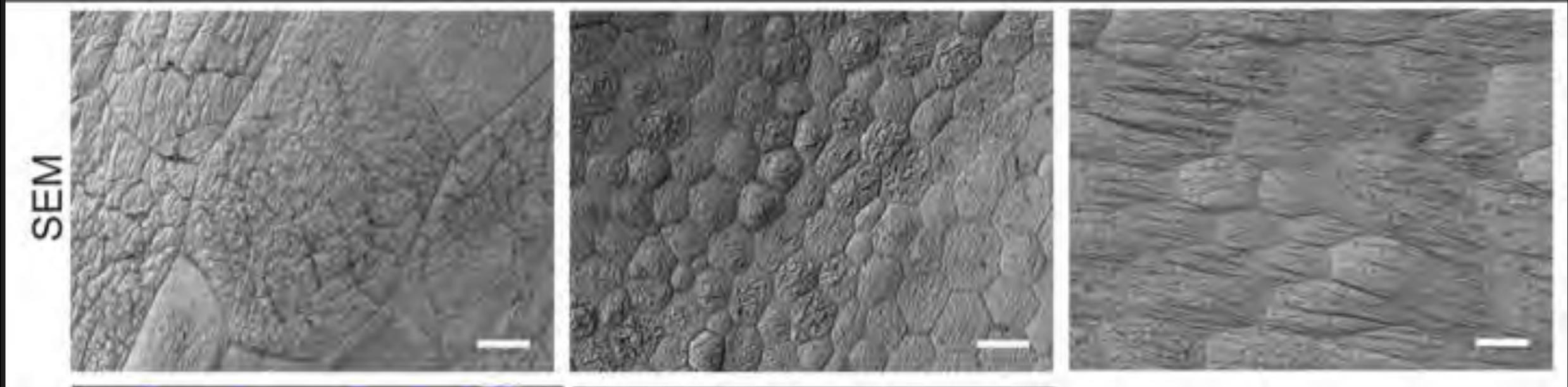


# An infection can leave a molecular imprint on the bladder sensitizing it to

Naive

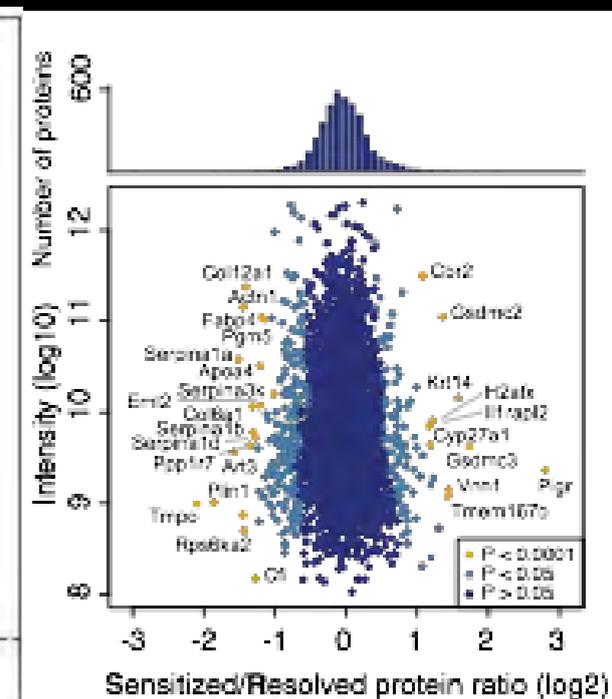
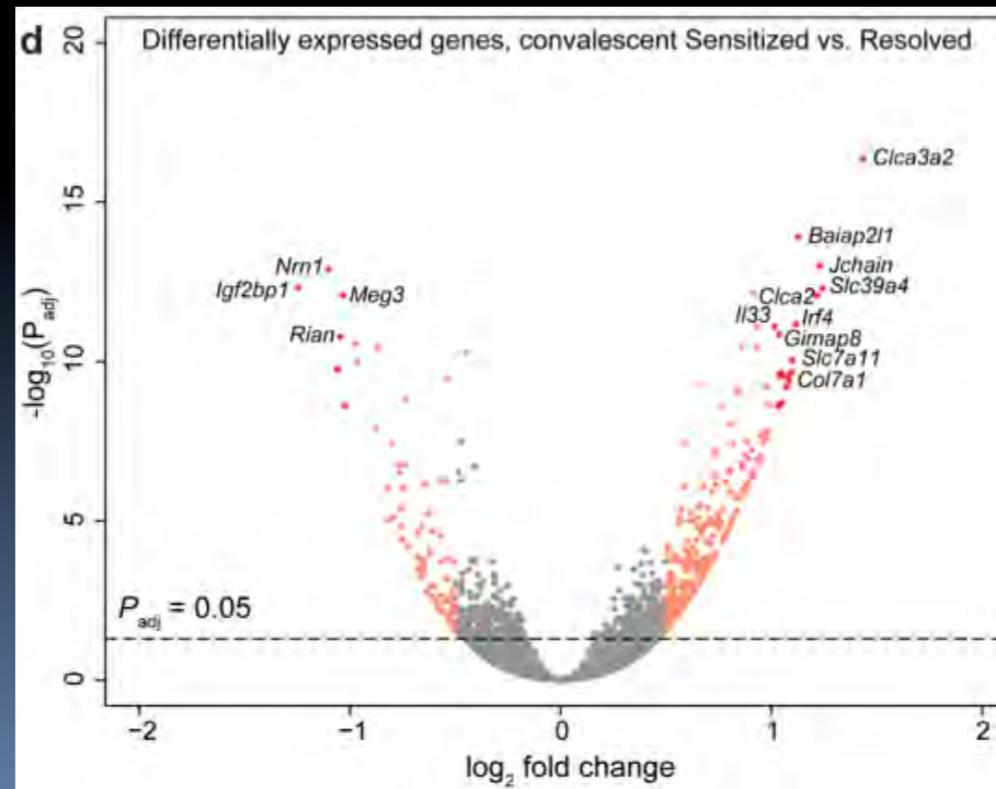
Sensitized

Resolved



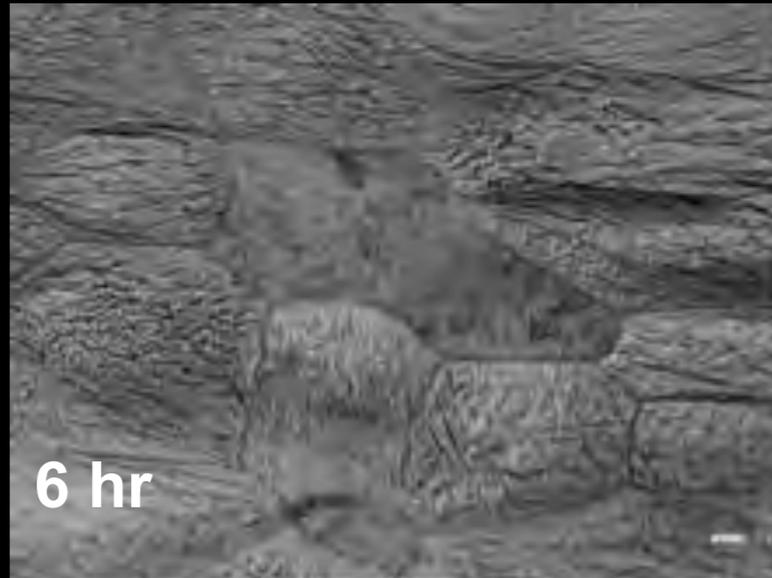
- Lipid metabolism
- Protease Inhibitors
- Cell-cell junctions/ECM
- Cytoskeleton
- Oxidative stress
- Tissue morphology
- Cellular development
- Cellular growth and proliferation

Suggests that the “sensitized” bladder epithelium is more sensitive to neutrophil damage as a consequence of inflammation

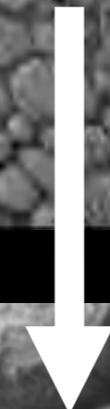
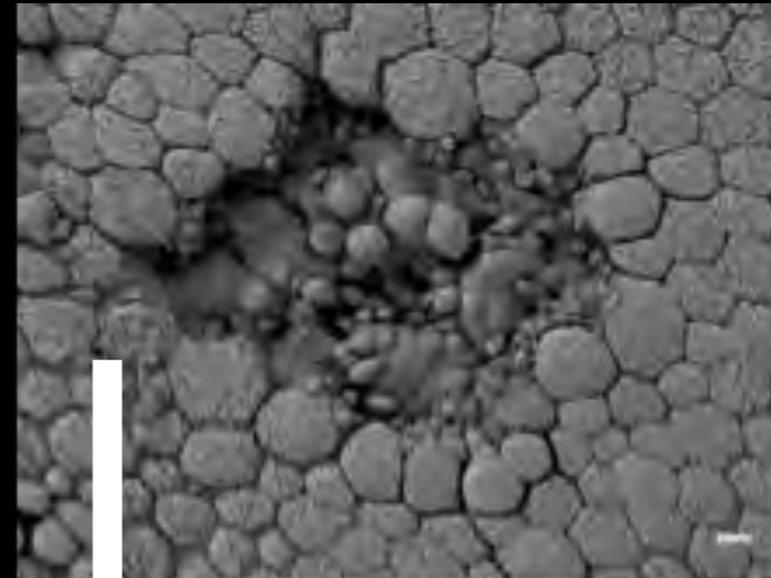


# This molecular imprint predisposes to rUTI

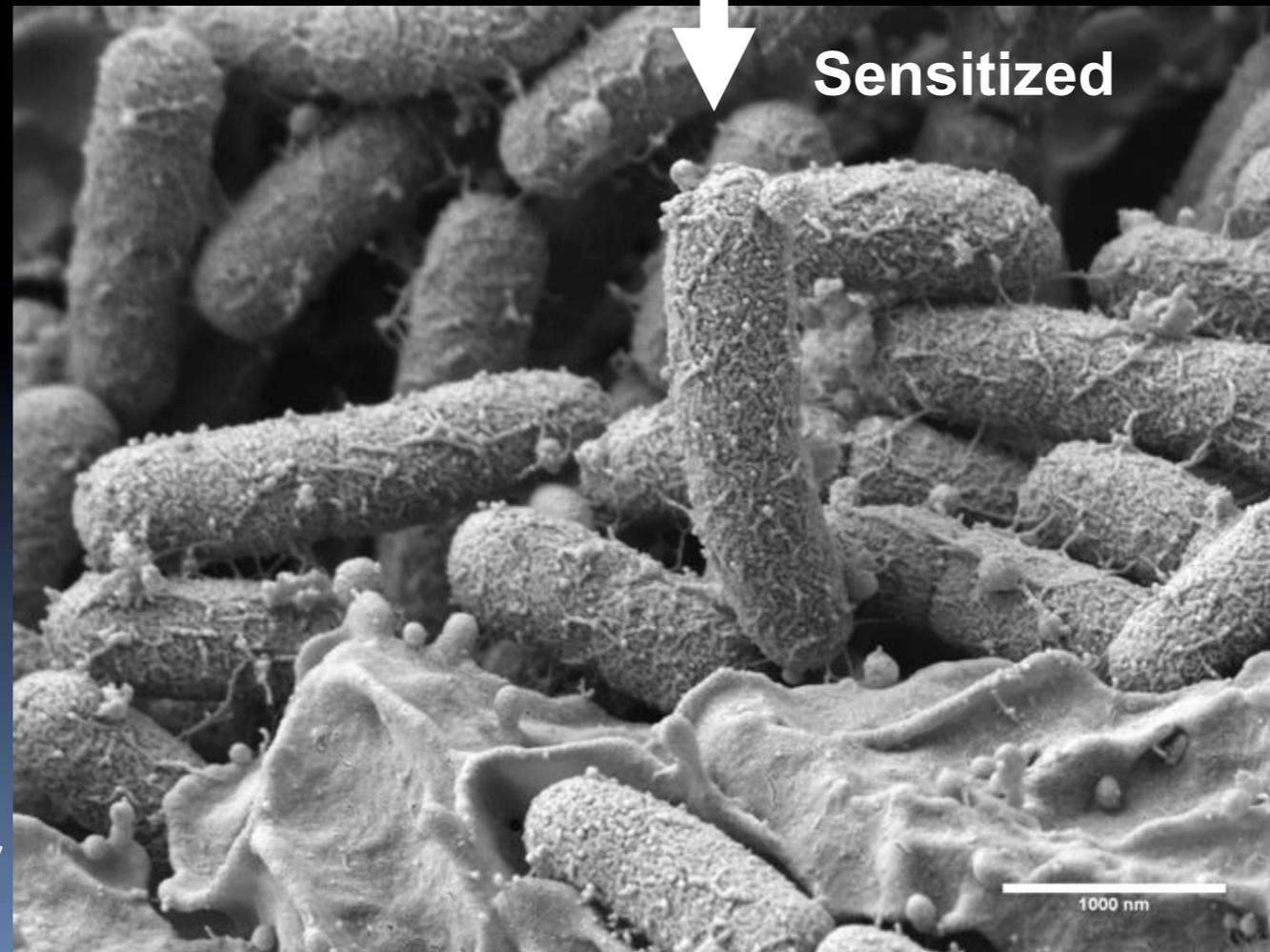
Naive



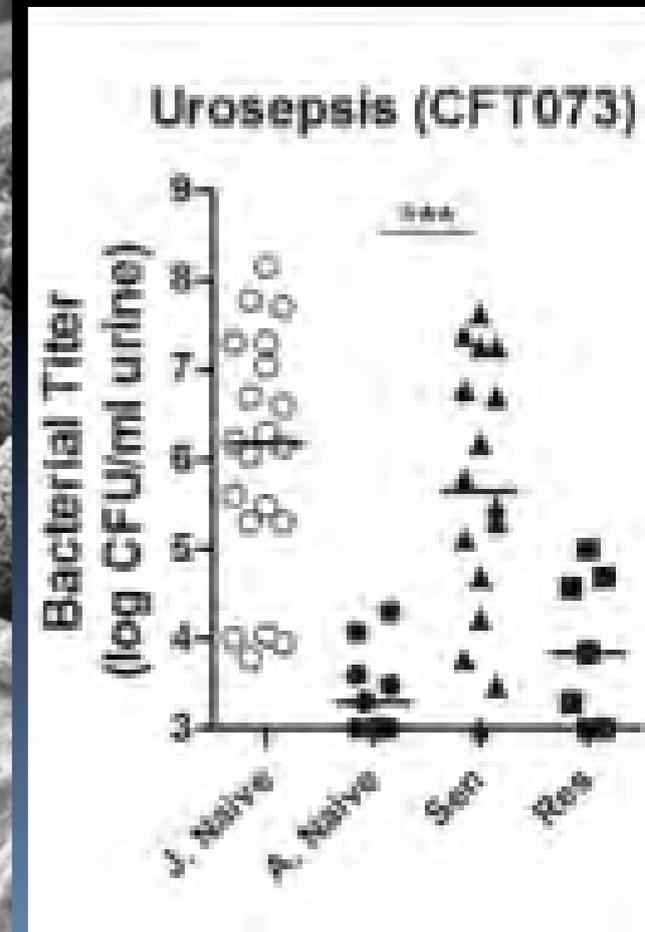
Sensitized



Sensitized

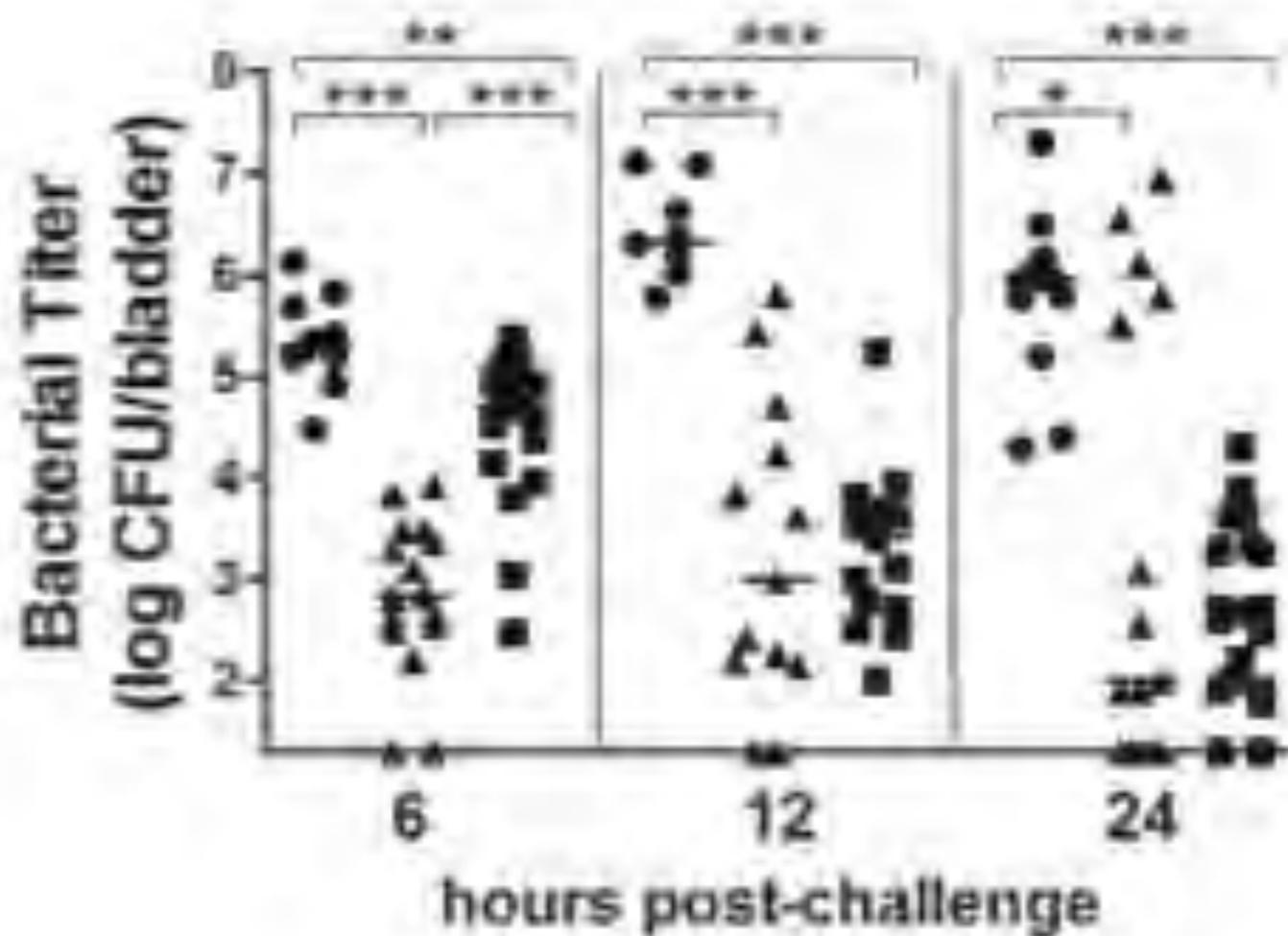


Sensitization (caused by prior infection) leads to long-lasting remodeling that increases vulnerability to subsequent infections.

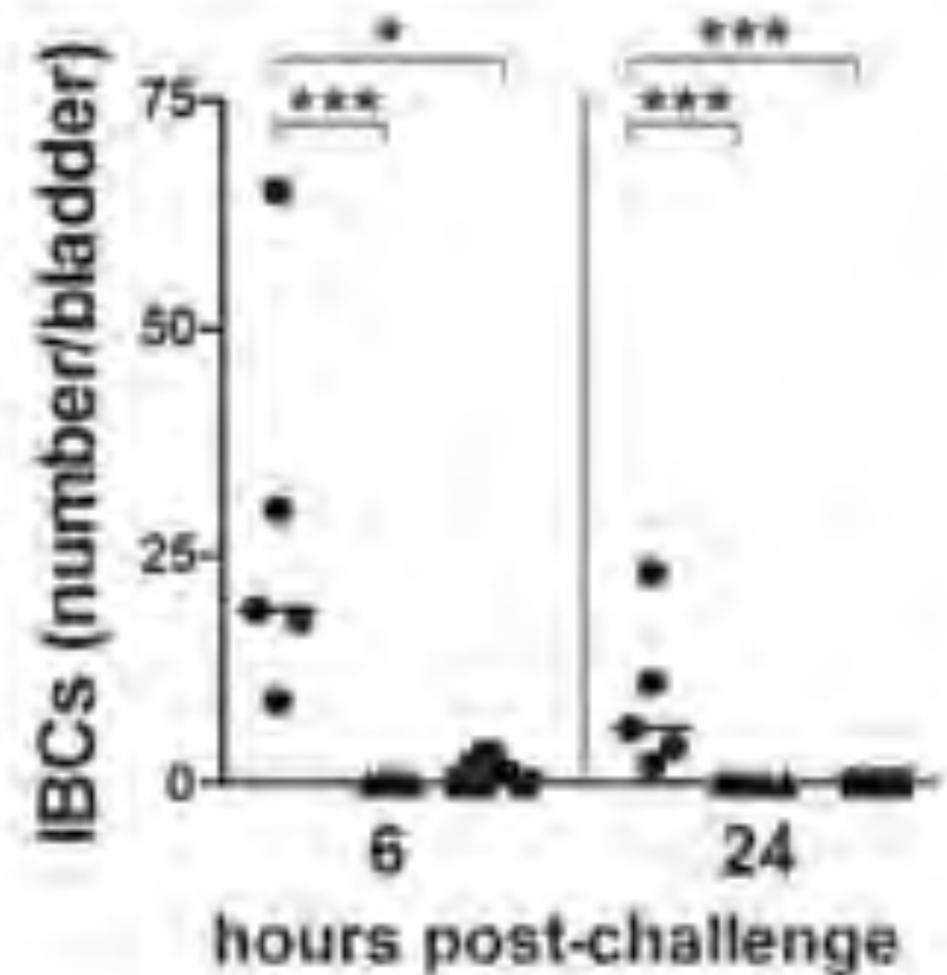


# Altered host-pathogen interactions - Colonization resistance

## Bladder colonization

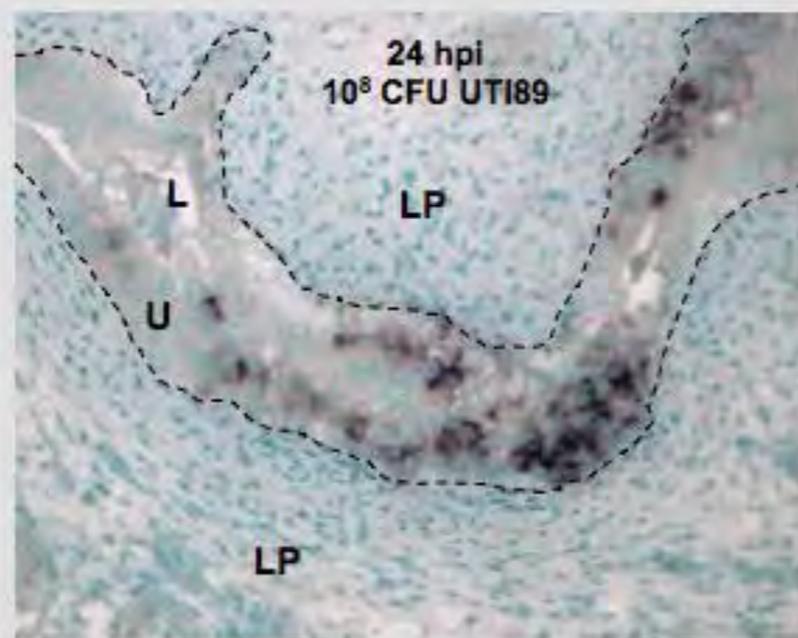
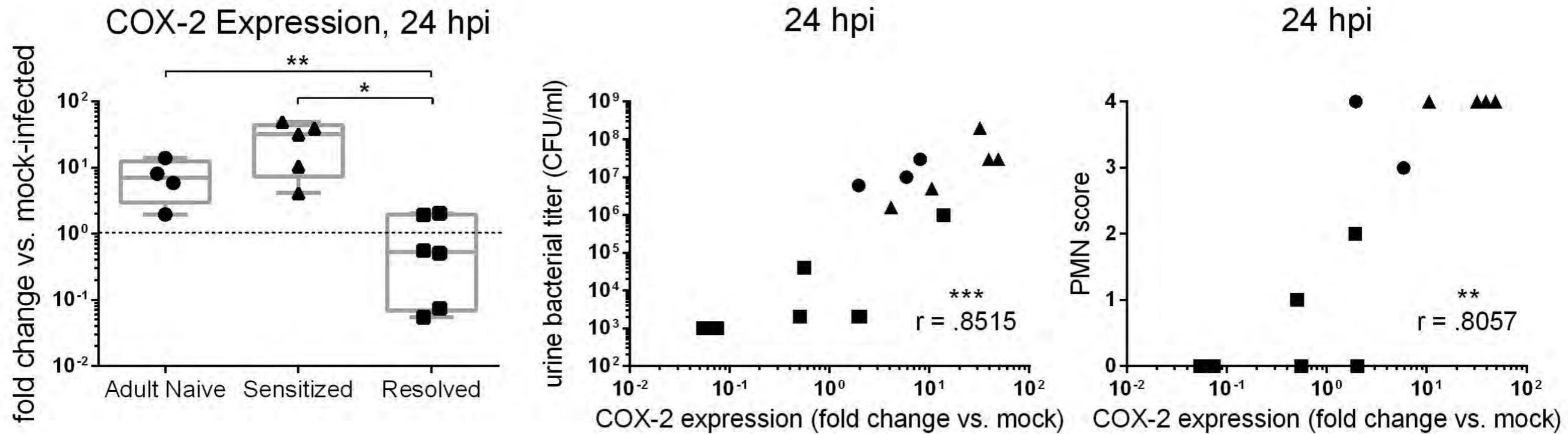


## IBC formation

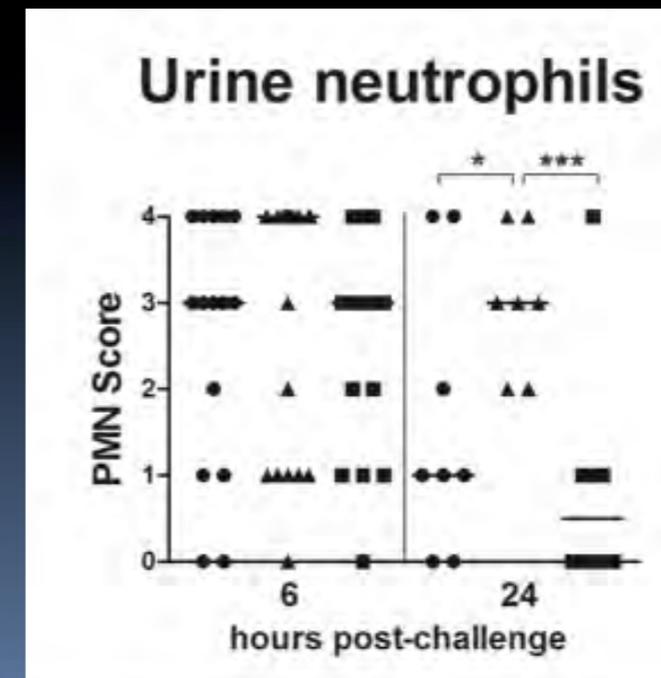


● Adult Naive    ▲ Sensitized    ■ Resolved

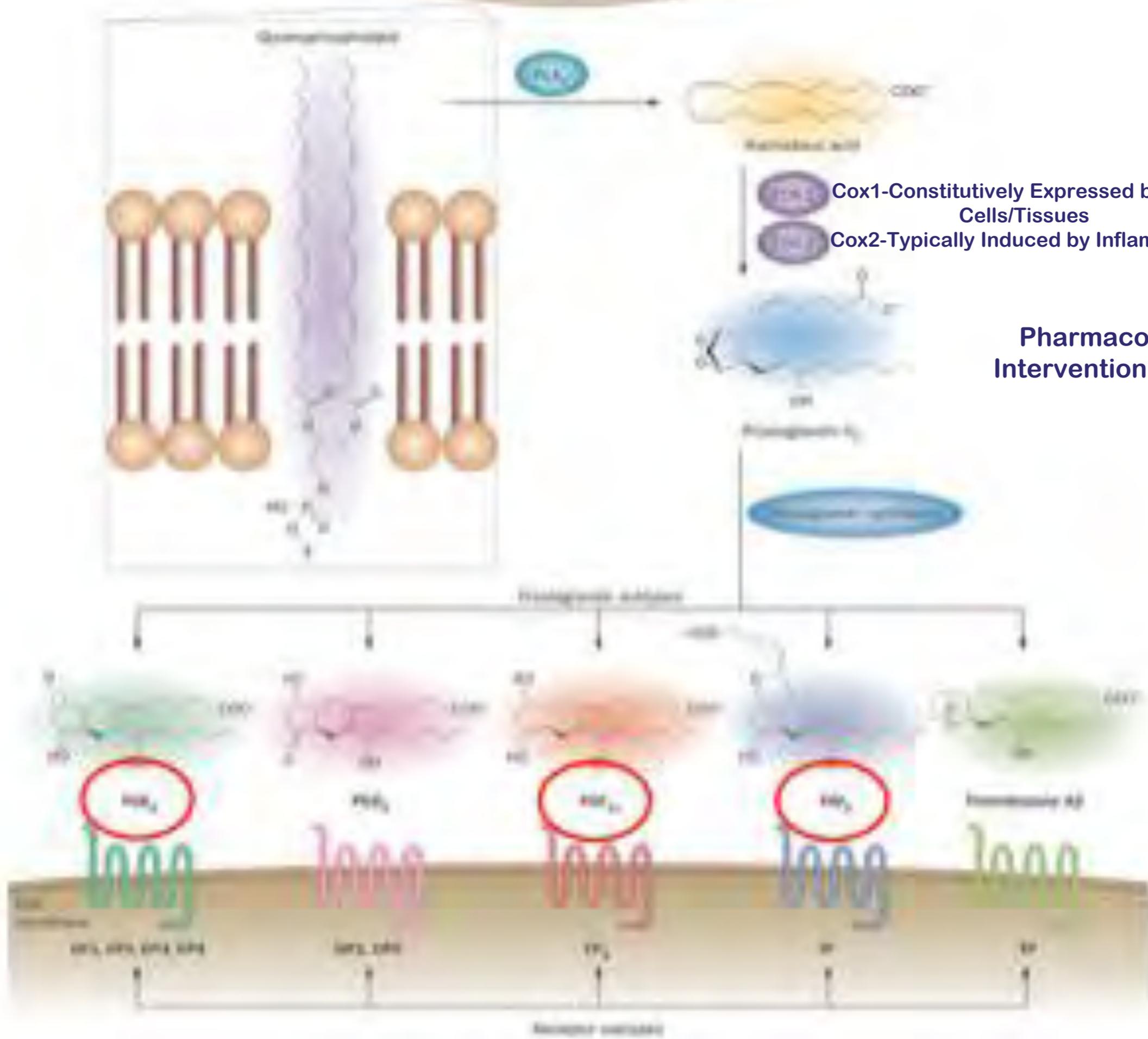
# Enhanced COX-2 Expression in Sensitized Mice



*in situ* hybridization for *Cox-2* transcript in mouse bladder



● Adult Naive ▲ Sensitized ■ Resolved



Cox1-Constitutively Expressed by many Cells/Tissues  
 Cox2-Typically Induced by Inflammation

Pharmacological Intervention: NSAIDs

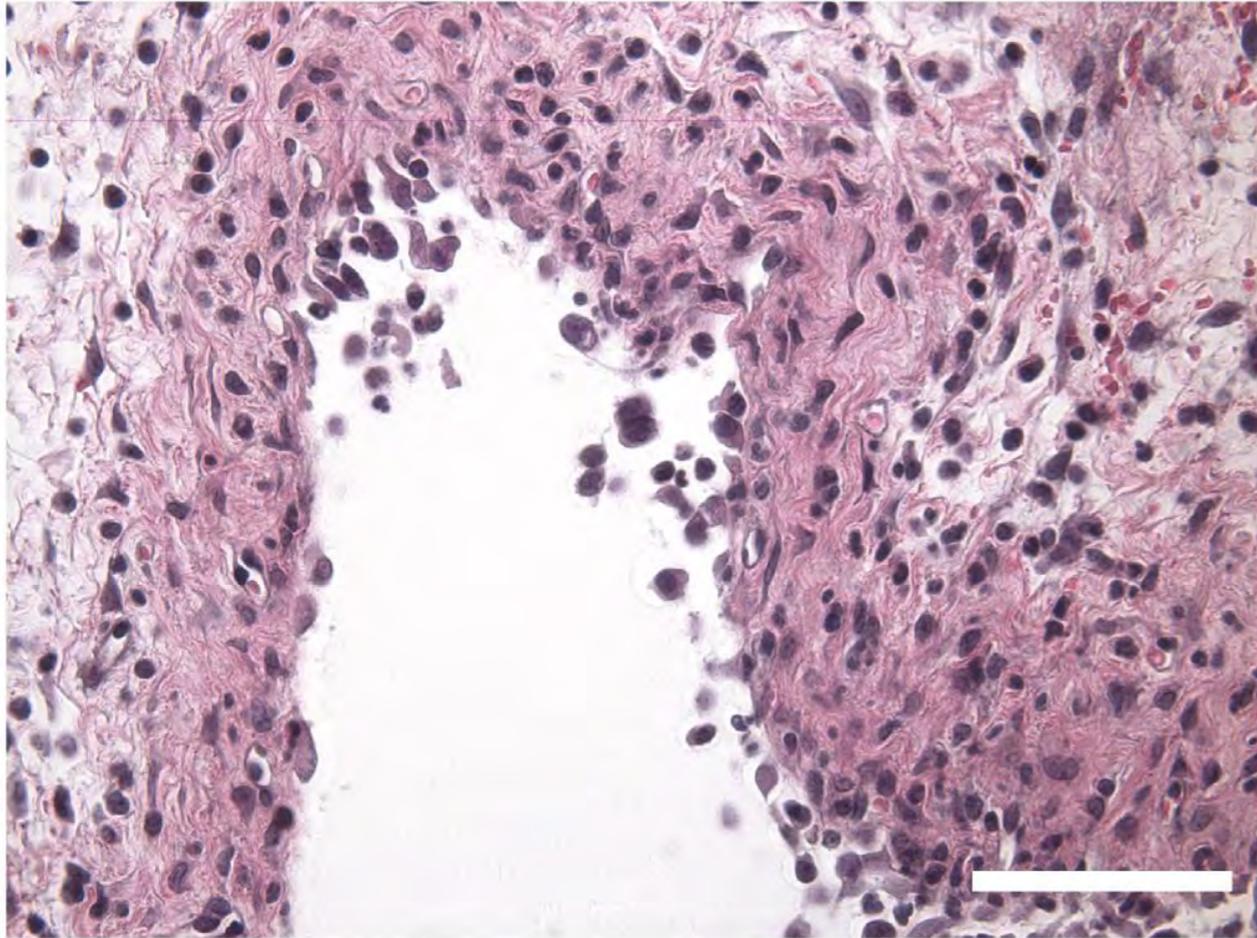
**Clinical Data and Biomarkers Suggest that an Over-exuberant Inflammatory Response Predisposes to rUTI.**

**Dexamethasone Protected Against Chronic Cystitis**

**Question: Could Immunomodulatory Therapy Alone Alter the Outcome of rUTI?**



## 2 Inhibition Suppresses Epithelial Transmigration by Neutrophils and Bladder



Mock



SC-236

Indomethacin (Indo): inhibits both COX-1 & 2  
SC-236 selectively inhibits COX-2  
SC-560 selectively inhibits COX-1

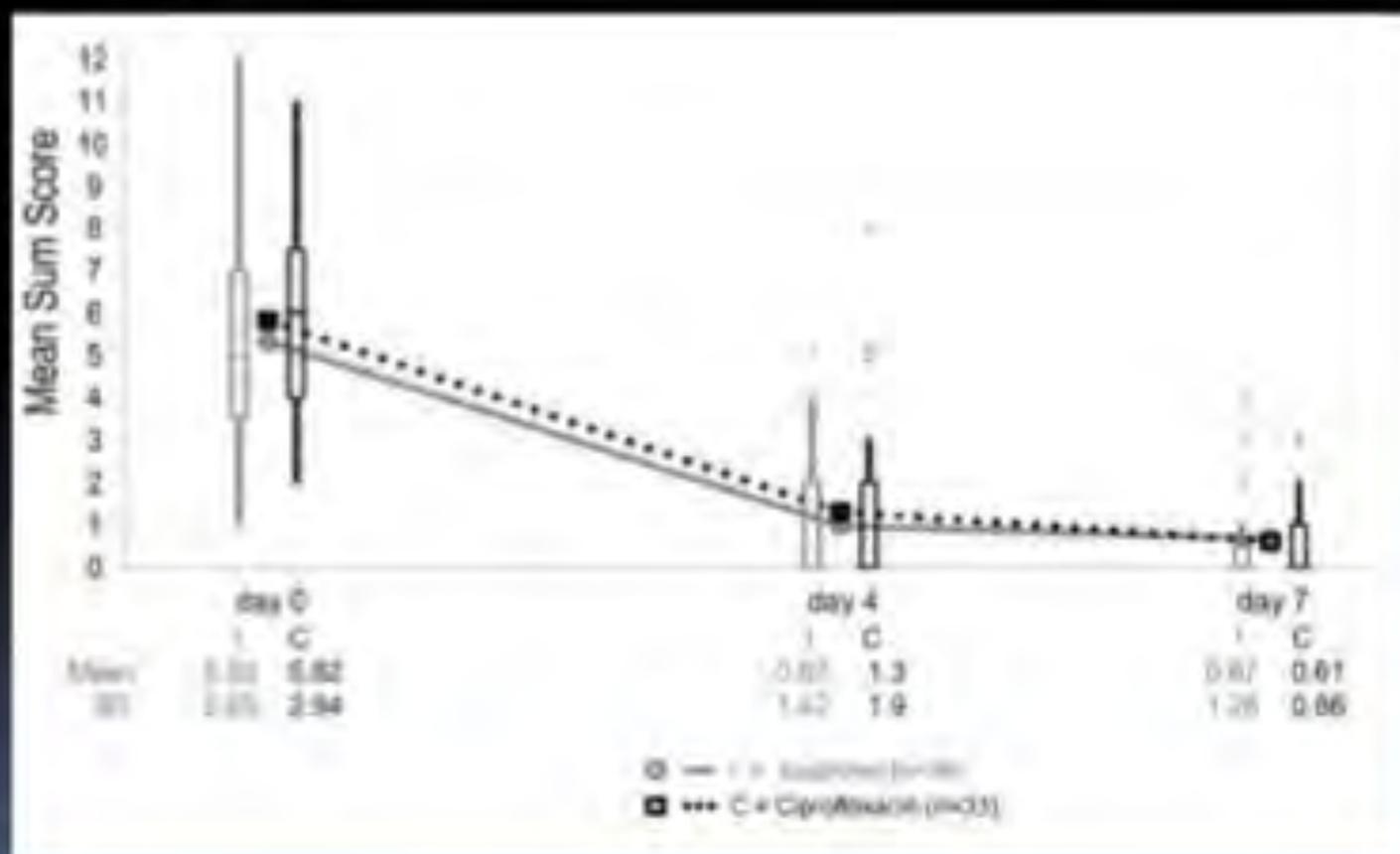
Tom Hannan

RESEARCH ARTICLE

Open Access

# Symptomatic treatment (ibuprofen) or antibiotics (ciprofloxacin) for uncomplicated urinary tract infection? - Results of a randomized controlled pilot trial

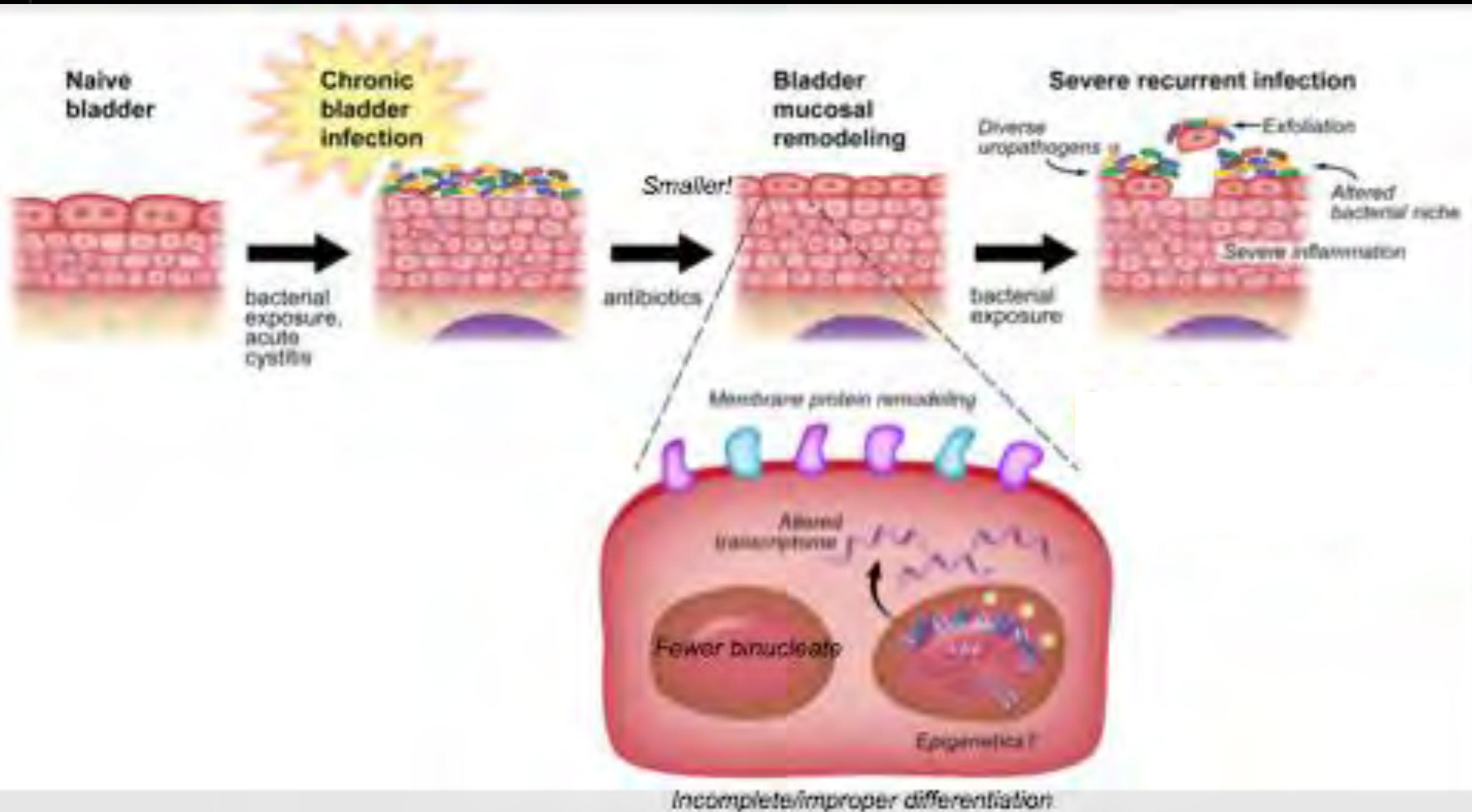
Katja Bleddin<sup>1\*</sup>, (Birk) Gágyor<sup>2,3</sup>, Michael M Kocjencil, Karl Wegscheider<sup>4</sup> and Eva Hummers-Pradler<sup>1</sup>



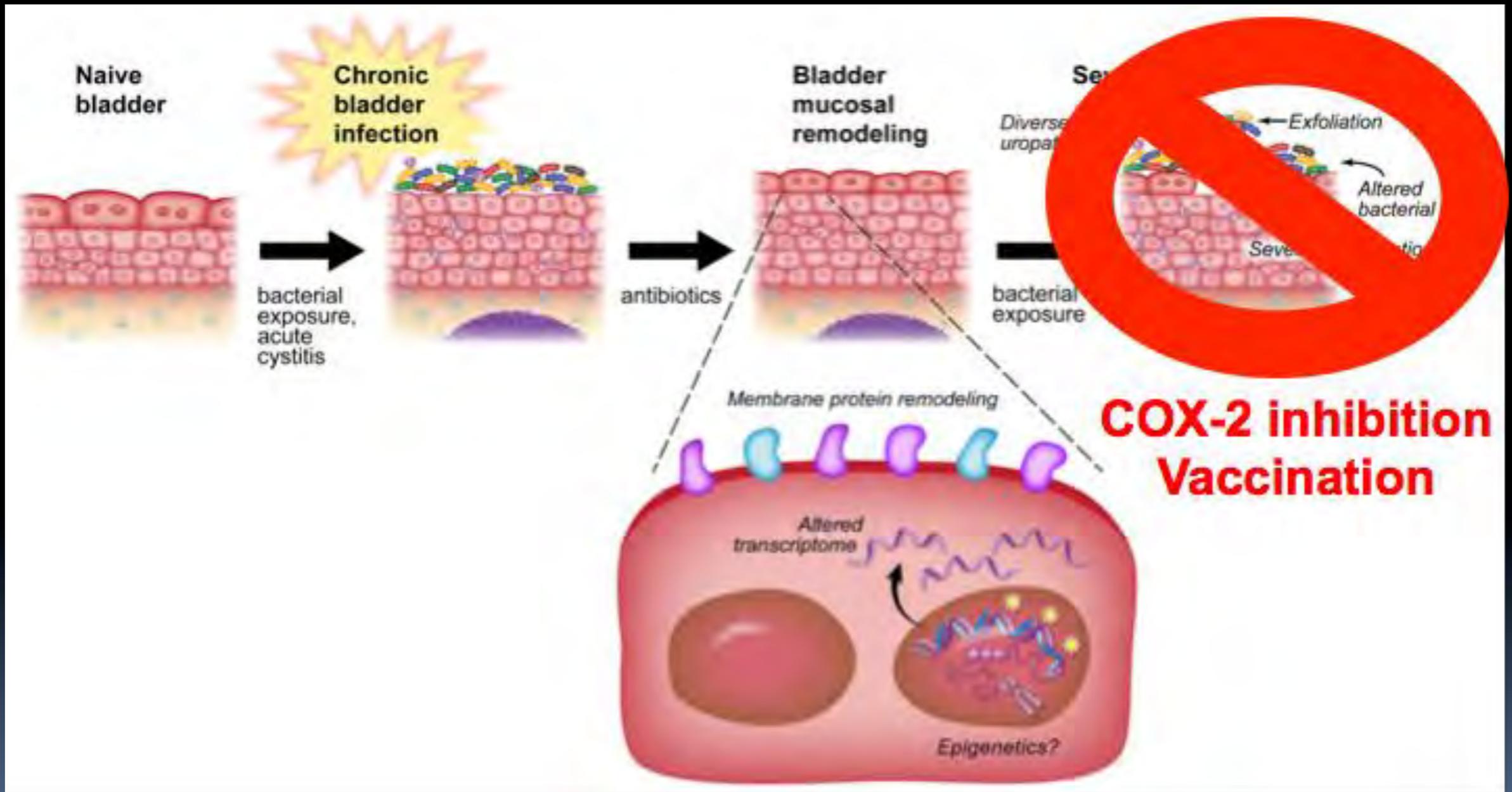
Although patients given only a 3 day course of drug, a similar clinical outcome was realized at days 4 and 7

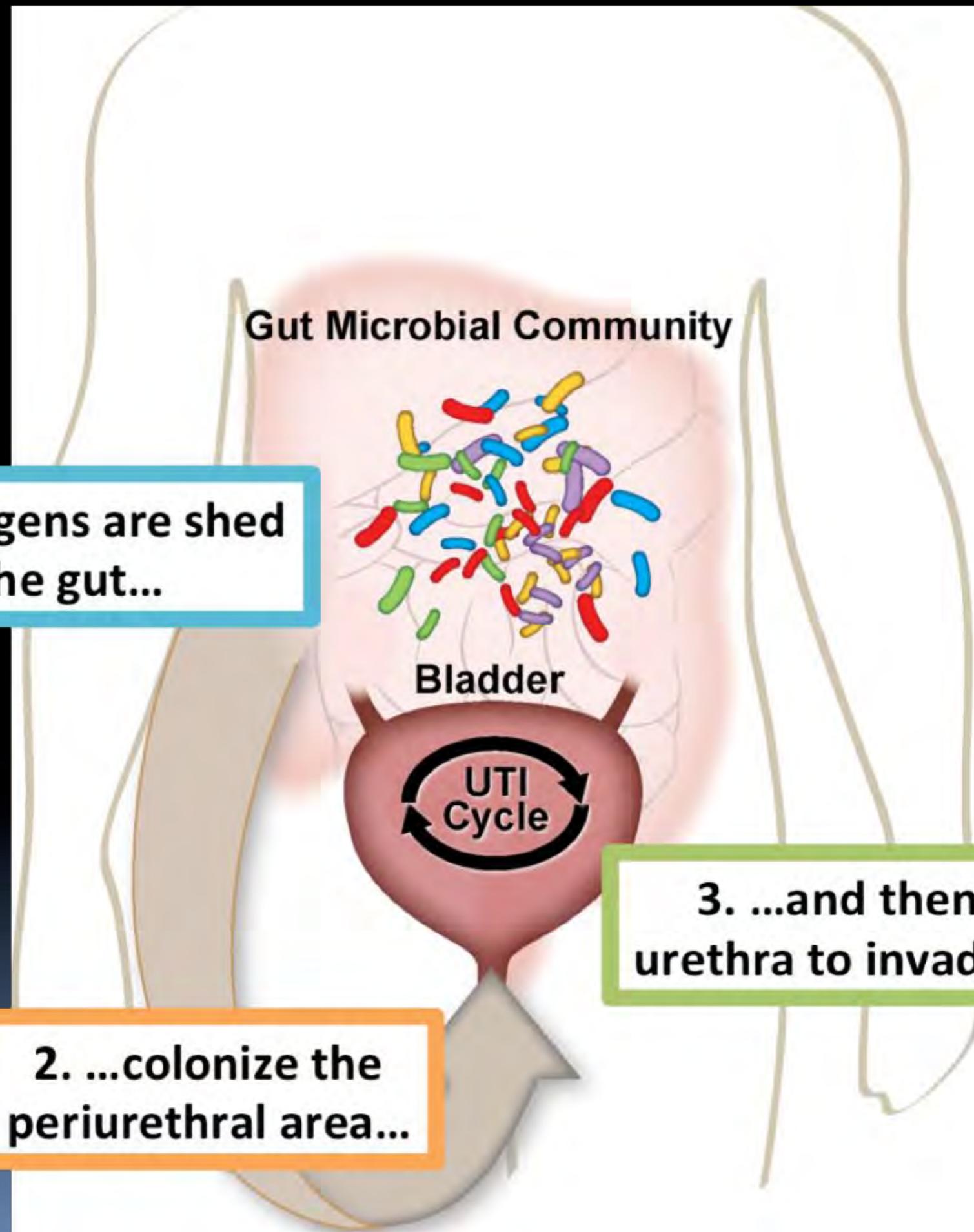
This suggests that NSAIDs do not just mask symptoms, but also alter the course of infection!

# An infection can leave a molecular imprint on the bladder sensitizing it to



# NSAIDs can protect against recurrent infections





**1. Uropathogens are shed from the gut...**

**2. ...colonize the periurethral area...**

**3. ...and then ascend the urethra to invade the bladder.**



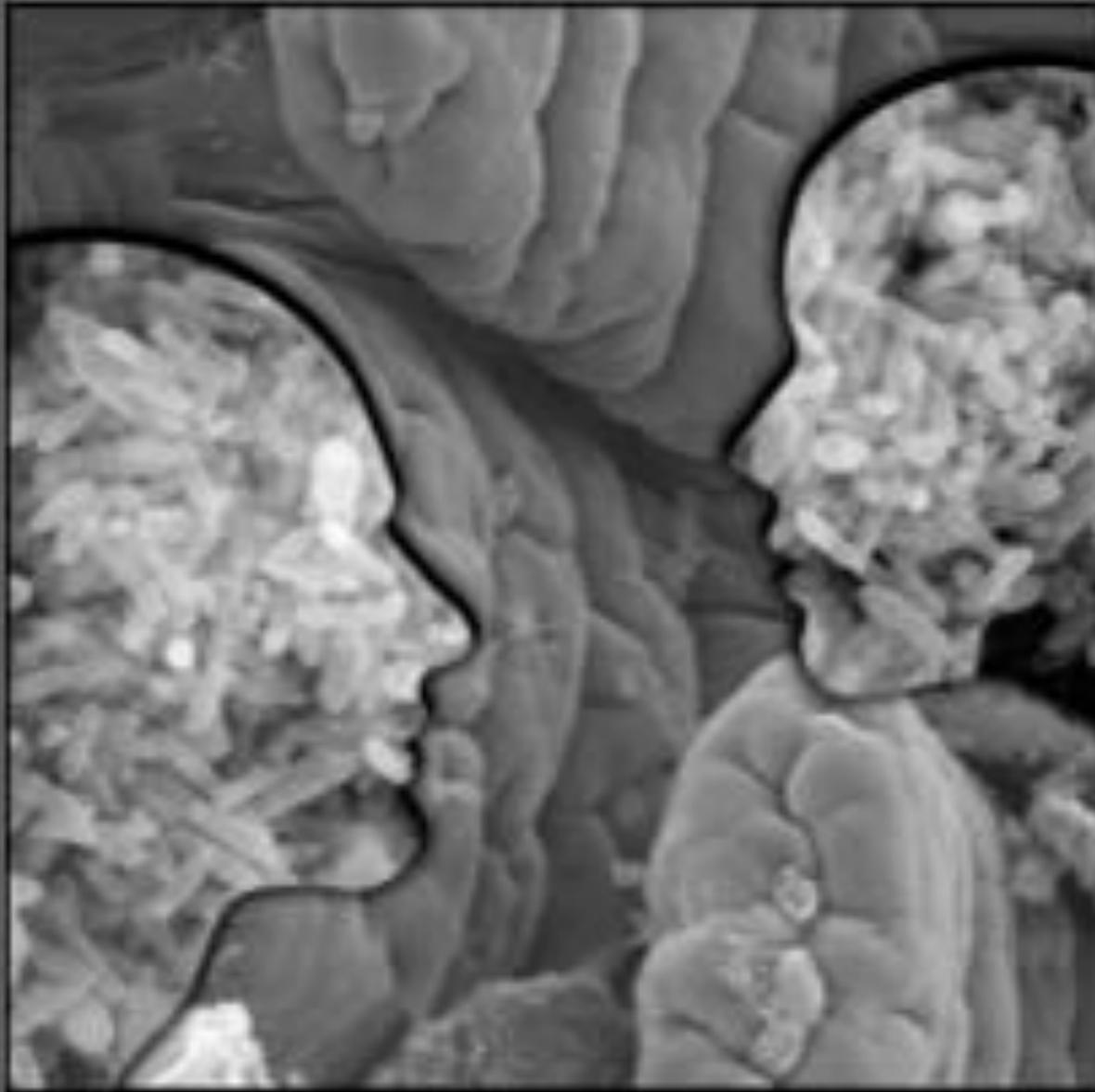
**What are the population dynamics of UPEC in the gut before, during, and after UTI?**

**How does the gut microbiota influence UTI susceptibility?**



**2. ...colonize the periurethral area...**

**3. ...and then ascend the urethra to invade the bladder.**



## Molecular basis of the gut-urinary tract axis in urinary tract infection

## Role of the Human Microbiome

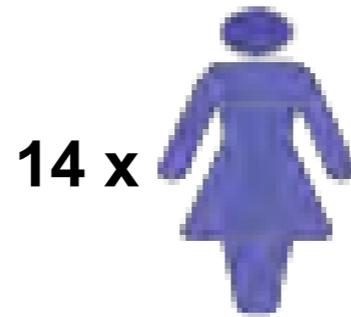
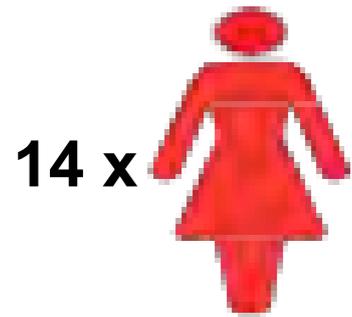
The makeup of the microbiota interacts with the host in such a way that it determines normal and/or abnormal nutrition abnormalities (disease, obesity, malnutrition, etc).

Our indigenous gut microbial communities endow us with physiological and metabolic attributes we have not had to evolve on our own.

A healthy microbiota in the gastrointestinal tract (GIT) serves an important function in the breakdown and absorption of essential dietary vitamins and nutrients. Additionally, it serves a role in the generation and maintenance of an immune balance that limits inflammation while combating colonization from unwanted pathogens. Antibiotic treatments are thought to expose individuals to an increased risk of opening up niches in the GIT which allows pathogens to expand.

# UMB Cohort, Study Design, and Collections

## Cohort Recruitment

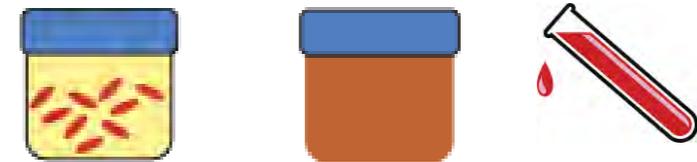


**Women with frequent recurrent UTI (rUTI)**  
*>3 UTI in past year*  
*No recent Abx*  
*No chronic illnesses*  
*No urological abnormalities*

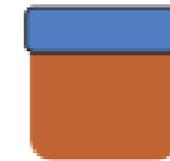
**Demographically-matched Healthy Controls**  
*0-1 UTI in lifetime*  
*No recent Abx*  
*No chronic illnesses*  
*No urological abnormalities*

## 12 Month Longitudinal Study Design Collections at:

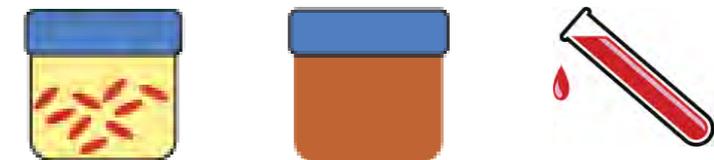
### 1. Enrollment



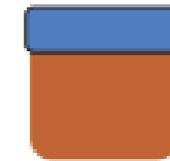
### 2. Monthly Time Points



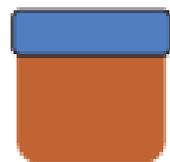
### 3. UTI Episodes



### 4. Post Antibiotic Treatment



## Total Collection:



387 fecal samples

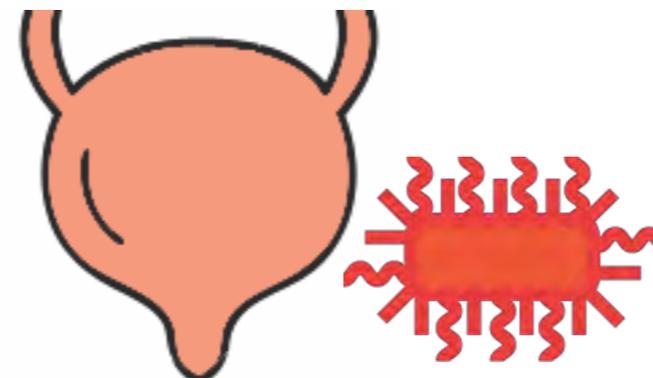


47 urine samples



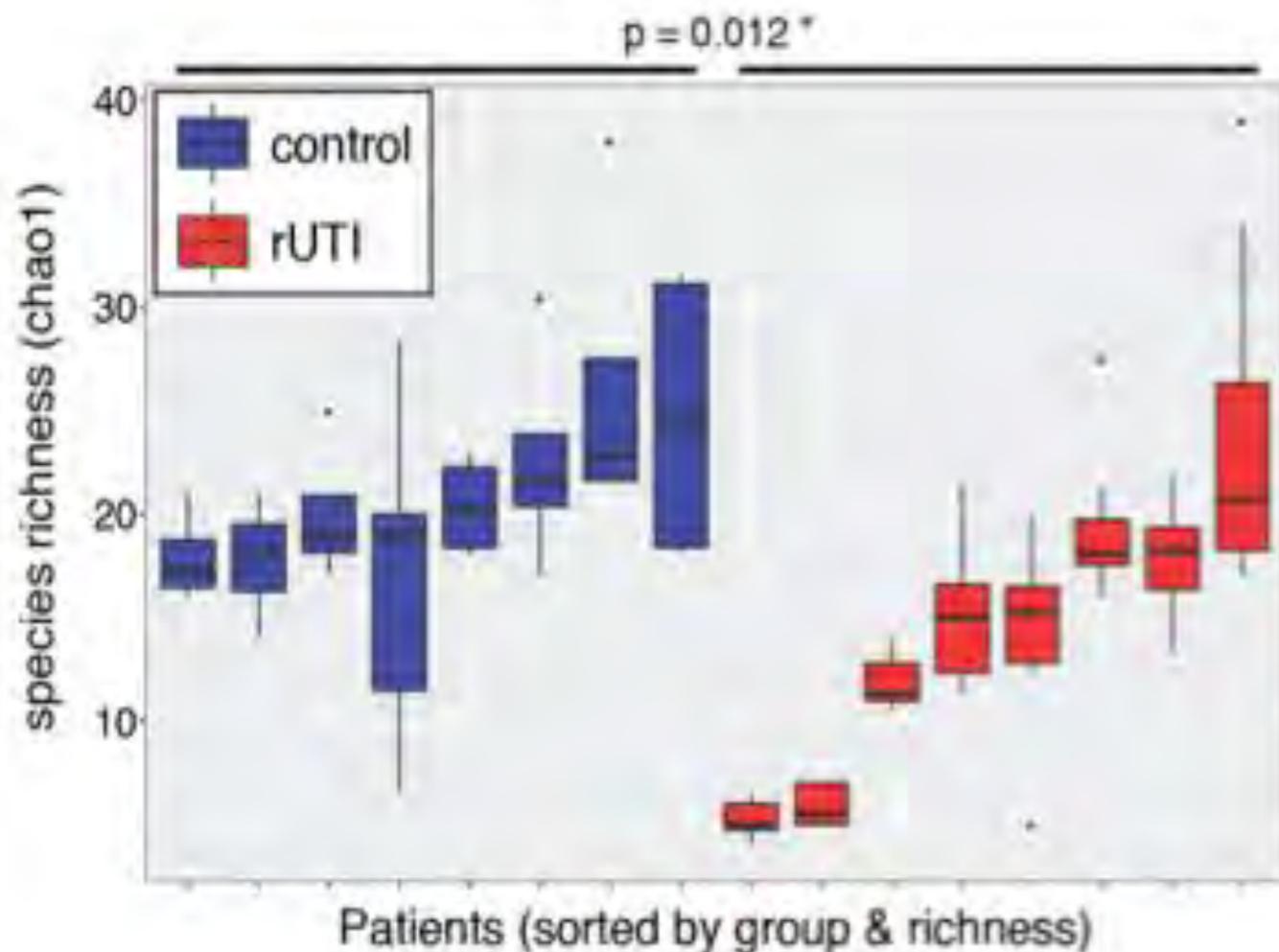
47 blood samples

19x UTI events

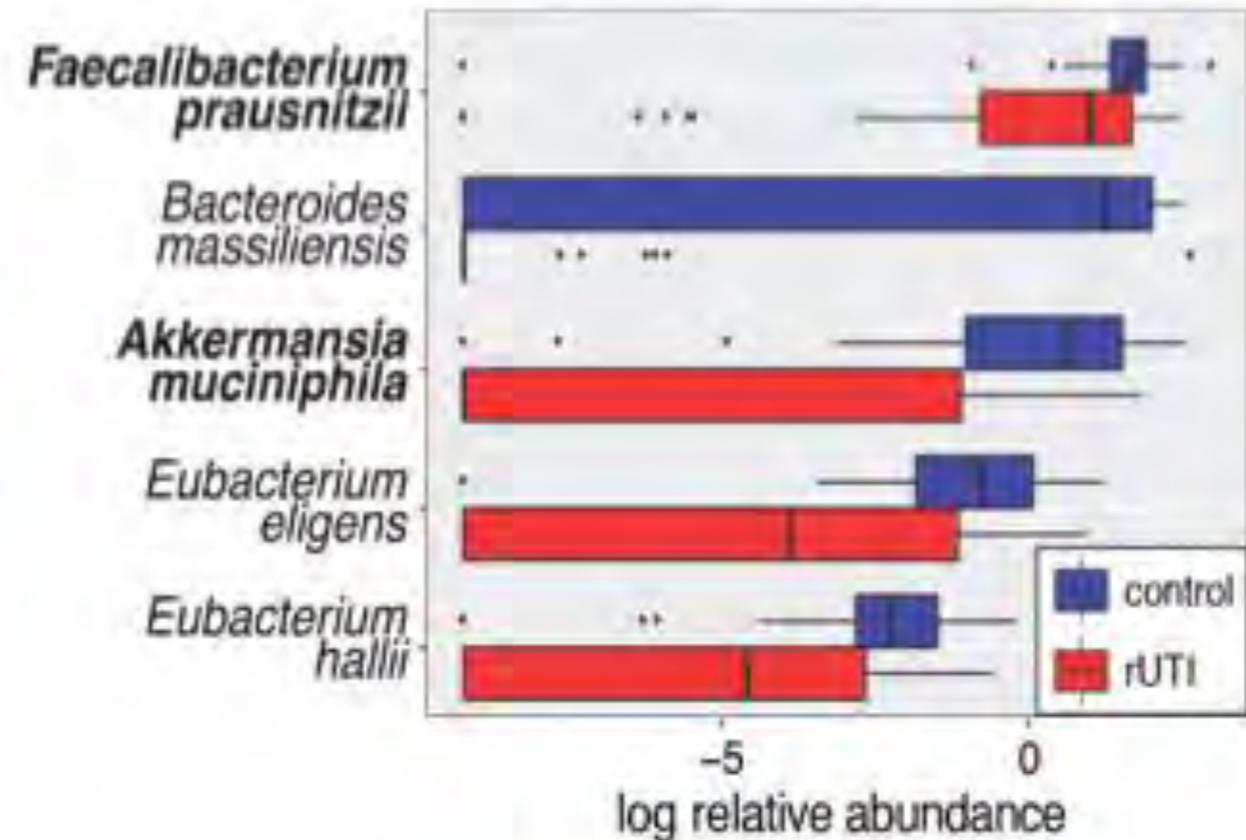


# rUTI women have lower-diversity microbiota

## Overall diversity



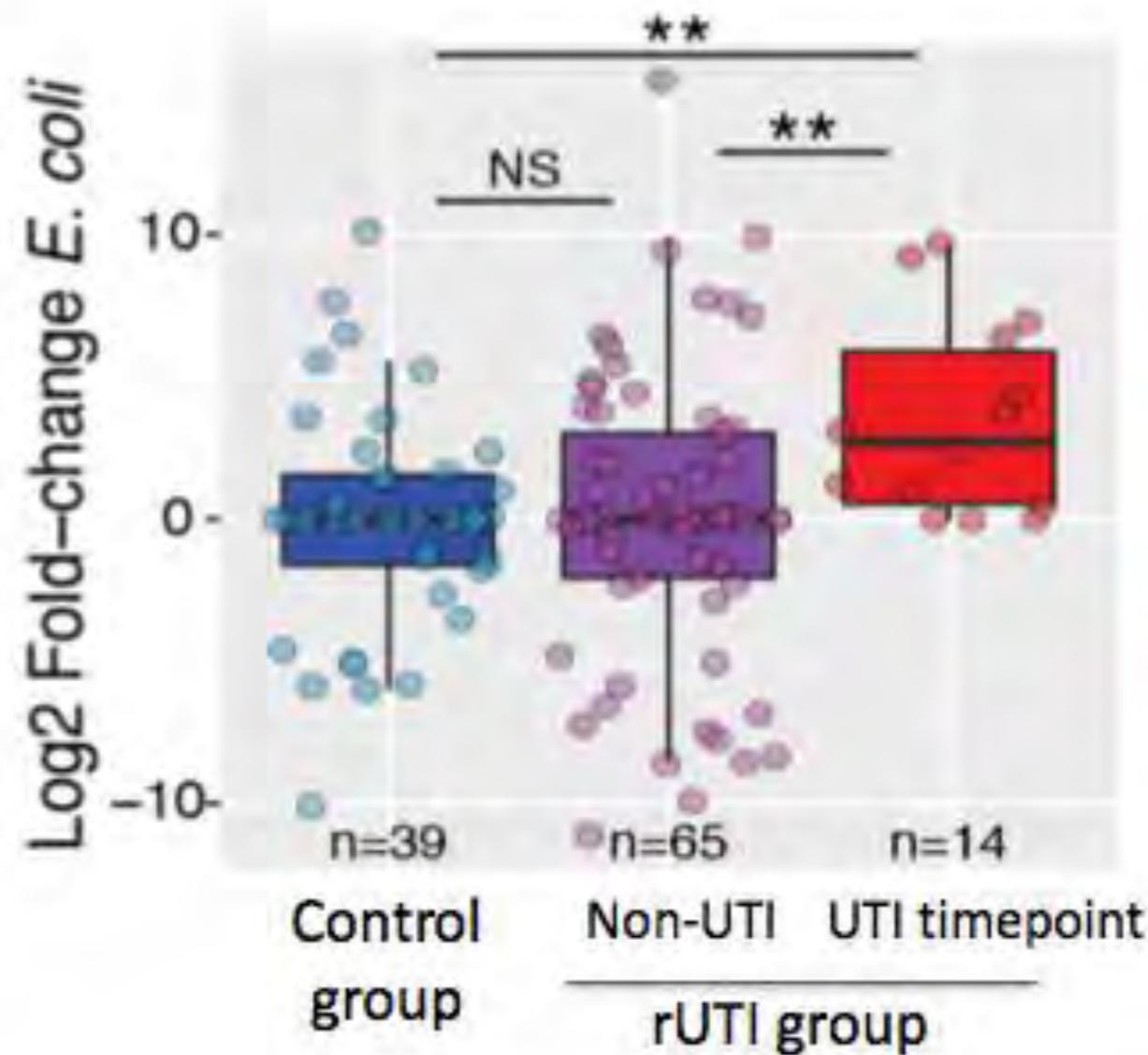
## Select species



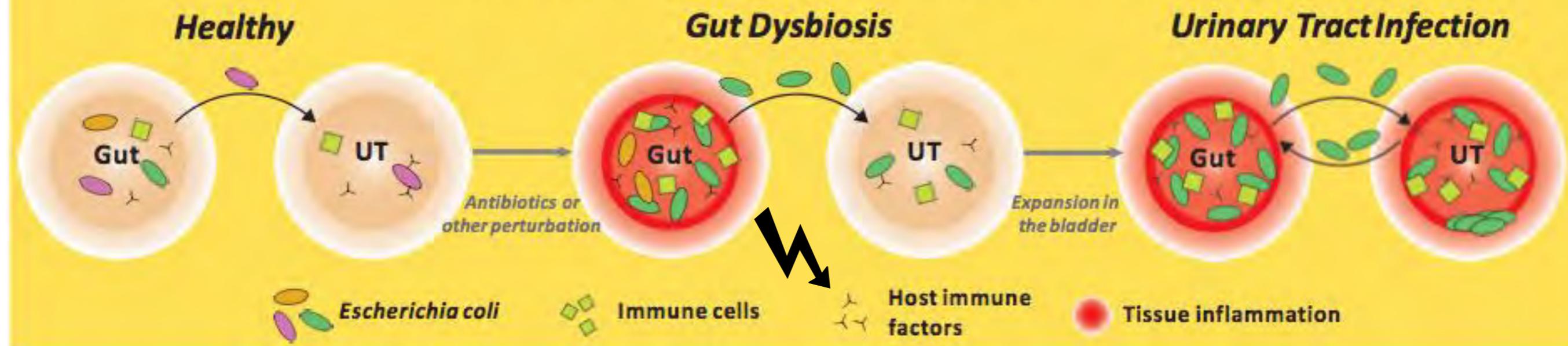
Thus, rUTI appears to be one of the growing number of human diseases associated with imbalance of complex microbial GIT communities.

# *E. coli* blooms in the gut coincide with UTI

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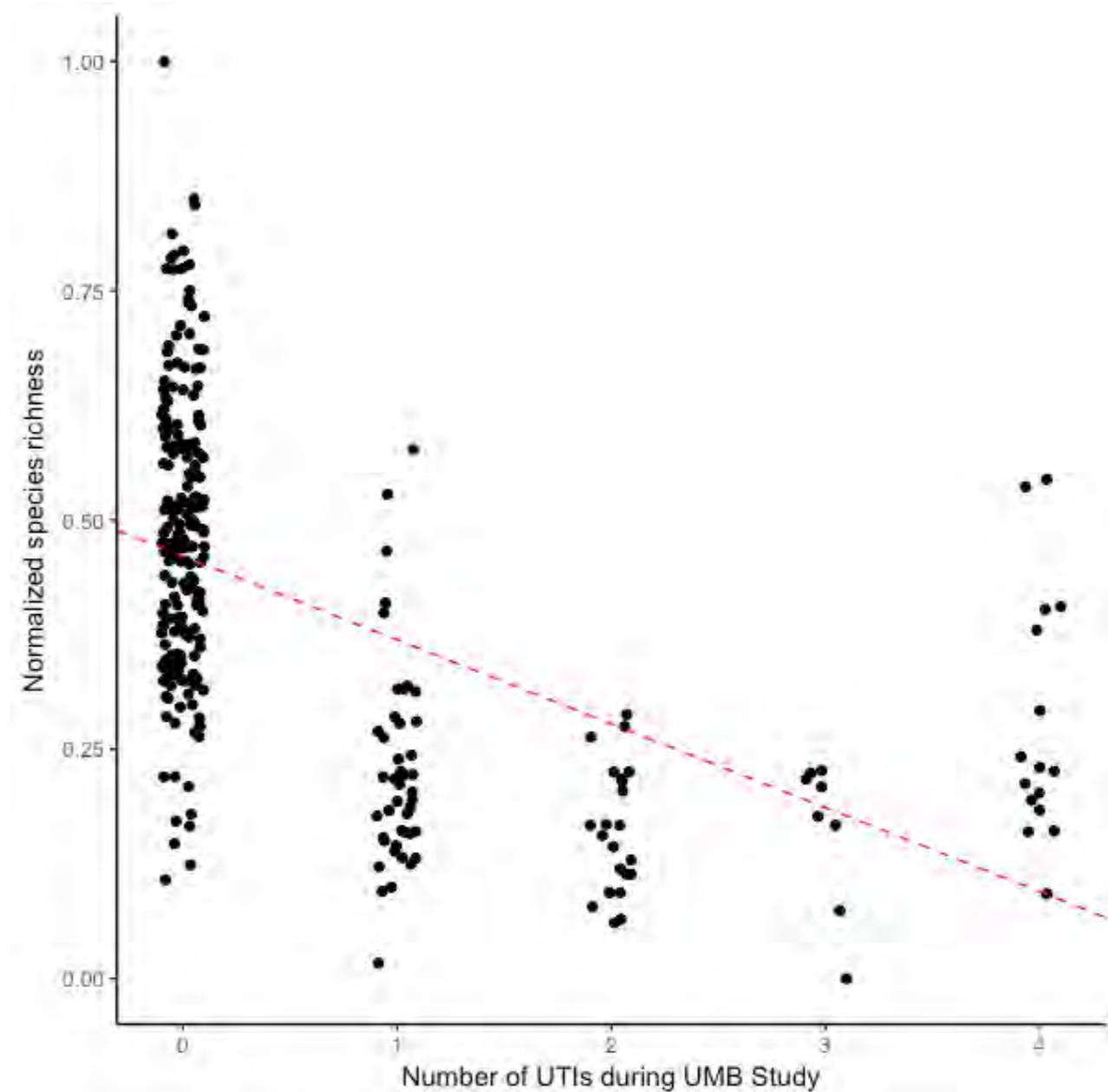


# Model of gut-bladder axis in UTI

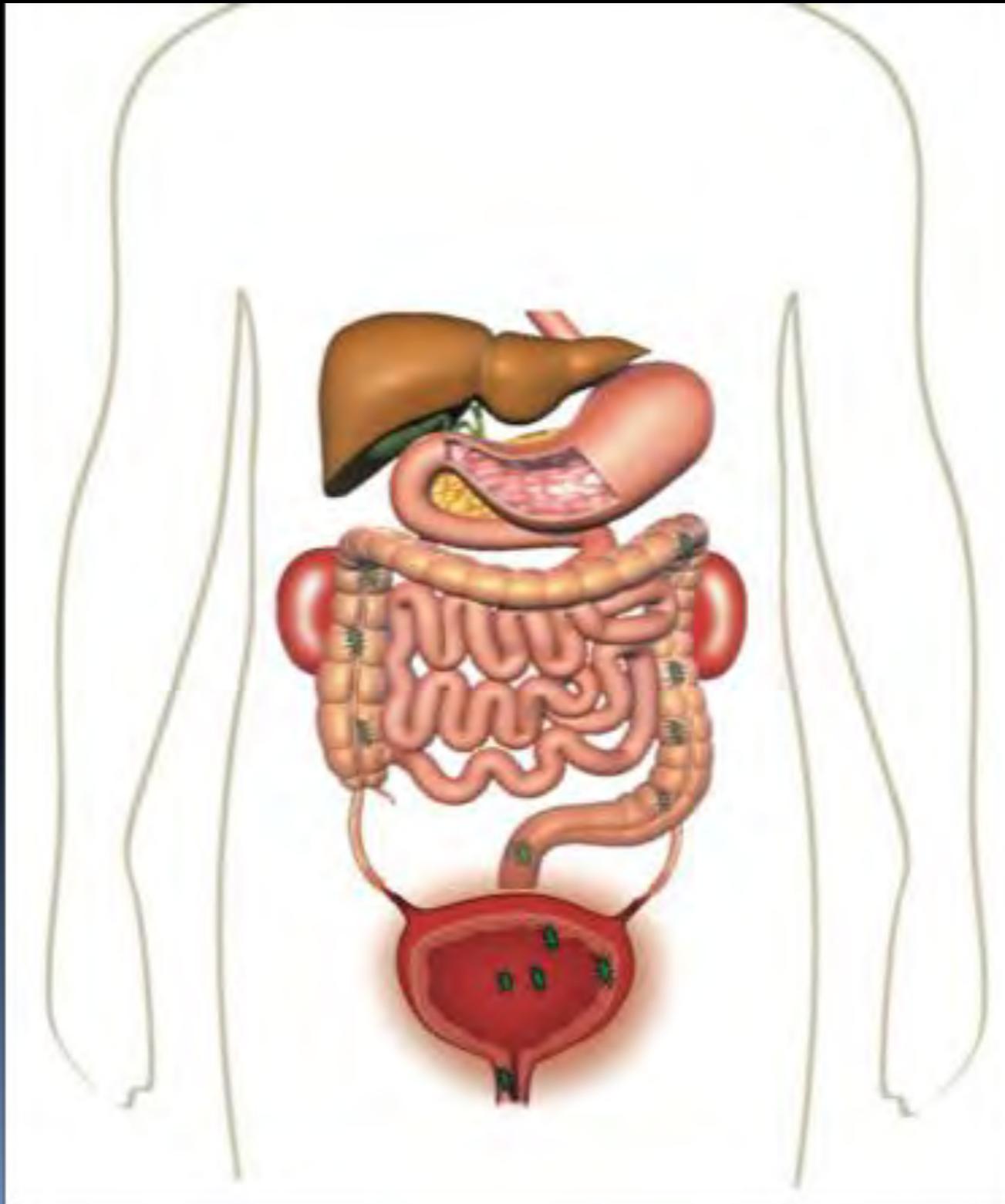


- The gut microbiotas of women with rUTI were significantly less rich (contain fewer species) than community- and age-matched healthy controls
- Several bacterial species associated with “healthy guts” were depleted in rUTI, including:
  - *Faecalibacterium prausnitzii*
  - *Akkermansia muciniphila*

**Allows UPEC Expansion: Seeds rUTI**



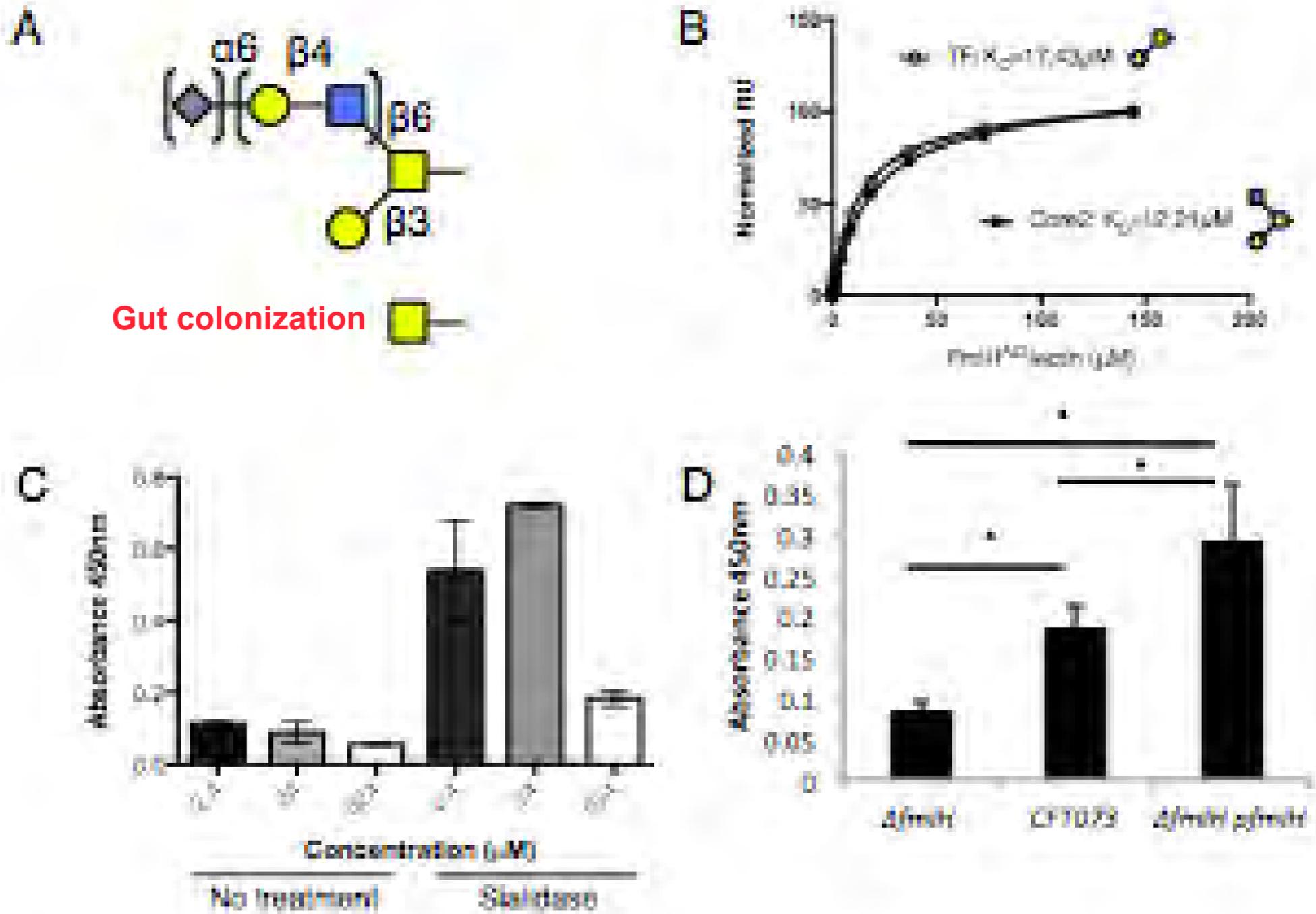
# Gut Reservoir



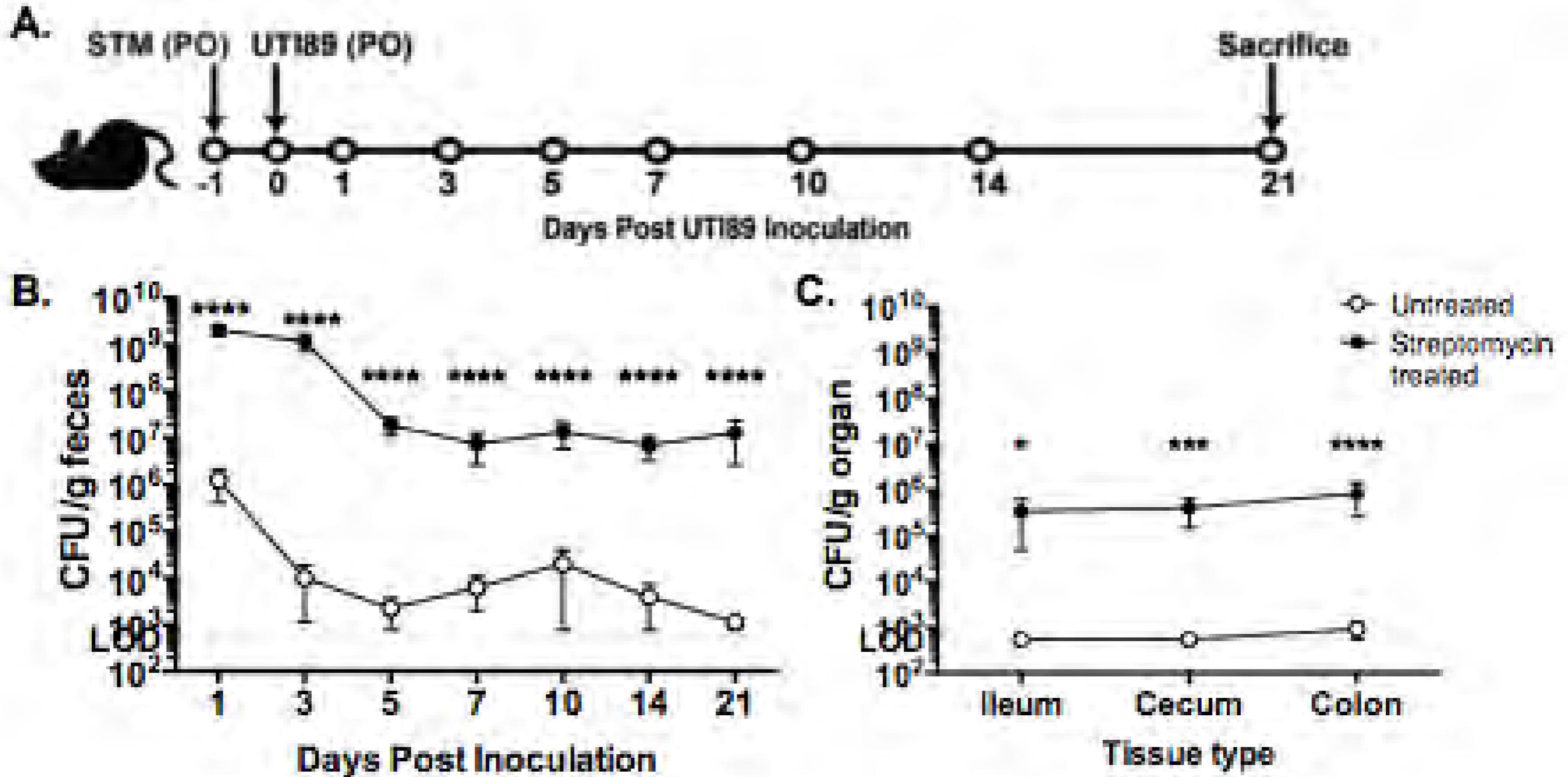
Caitlin Spaulding

# Chaperone-usher pathway pili (CUPs)

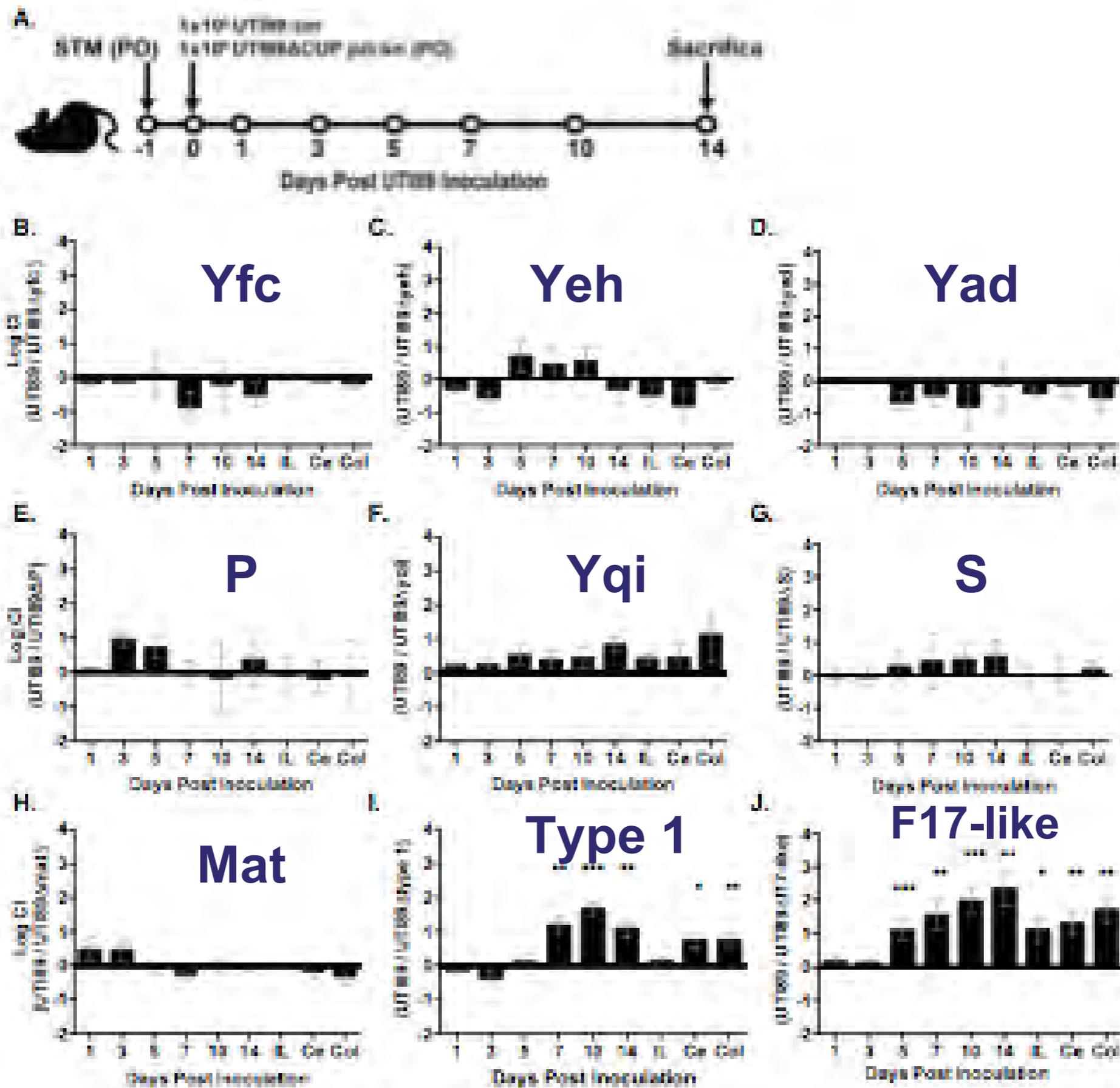
Figure 2



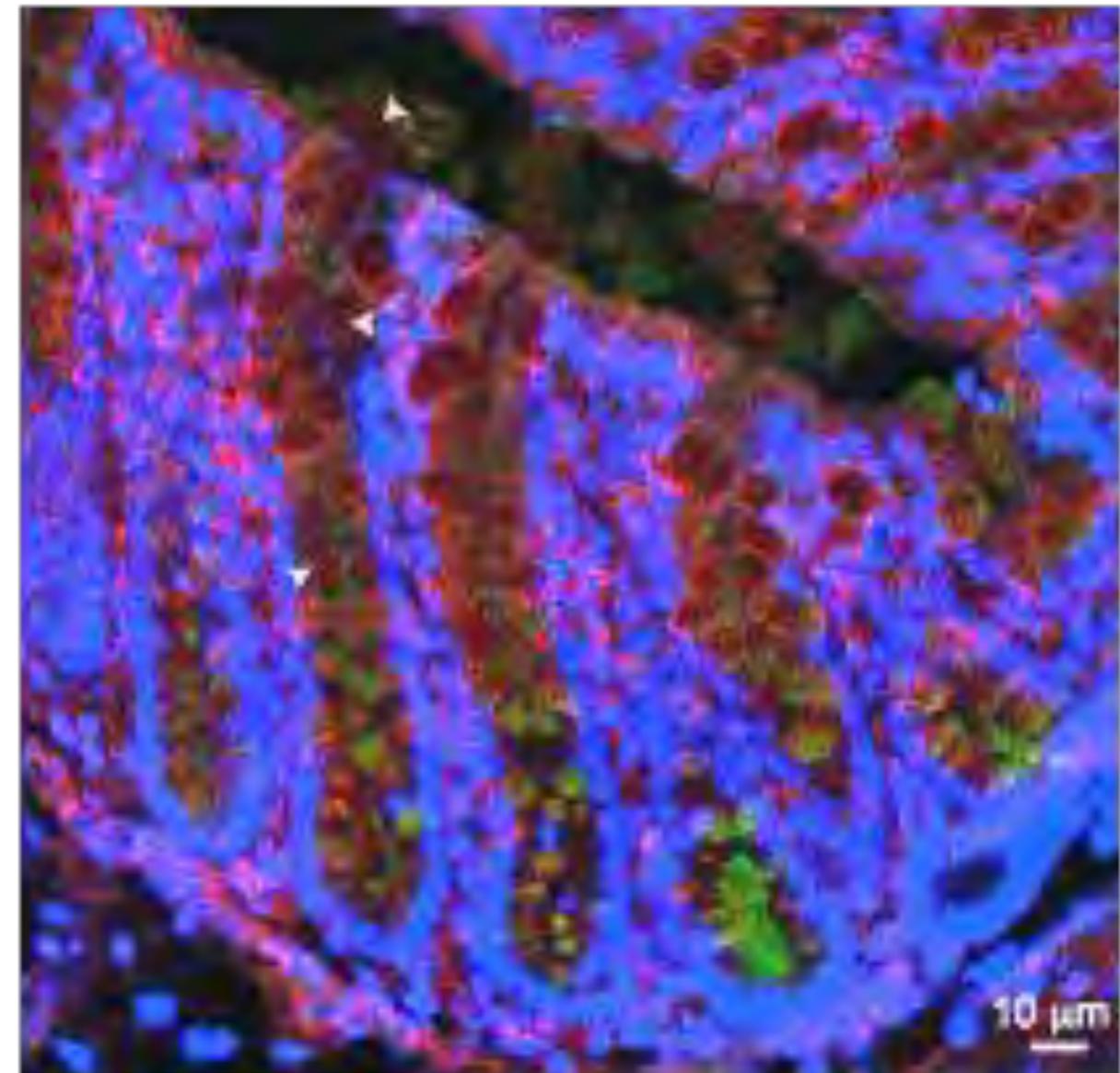
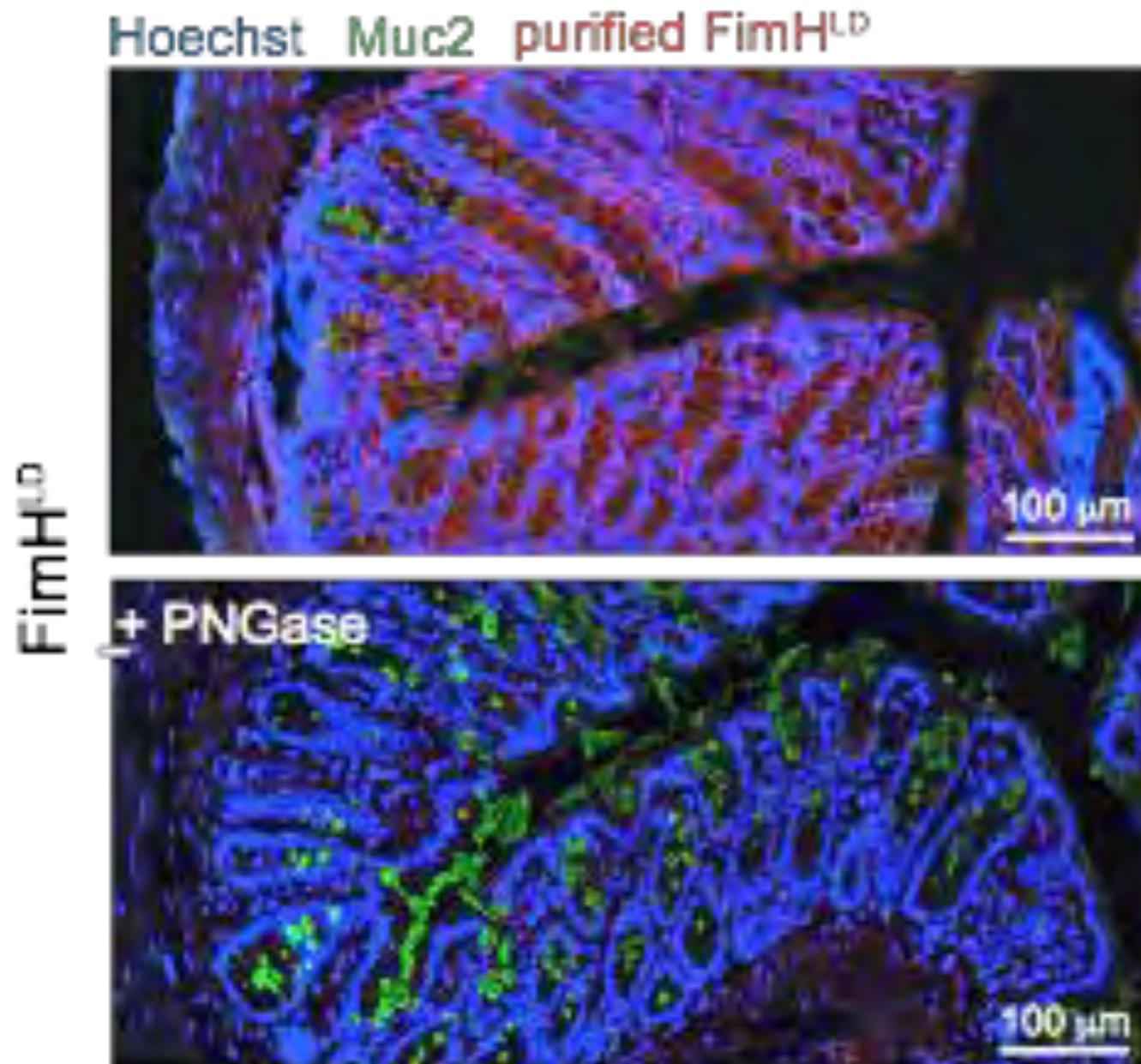
# STM Model of UPEC GIT Colonization



# UPEC Fitness Factors in GIT Colonization

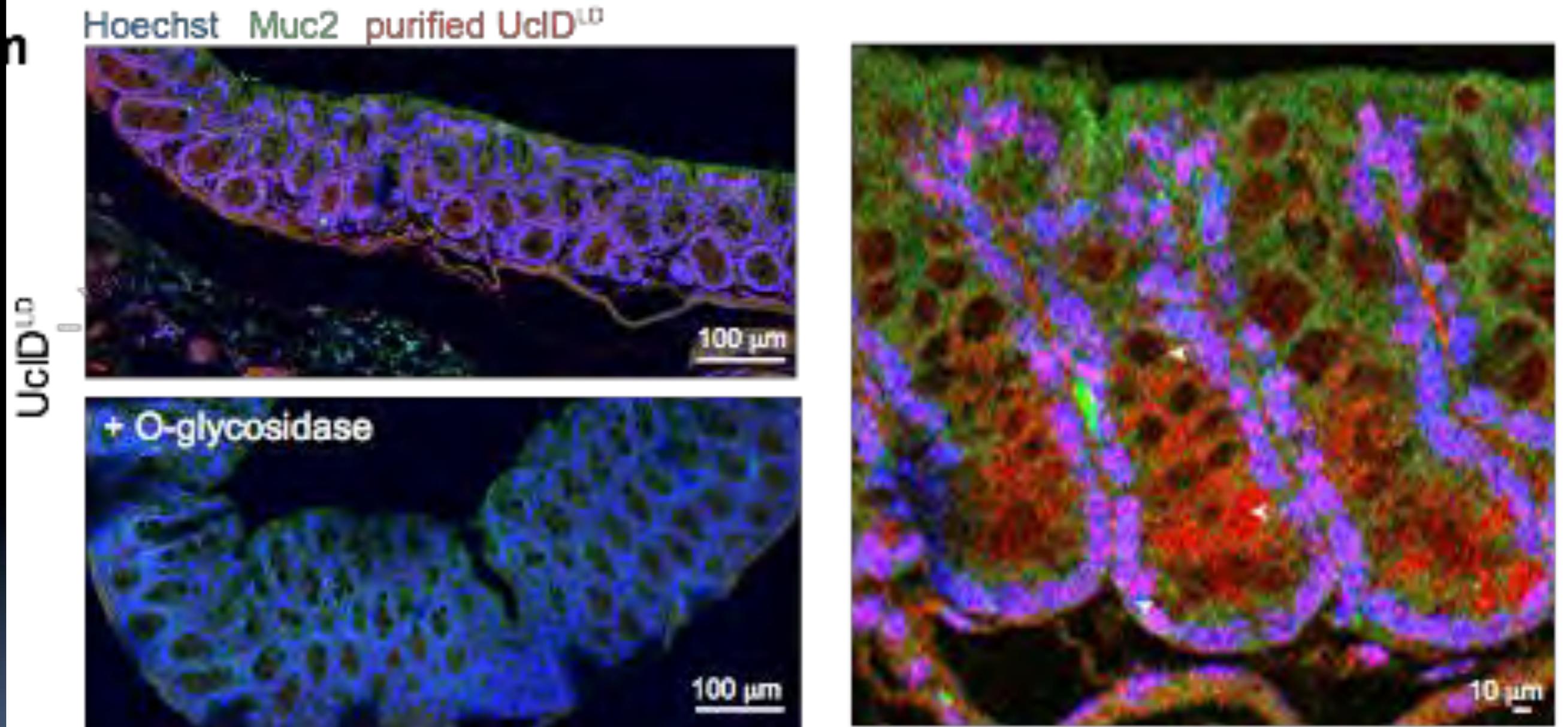


# FimH binds N-linked Oligosaccharides of the Upper Crypts



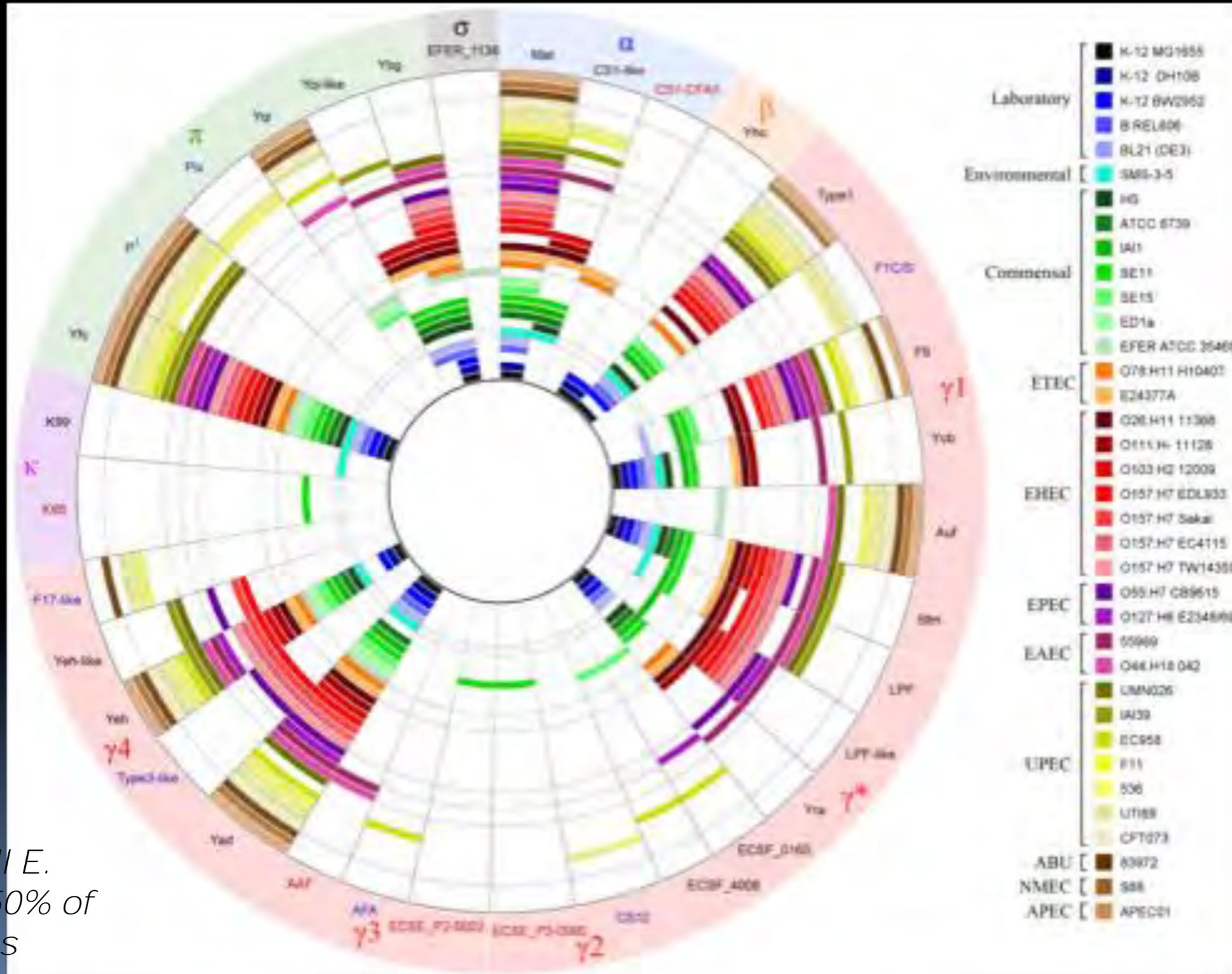
Segolene Ruer

# Uc1D binds O-linked Oligosaccharides of the Lower Crypts



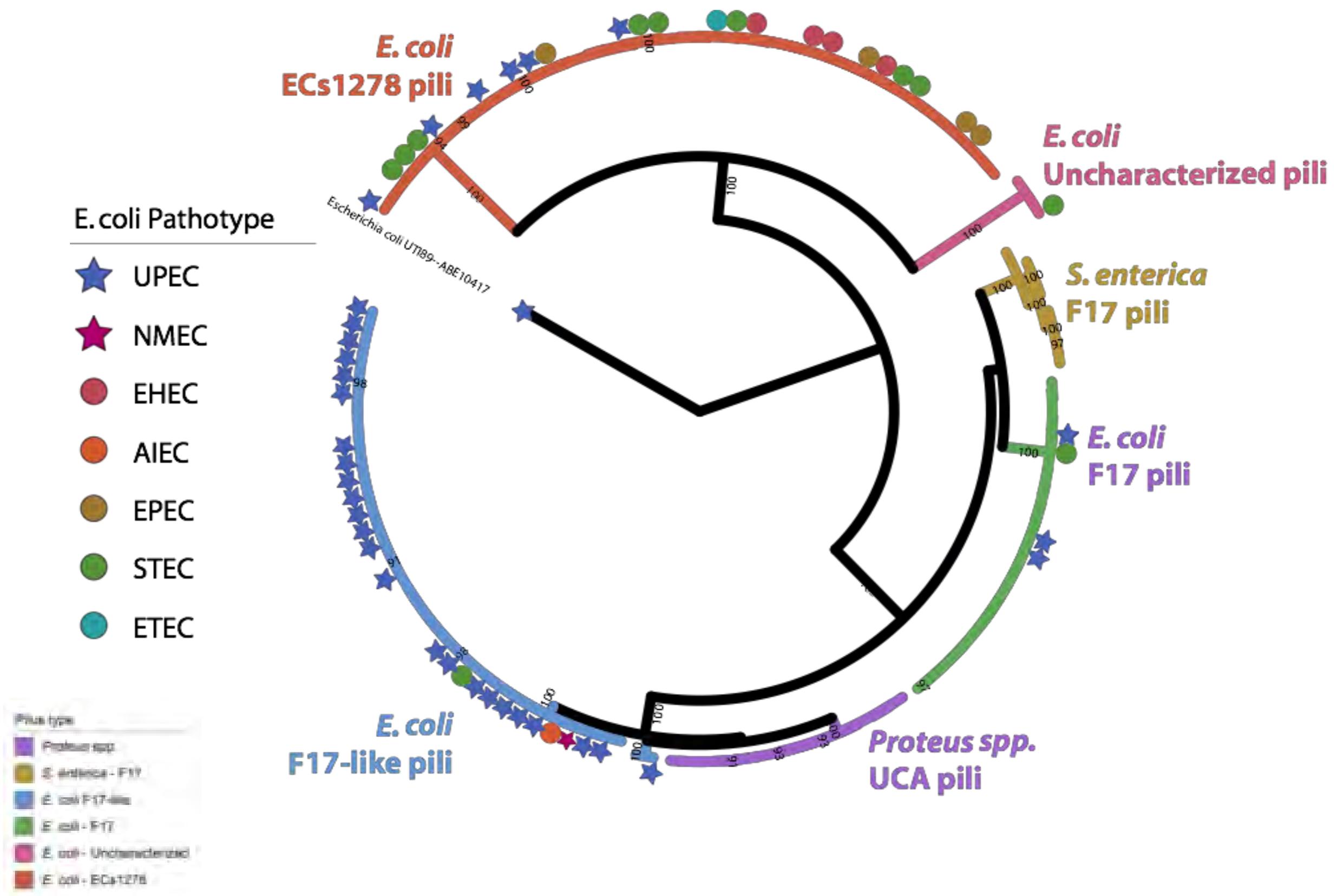
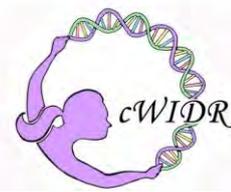
Crypt colonization provides a less competitive environment with regards nutrient competition with the microbiota in the lumen.

# F17-like Pili Restricted to Extra-intestinal *E. coli*

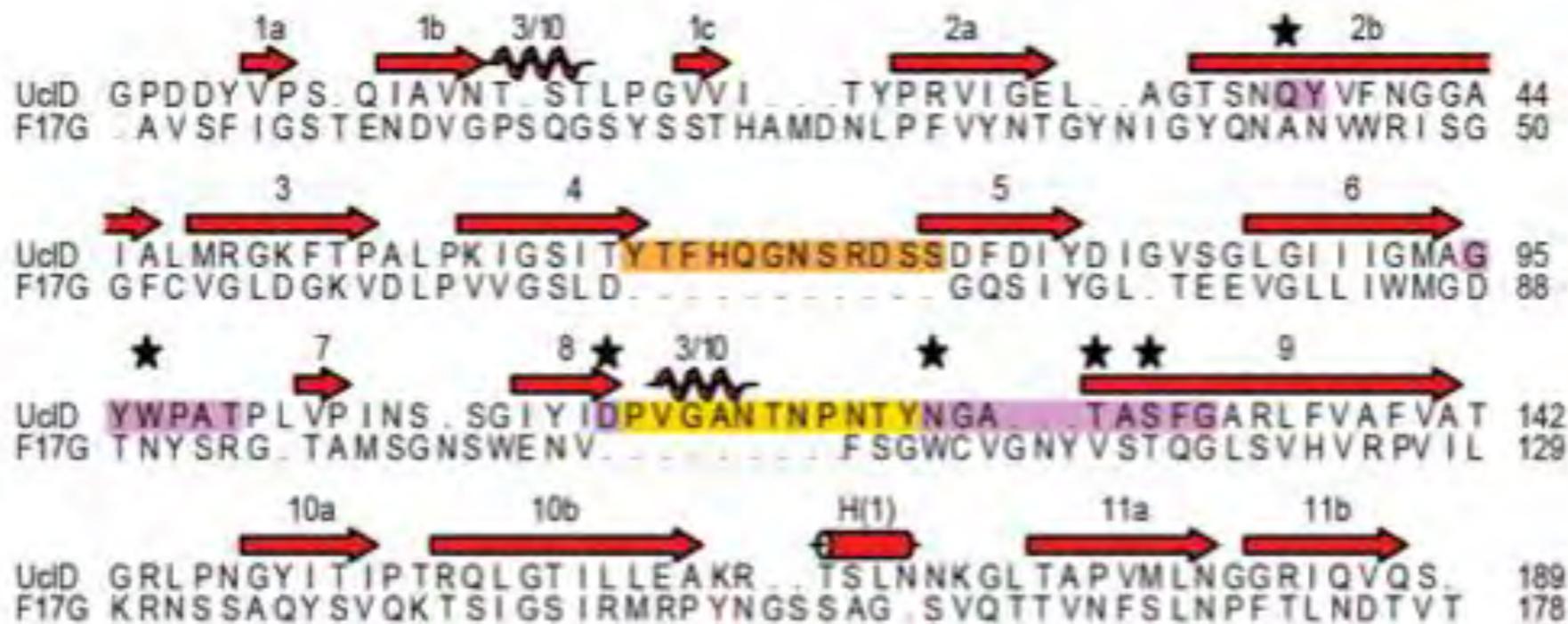
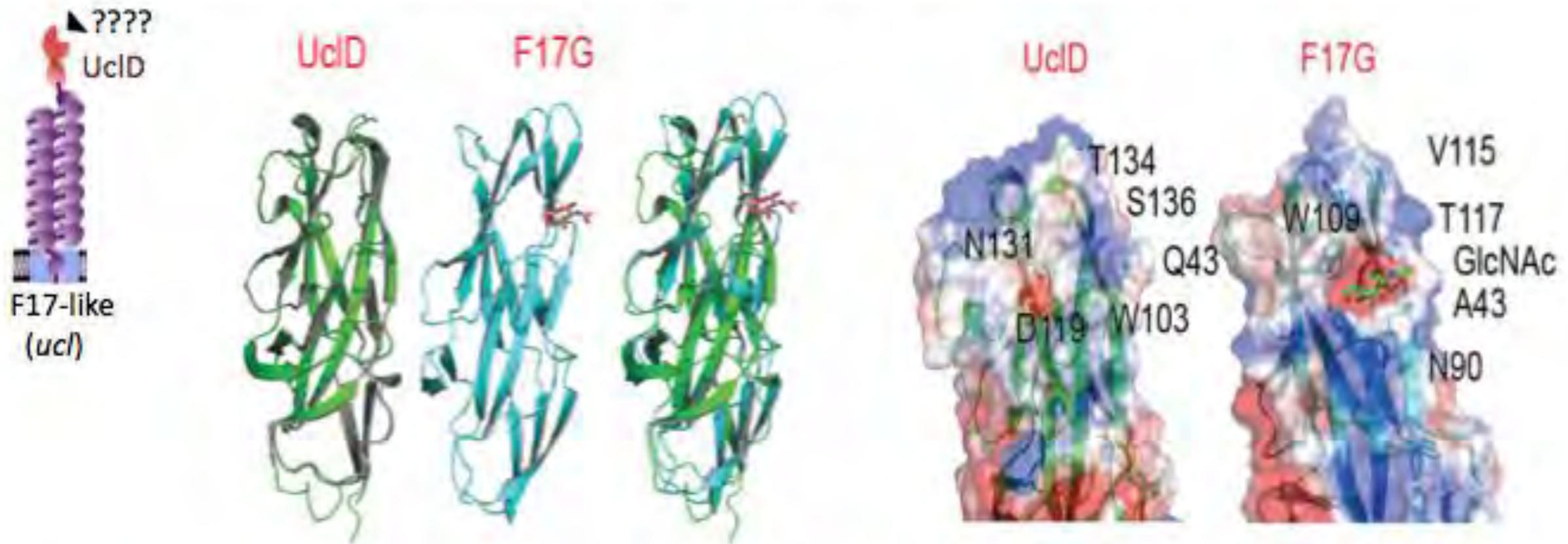


- ▶ 11% of all *E. coli* but 50% of B2 strains

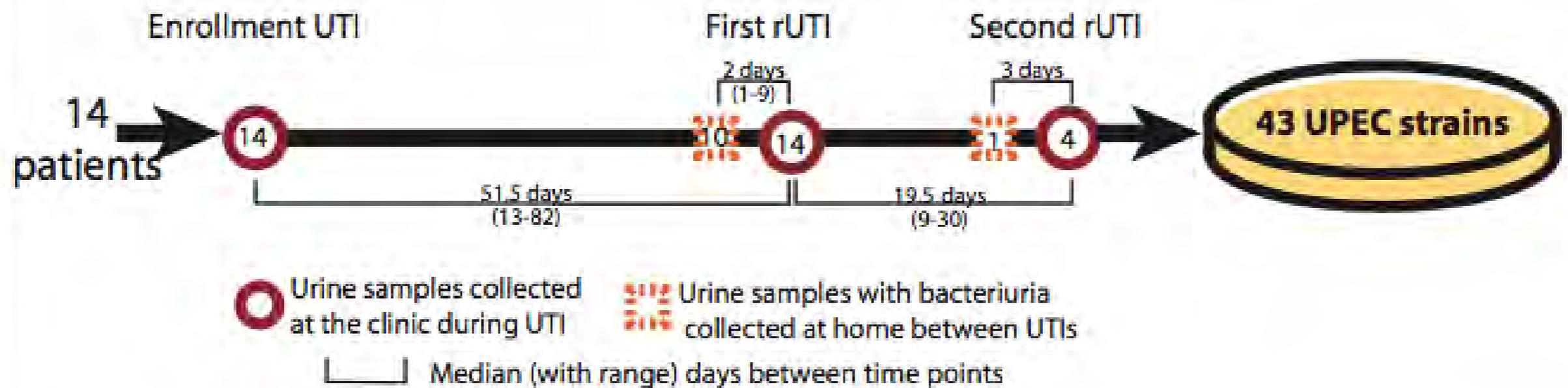
# B2 UPEC acquired F17-like pili from intestinal pathogens



# UclD has same structure as F17G



## F17-like carriage in UPEC from patients with rUTI



### Carriage of F17-like pili in *E. coli*

11% of *E. coli* strains encode F17-like pili

50% of B2 strains encode F17-like pili

### Carriage of F17-like pili in UPEC

13/14 women with rUTI caused by a B2 *E. coli* strain encode F17-like

**F17-like pili might be associated with UPEC persistence in women with rUTI by promoting the maintenance of the UPEC intestinal reservoir.**

- **Translate basic science advances into new and better antibiotic-sparing therapeutics**
- **Antibiotic resistance rising at an alarming rate**
- **Reaching a tipping point**

# Development of Anti-Virulence Therapeutics

- Mannosides
- UTI vaccine
- FmID inhibitors
- PapG inhibitors
- Pilicides (Assembly)
- CAUTI vaccine

| ANTIBIOTIC                   | NUMBER OF PRESCRIPTIONS |
|------------------------------|-------------------------|
| CIPROFLOXACIN                | 4,274,000               |
| LEVOFLOXACIN                 | 1,334,000               |
| SULFAS & TRIMETH             | 3,135,000               |
| NITROFURANTOIN               | 2,069,000               |
| CEPHALOSPORINS & PENICILLINS | 735,000                 |

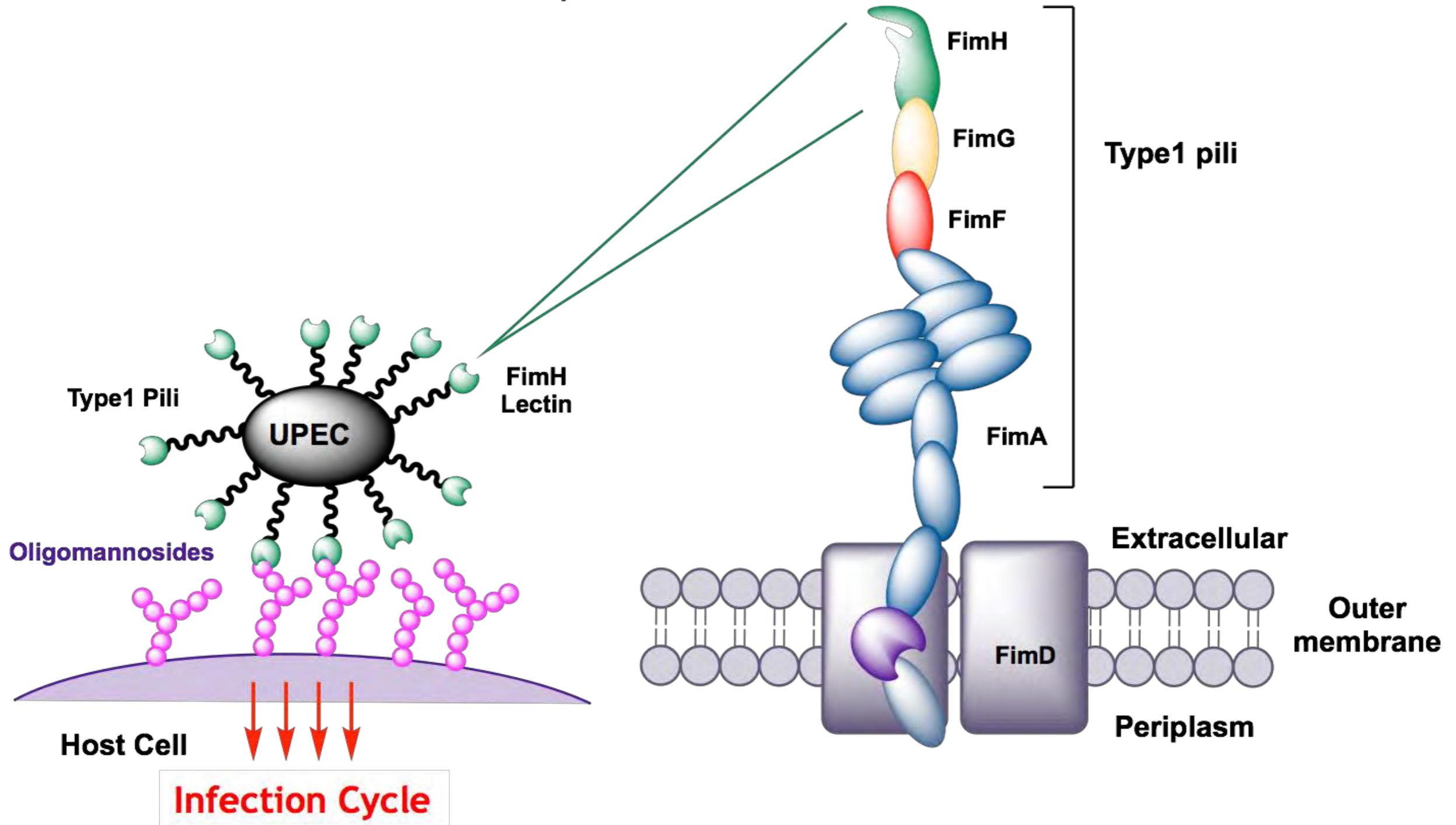
National Disease and Therapeutic Index™ IMS Health 2008

**Need Antibiotic-Sparing Agents**

# Molecular Basis of FimH Vaccine

## FimH Lectin

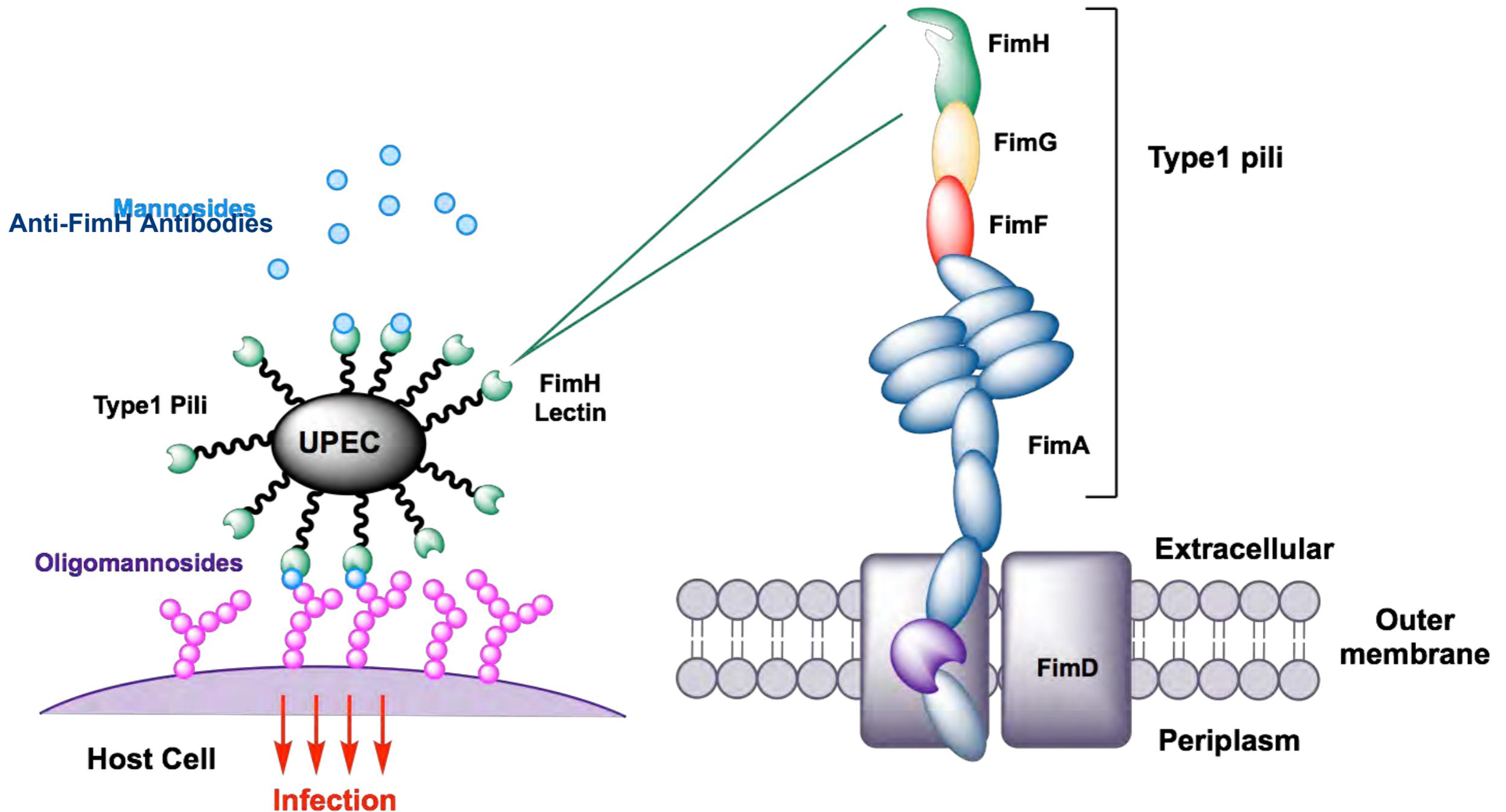
- Is a known UPEC virulence factor
- Plays an important role in all aspects of the infection cycle
- Mannose-specific



# Molecular Basis of FimH Vaccine

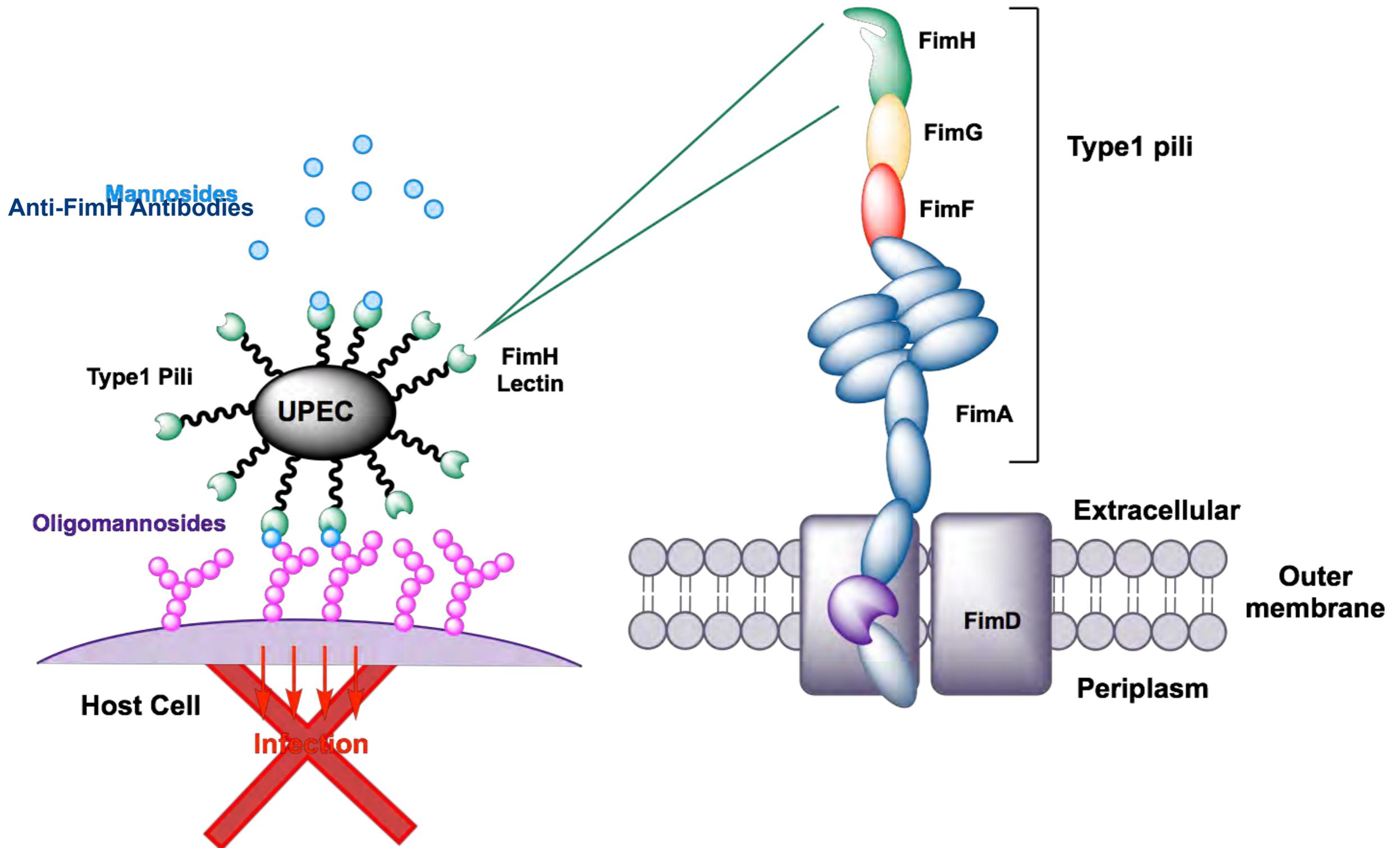
## Anti-FimH Antibodies Inhibit Function of FimH

- prevents UPEC from causing further infection or from propagating an existing infection



# Anti-FimH Antibodies Inhibit Function of FimH

- prevents UPEC from causing further infection or from propagating an existing infection



# Phase 1A/1B FimH Vaccine Study

▶ Objectives of this study were to:

- Assess safety and tolerability
- Measure the serum IgG response to the FimH lectin domain
- Measure the duration and sustainability of the IgG response

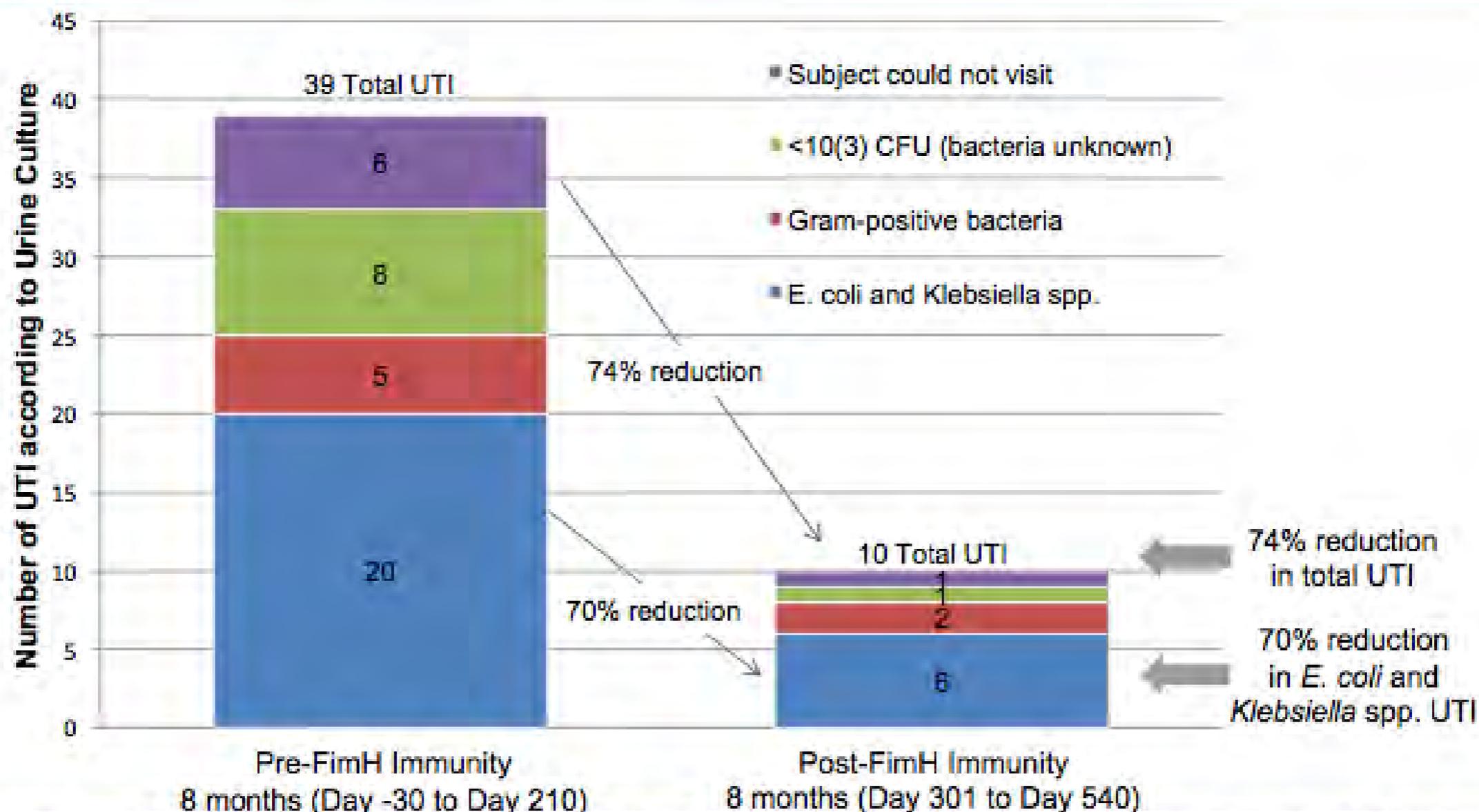
▶ Study design:

- Included 67 women, ages 21-64, in 6 cohorts; dose escalation design
- Conducted at 5 clinical sites with monitoring by a Safety Review Committee
- Subjects in cohorts 1 to 4 (Phase 1A) did not have a history of UTI in the previous 24 months prior to enrollment into the study. Subjects in cohorts 5 and 6 (Phase 1B) had  $\geq 5$  documented UTI in the last 24 months, including at least 1 with *E. coli*. Doses for cohorts 5 and 6 were based on safety data and antibody responses from cohorts 1 to 4.
- Intramuscular (IM) dosing on days 0, 30, 90 and 180; end of study was 12 months after last vaccination

| Cohort | Subjects | FimCH ( $\mu\text{g}$ ) | Adjuvant ( $\mu\text{g}$ ) | UTI Status |
|--------|----------|-------------------------|----------------------------|------------|
| 1      | 5        | 107                     | 0                          | None       |
| 2      | 8        | 50                      | 10                         | None       |
| 3      | 16       | 50                      | 20                         | None       |
| 4      | 8        | 50                      | 40                         | None       |
| 5      | 16       | 50                      | 40                         | Recurrent  |
| 6      | 14       | 107                     | 43                         | Recurrent  |

# Incidence of Recurrent UTI among the 13 Subjects of Cohort 5

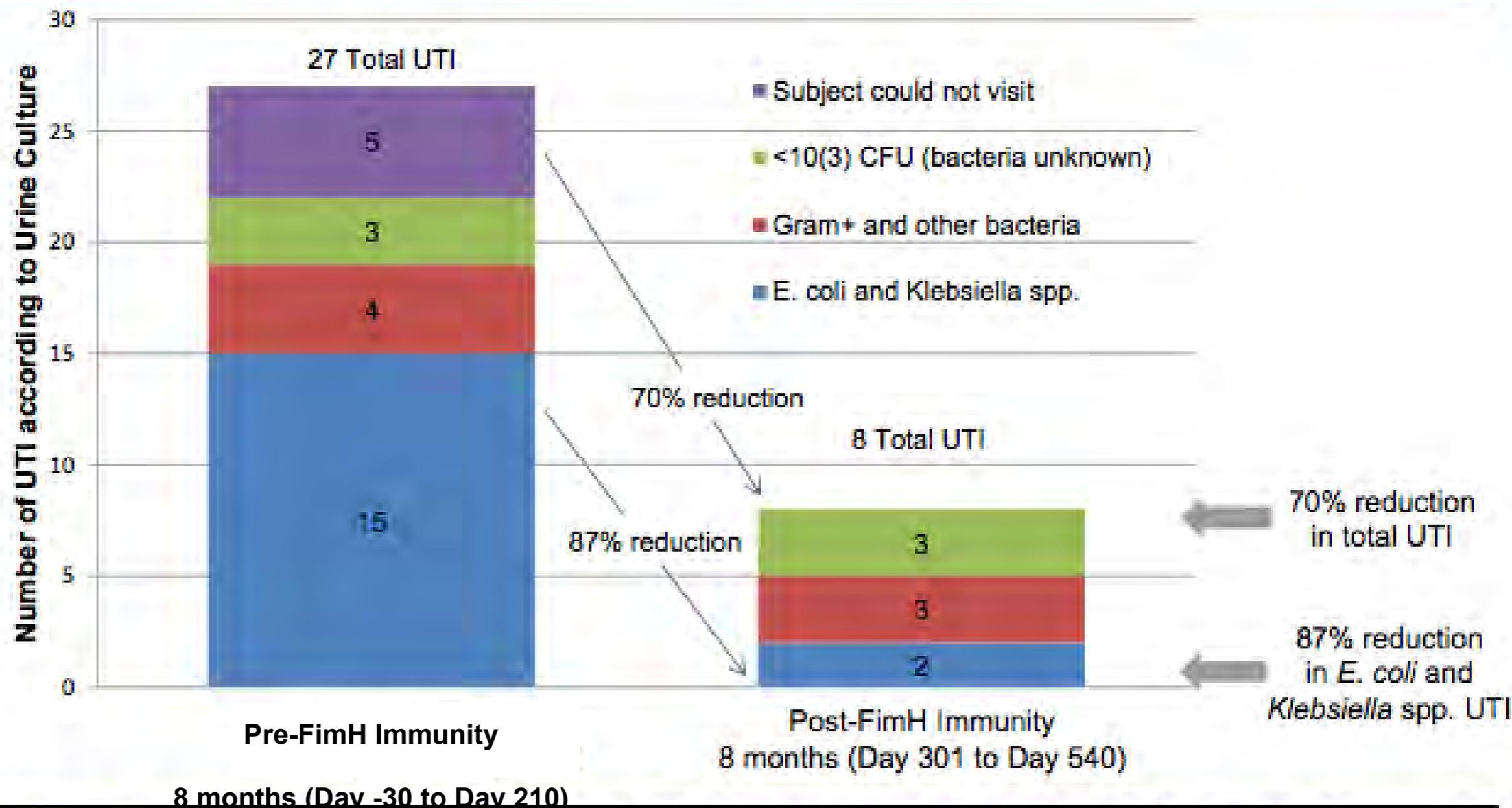
## First 8 study months compared to the last 8 study months



The Phase 1B results show trends of a 74% reduction in total UTI and 70% reduction in *E. coli* and *Klebsiella* spp UTI. 6 of these subjects did not have any UTI during the last 8 months of the study. These preliminary data support conducting a randomized, placebo-controlled Phase 2 study.

# Incidence of Recurrent UTI among the 12 Subjects of Cohort 6

## First 8 study months compared to the last 8 study months



The Phase 1B results show trends of a 70% reduction in total UTI and 87% reduction in *E. coli* and *Klebsiella* spp UTI. 8 of these subjects did not have any UTI during the last 8 months of this study. These preliminary data support conducting a randomized, placebo-controlled Phase 2 study.

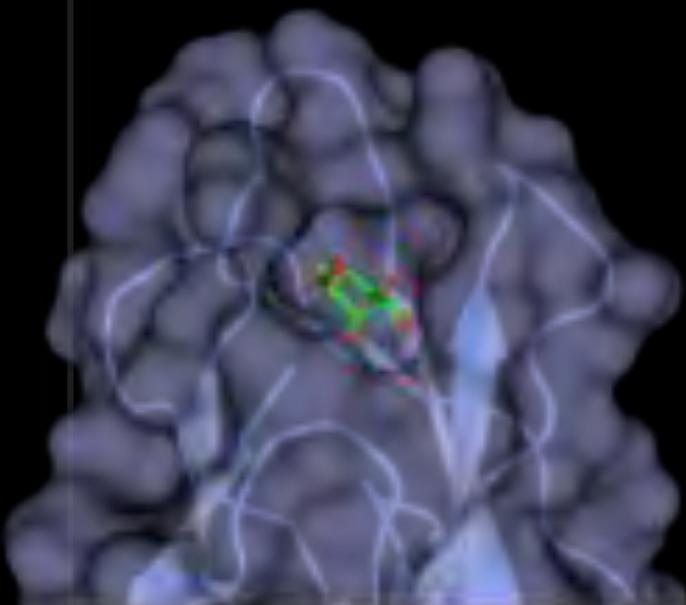
# Compassionate Use of Sequoia's Vaccine Approved by CBER / FDA in Q3 2016

## UTI History of a 73-year old woman

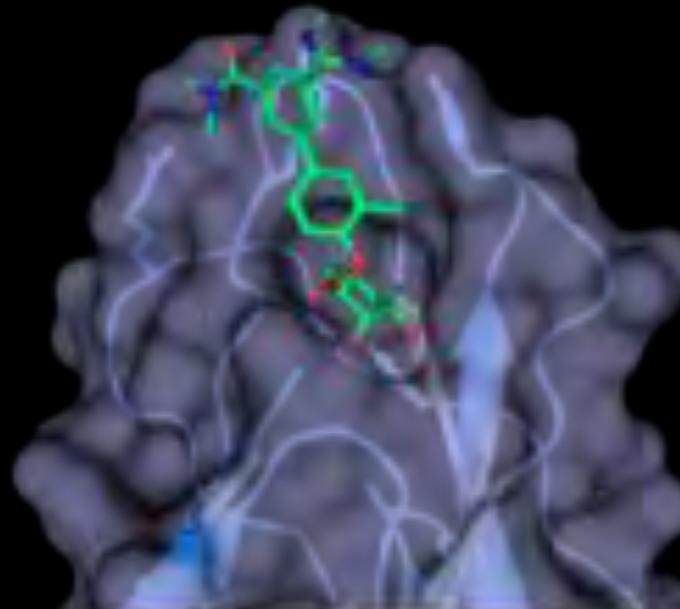
- Recurrent UTI caused by *E. coli* resistant to the standard of care
- Exhausted all therapeutic options requiring the last-line of defense carbapenem antibiotics
- ▶ *E. coli* identified in her urine during UTI symptoms
  - February, 2016 – failed prophylaxis with oral ampicillin
  - March, 2016 - resistant to fluoroquinolones and trimethoprim-sulfamethoxazole
  - March, 2016 - failed prophylaxis with amoxicillin / clavulanate
  - April, 2016 - failed prophylaxis with nitrofurantoin
  - May, 2016 - resistant to nitrofurantoin
  - May, June, and August, 2016 – identified extended-spectrum  $\beta$ -lactamase (ESBL)
  - Final option used throughout failures in 2015 to 2016 has been intravenous ertapenem for seven to twelve days to achieve clinical response

**Based on the first compassionate use experience above, Thomas Hooton, MD received approval to expand the compassionate use program in collaboration with Sequoia.**

# Mannosides Target Attachment, the First Step in the Pathogenic Cycle



**FimH-mannose**

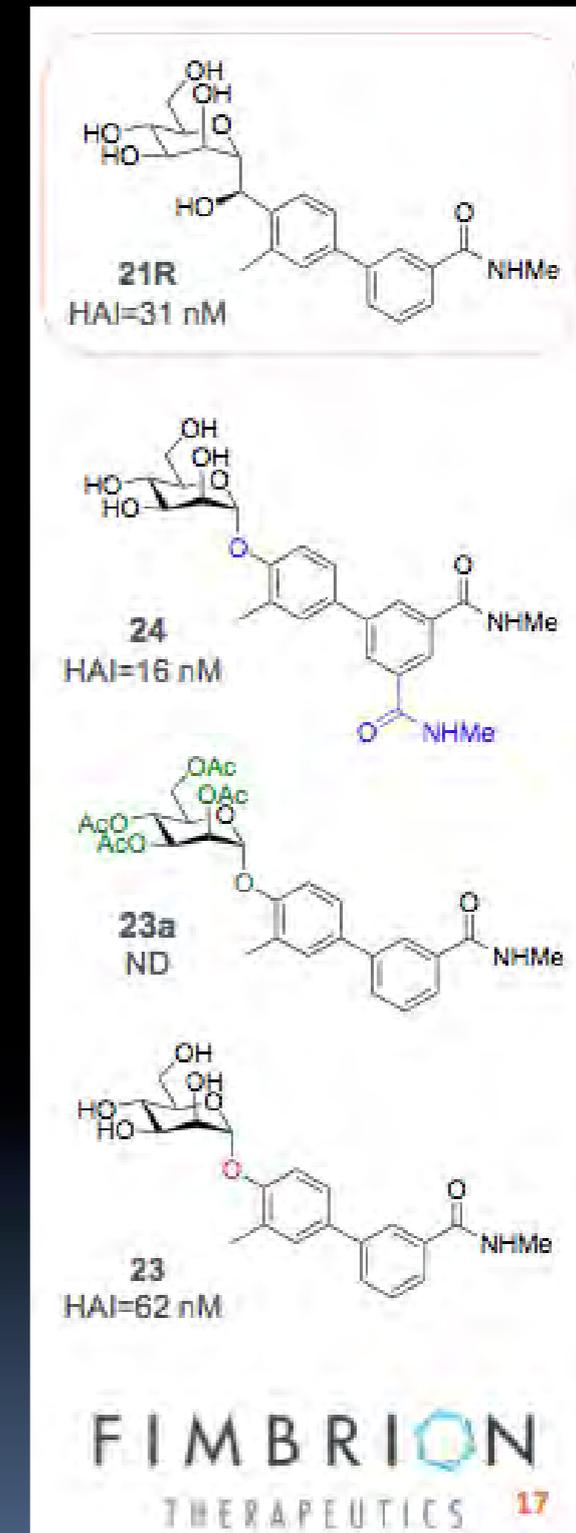


**FimH-mannoside**

Rationally Designed Mannosides to Make High Affinity Interactions with FimH

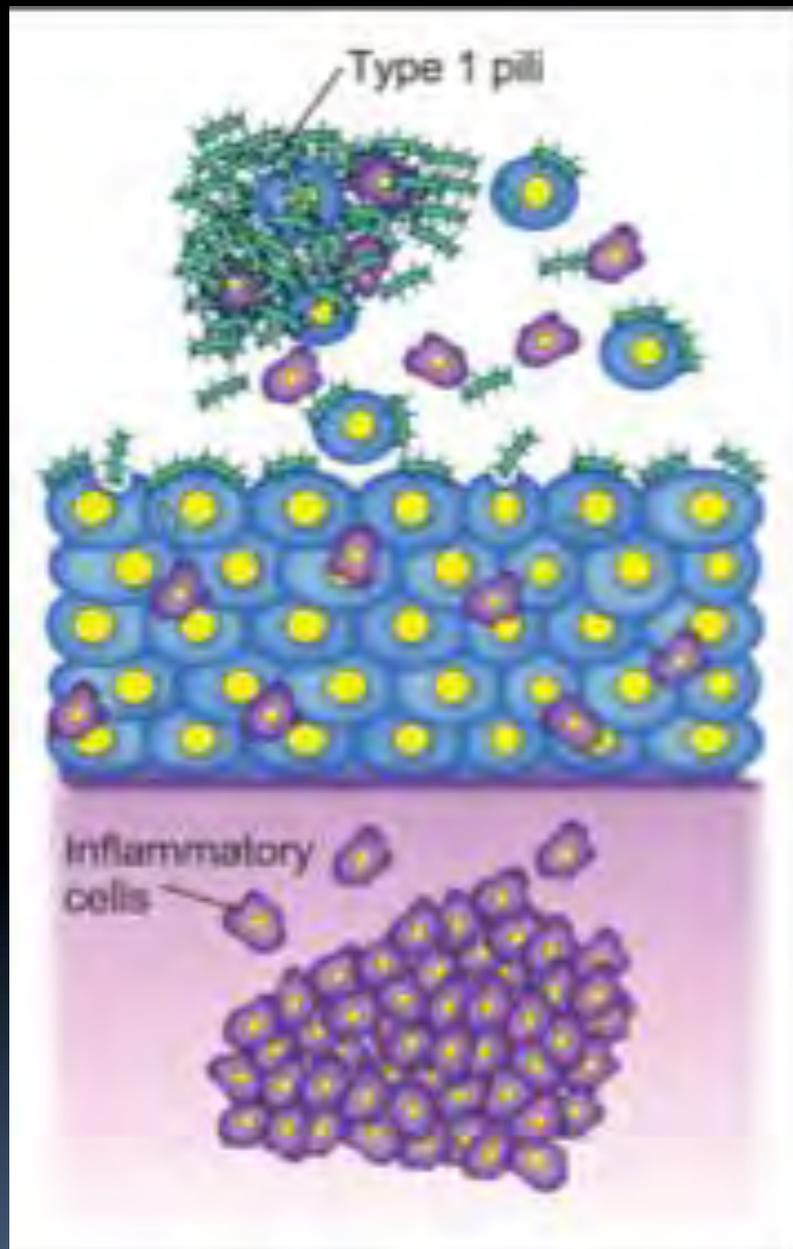
Lead Mannosides have >1,000,000 Times Increased Affinity for FimH over Mannose

Mannosides Effectively Block Bacterial Binding to the Bladder

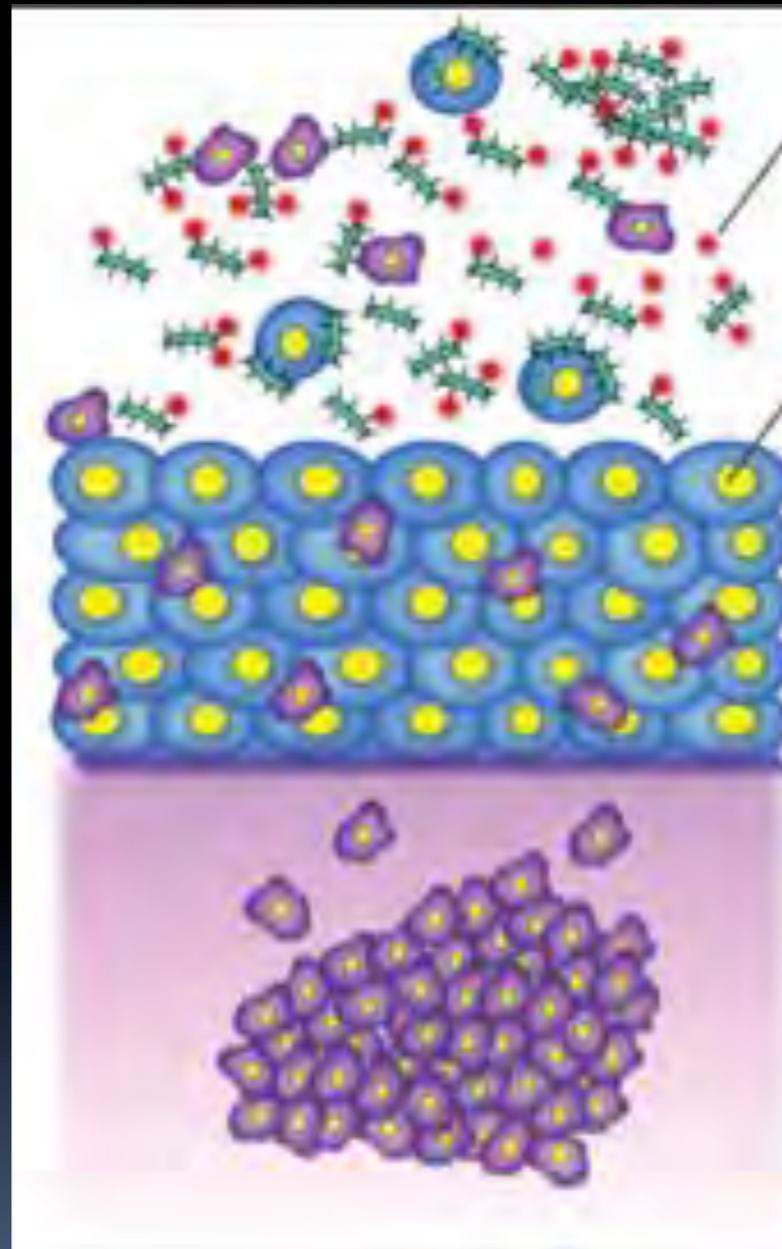


**Fimbrion-GSK**

# The Opportunity: Mannosides as Therapeutics



Bacteria Bind to Cells,  
Causing Infection



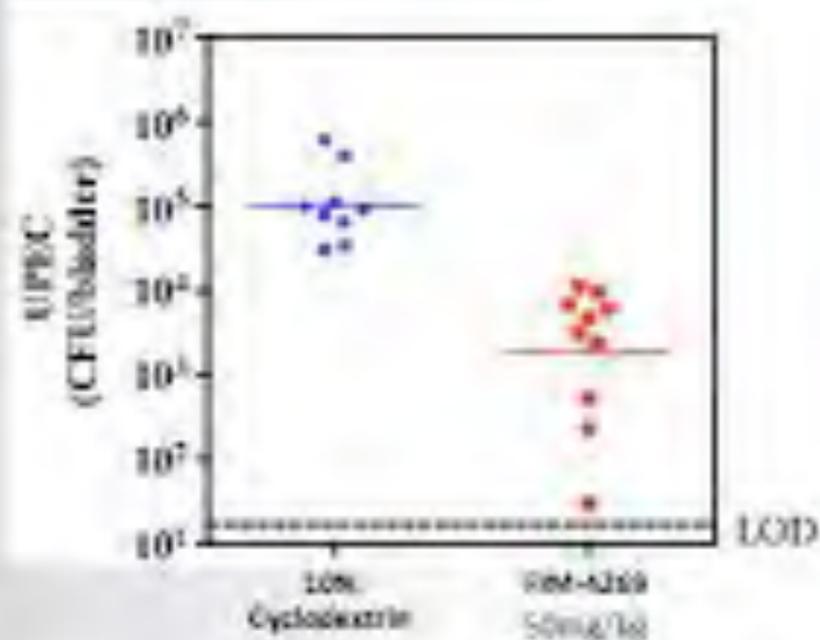
Mannosides Block FimH Mediated Binding,  
Preventing Adherence and Invasion into  
Bladder Epithelium

**Moving our lead  
compounds into  
clinical trials**

# Treating Bladder Infections with FIM-4269

## PROPHYLACTIC TREATMENT

3 Hours Prior to Infection

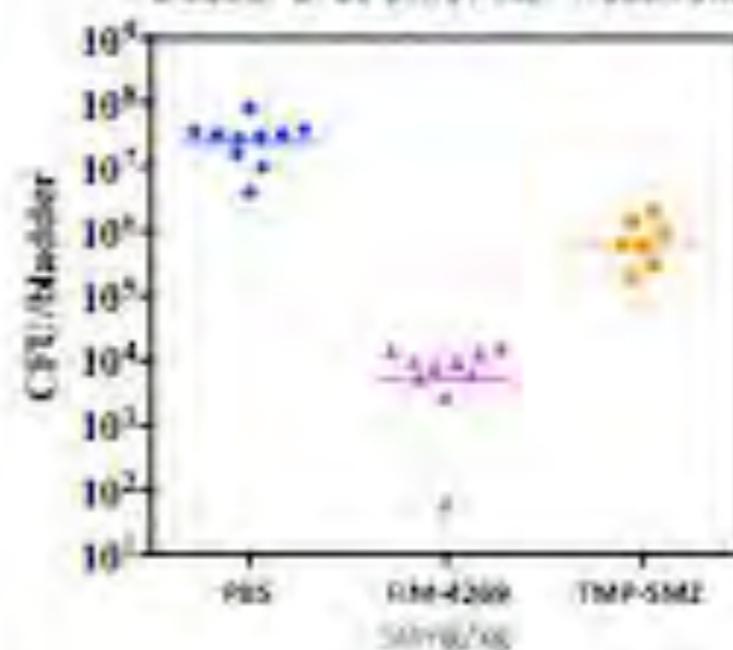


FIM-4269 Shows Protection 3 hours Prior to Infection, Reducing CFUs in the Bladder ~100 fold

## TREATMENT OF UTI INFECTION

Single Dose

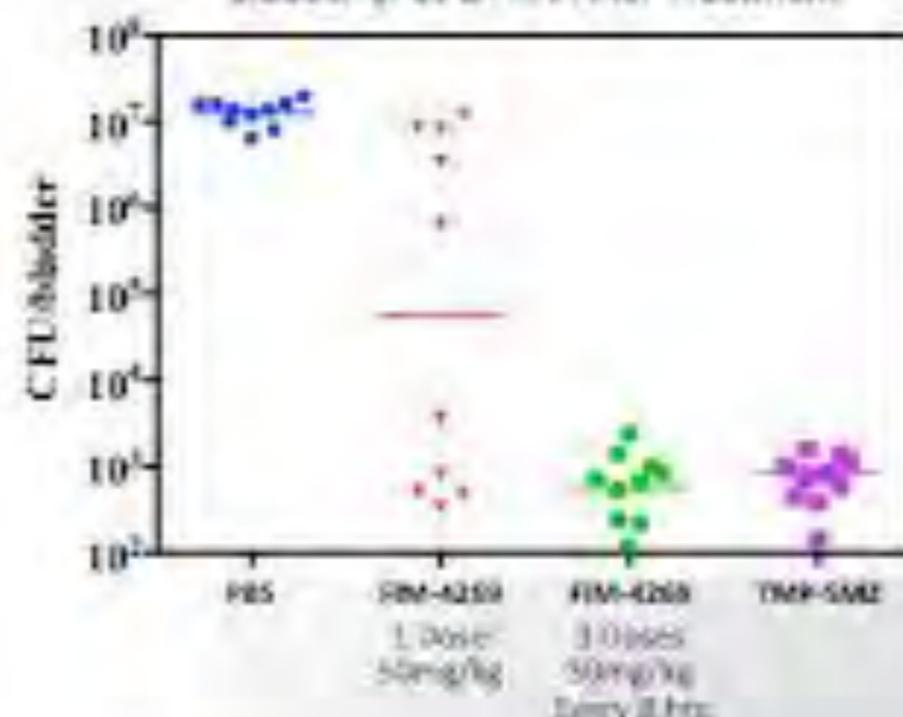
Bladder CFUs 6 hrs After Treatment



Treatment of *E. coli* Infected Mice with Mannosides Clears Bacteria from Bladder More Quickly Than TMP-SMZ

"Multiple" Doses

Bladder CFUs 24 hrs After Treatment

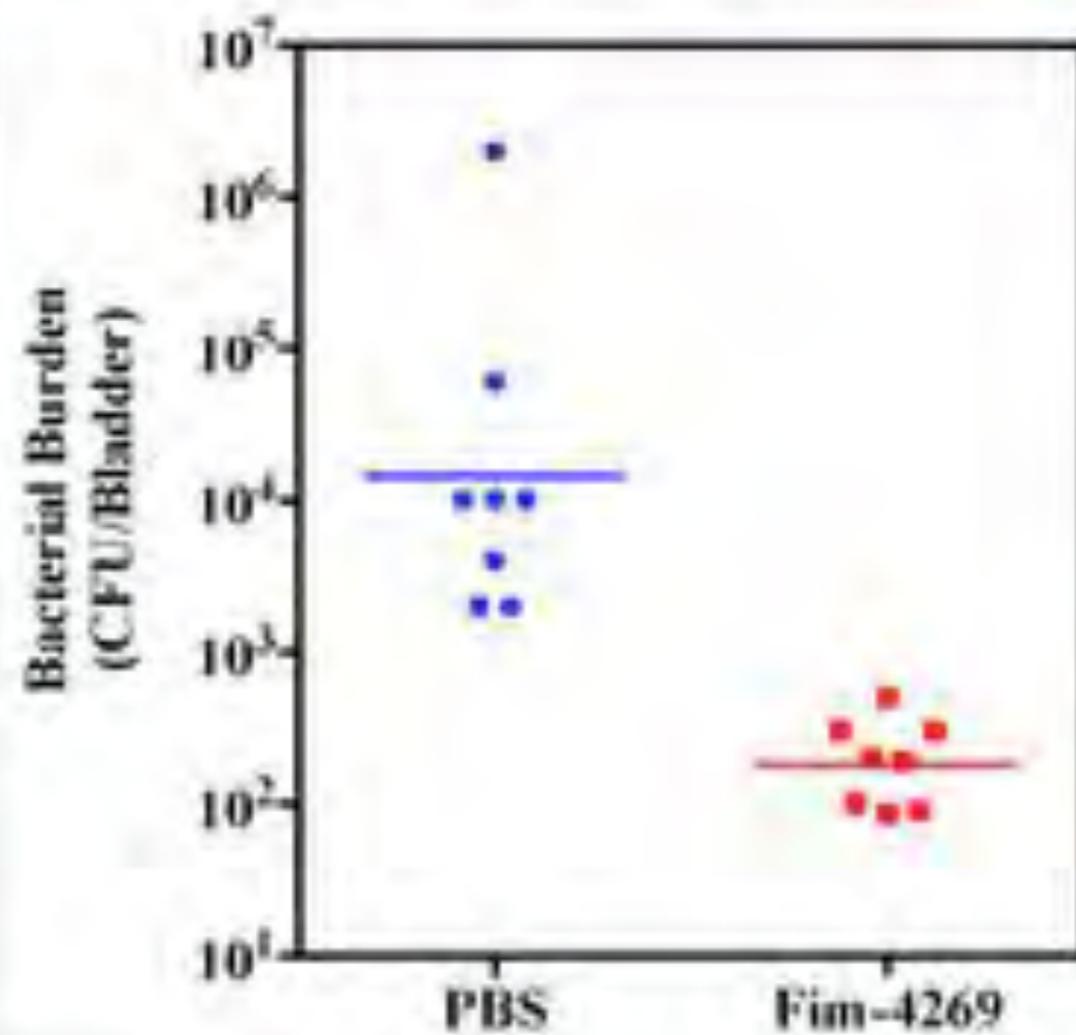


Treatment of an Infection Every 8 hrs Eliminates Bacteria from the Bladder and is Equivalent to TMP-SMZ

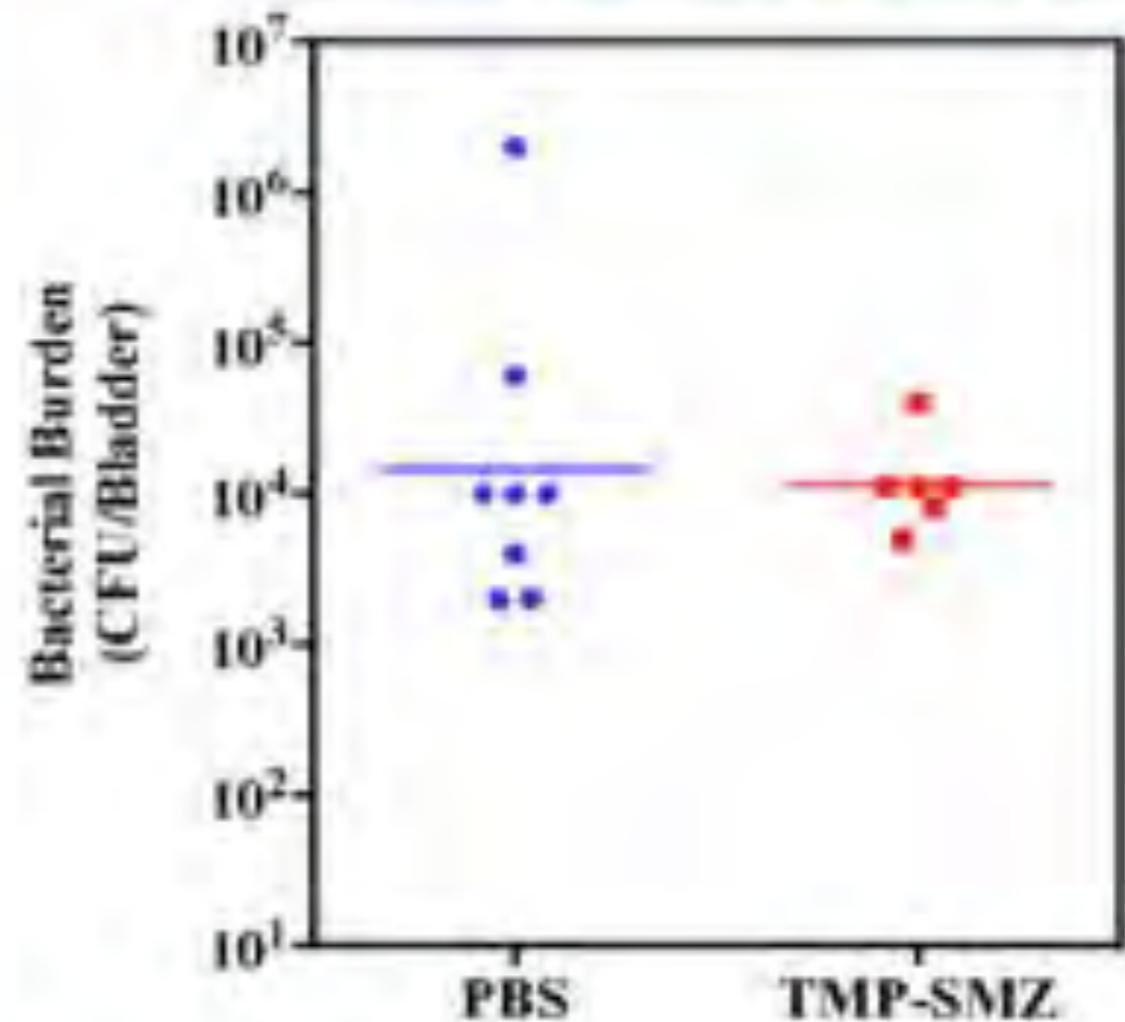
# Prevention of Multi-Drug Resistant ST131 Strain

## BLADDER CFUs 6 HRS AFTER TREATMENT

Mannoside Treatment of ST131 Strain



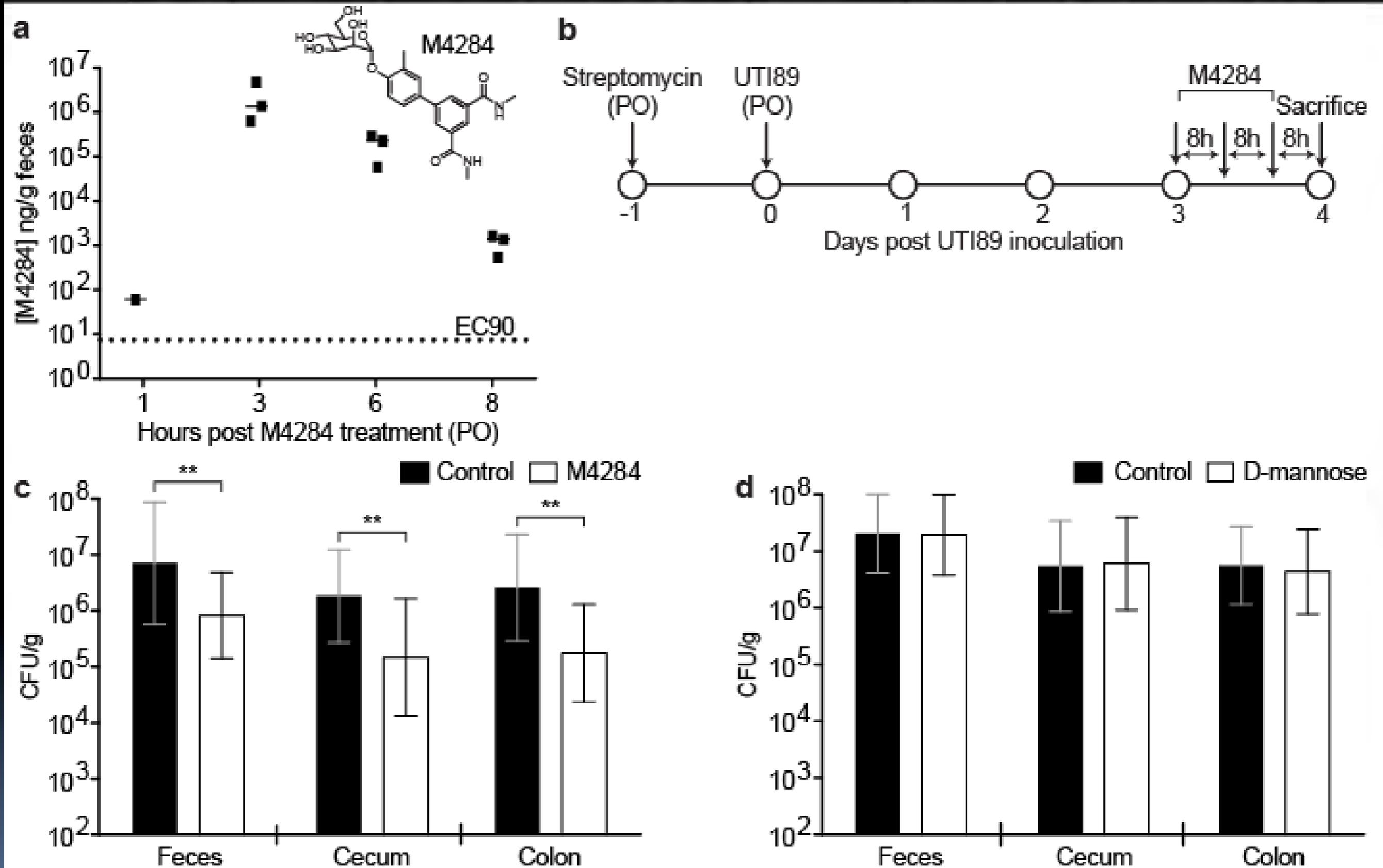
Antibiotic Treatment of ST131 Strain



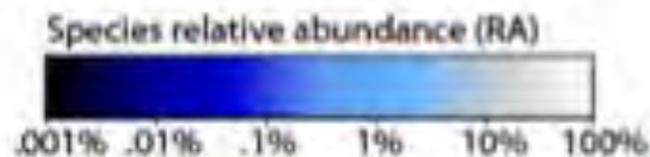
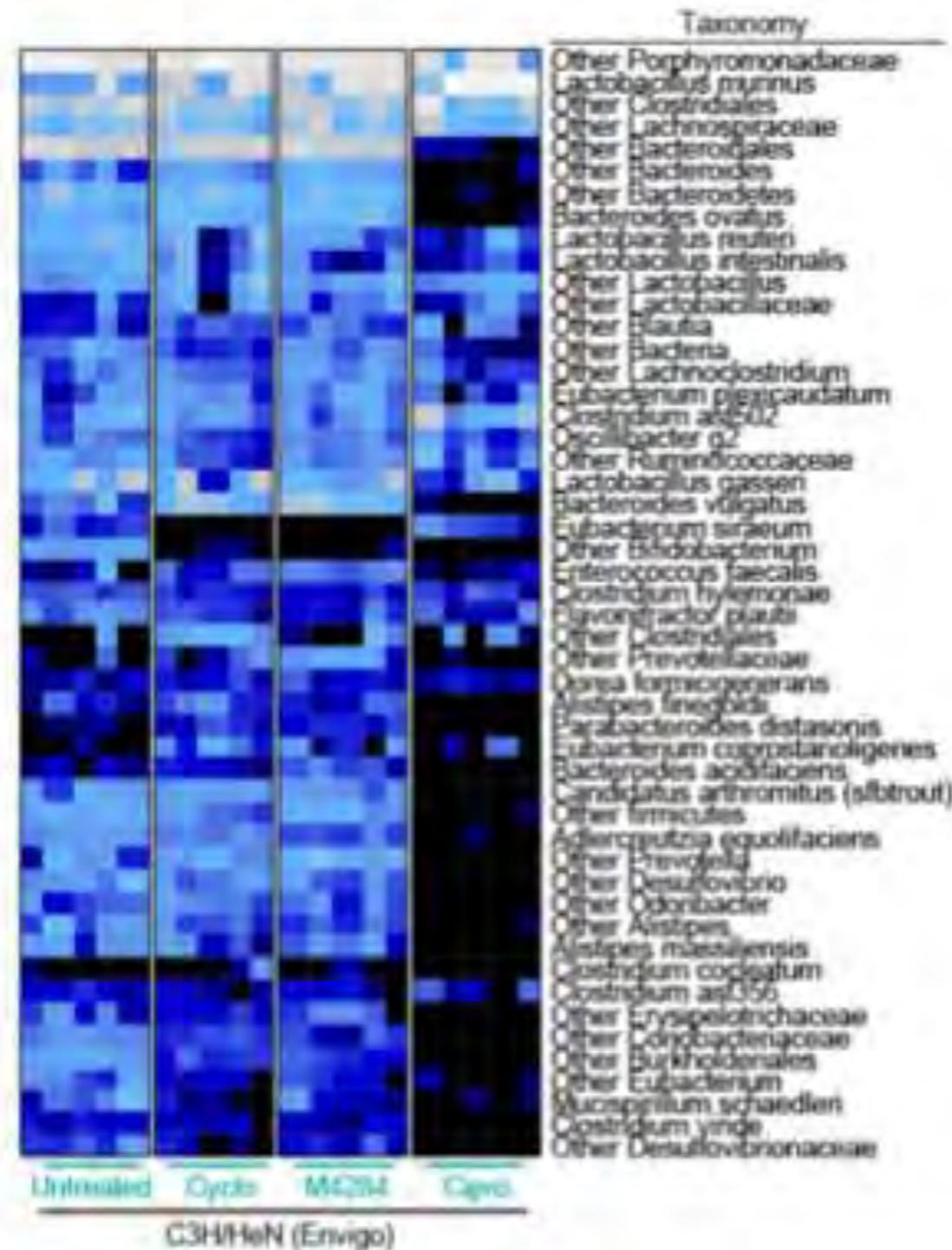
Treatment of Mice Infected with Multi-Drug Resistant *E. coli* Strain  
Much More Effective with Mannosides

Mice treated with TMP/SMX (54/270 ug/mL) for 3 days prior infection or with 50 mg/kg of 4269 30 min. prior infection

# Mannoside treatment reduces UTI89 intestinal colonization

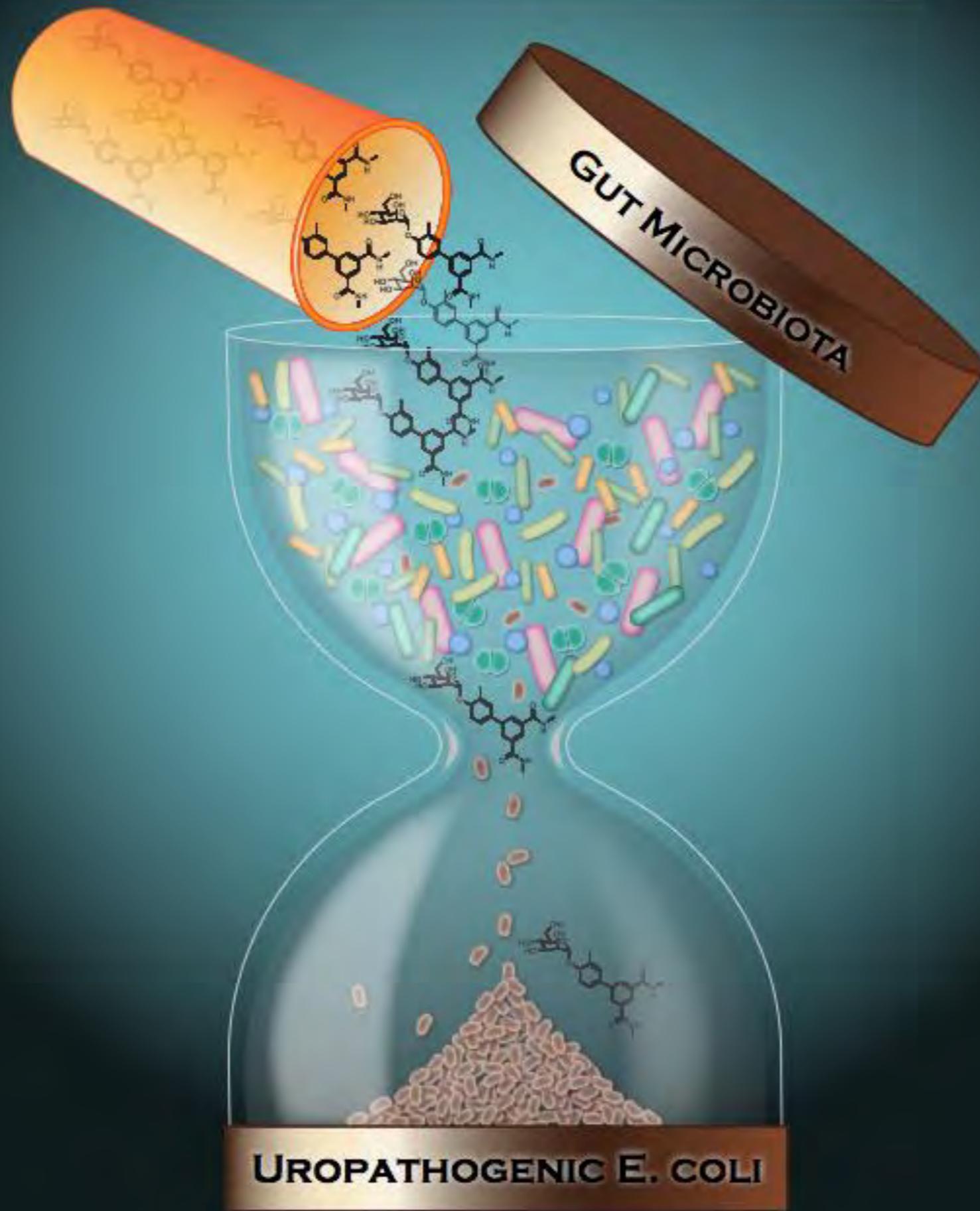


# M4284 treatment minimally alters the microbiota community structure of naïve C3H/HeN mice

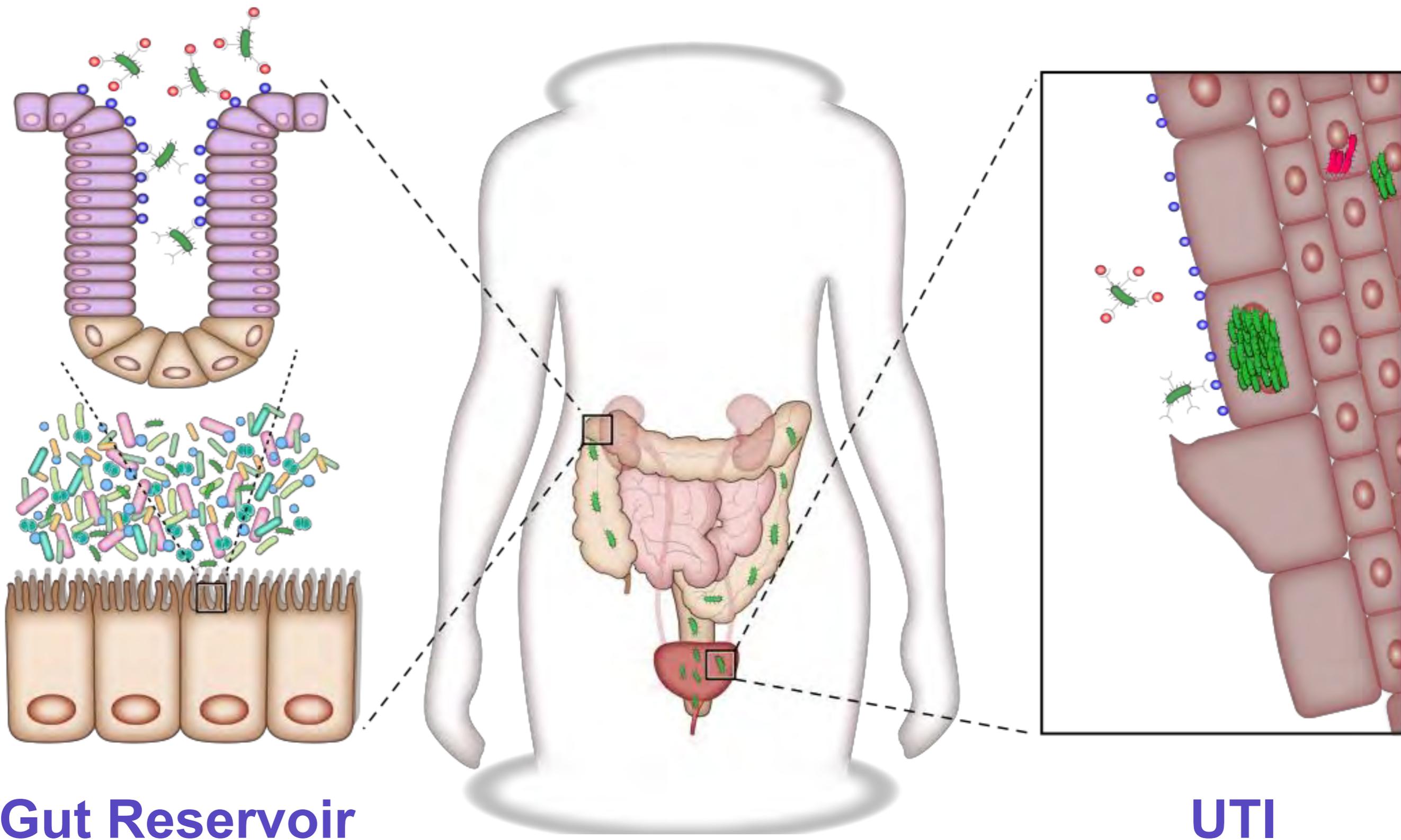


Taxon is significantly different ( $p < 0.05$ ) between:

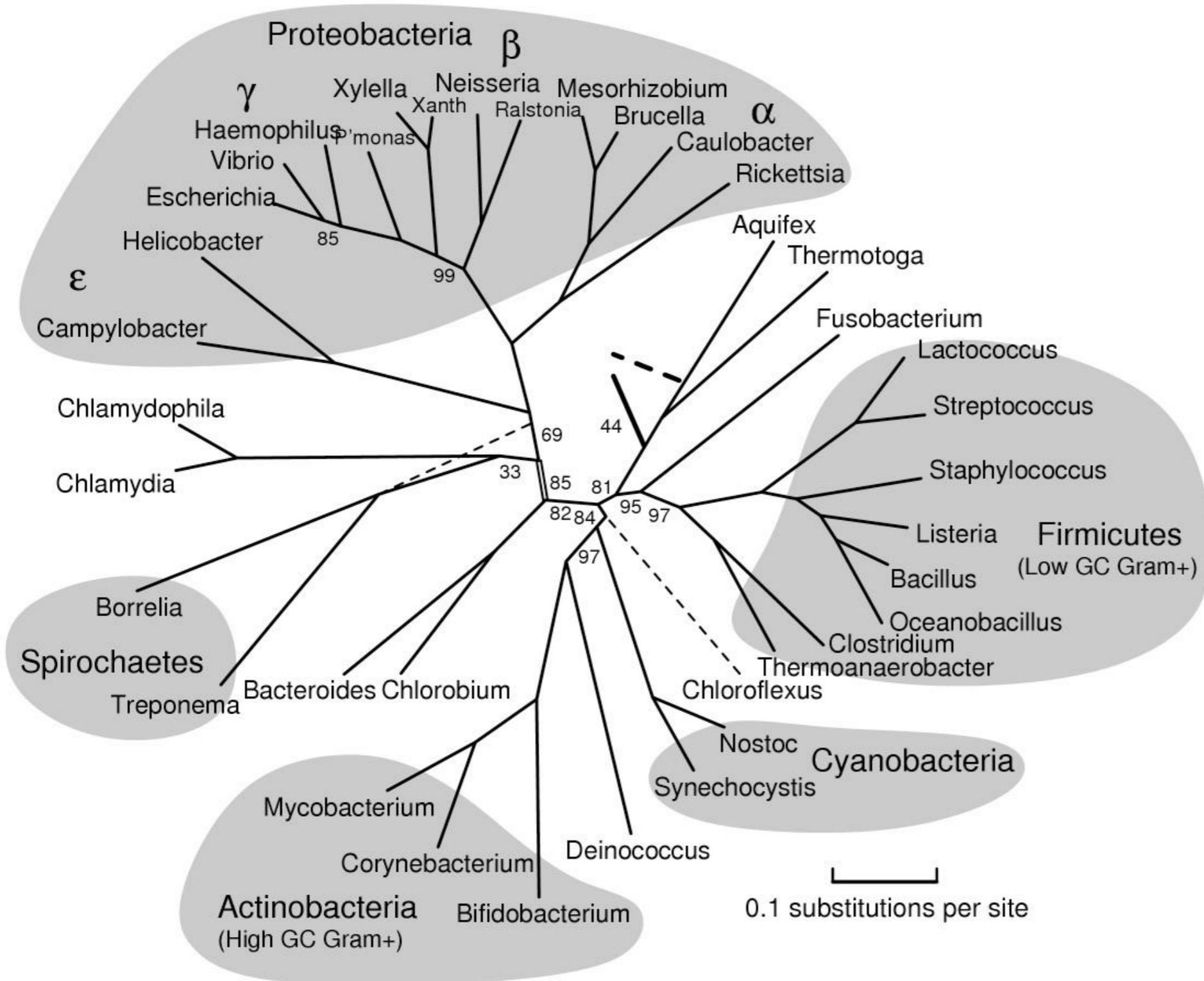
- Untreated vs Cipro treated in C3H/HeN from Envigo
- Untreated vs Cipro treated in C3H/HeN from CRL



# Mannosides Selectively Deplete Reservoir while Simultaneously Treating UTI

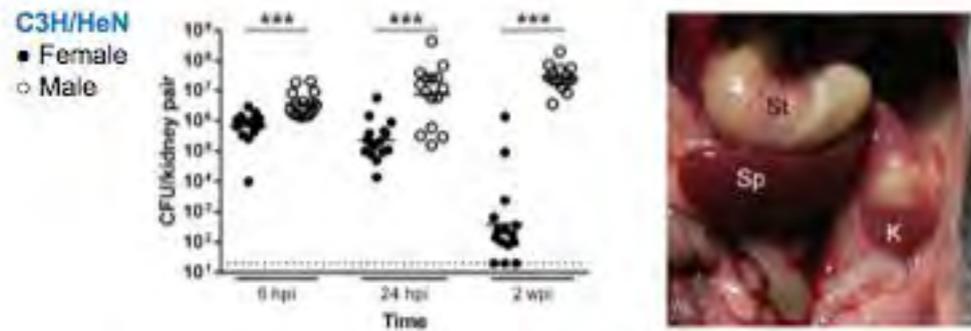


# Tree of Pathogenic Bacteria

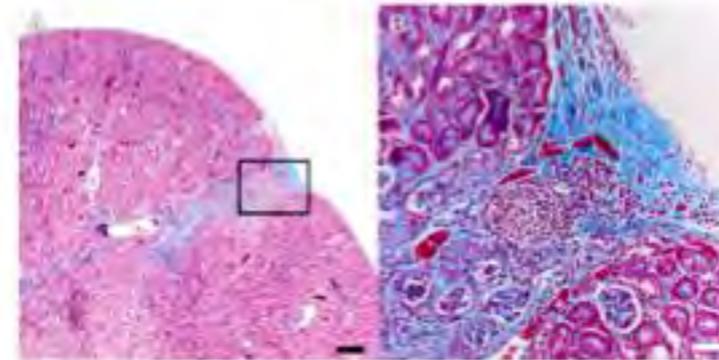


We are hoping to revolutionize the way bacterial infections are treated through dissection of the host-pathogen interface to produce antibiotic-sparing therapeutics.

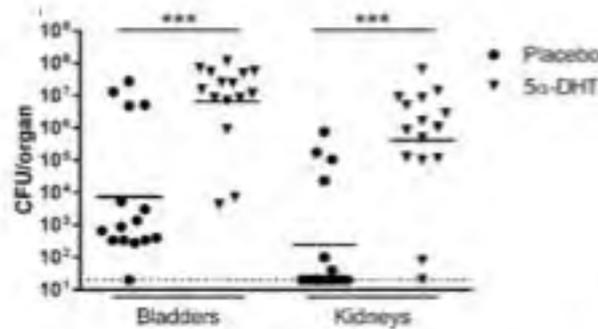
# Studying sex differences in UTI susceptibility and outcomes



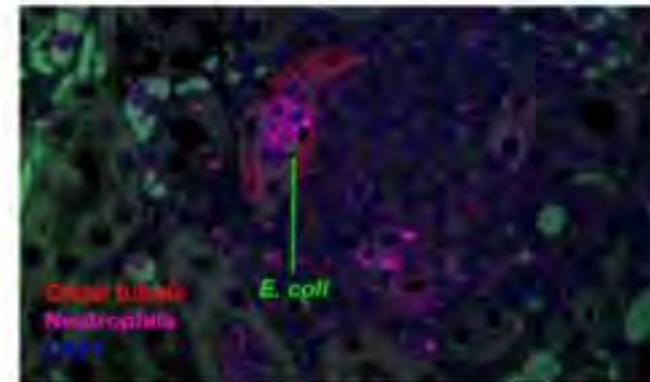
Males exhibit far more extensive kidney disease, including renal abscess formation



New model of renal scarring following antibiotic-treated pyelonephritis



Androgen exposure in female hosts provokes susceptibility to severe UTI outcomes



Dissecting UPEC – host interactions within renal tubules

Olson et al., 2016; Olson et al., 2017; Olson, McLellan et al., 2018

## Disclosure

I am a part owner of Fimbrion and may financially benefit if the company is successful in marketing the mannosides.



# Acknowledgements



## UTI outcome and sensitized model:

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\*Andrew Kau, MD, PhD

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\*Fredrik Almqvist, PhD

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Abigail McGuire, PhD  
\*Jonathan Livny, PhD

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Roger Klein  
\*Han Remaut  
Natalie Omattage  
\*Gabriel Waksman, PhD

Washington University School of Medicine University of Gothenburg Umea University The Broad Institute University College of London  
\*Team Leaders



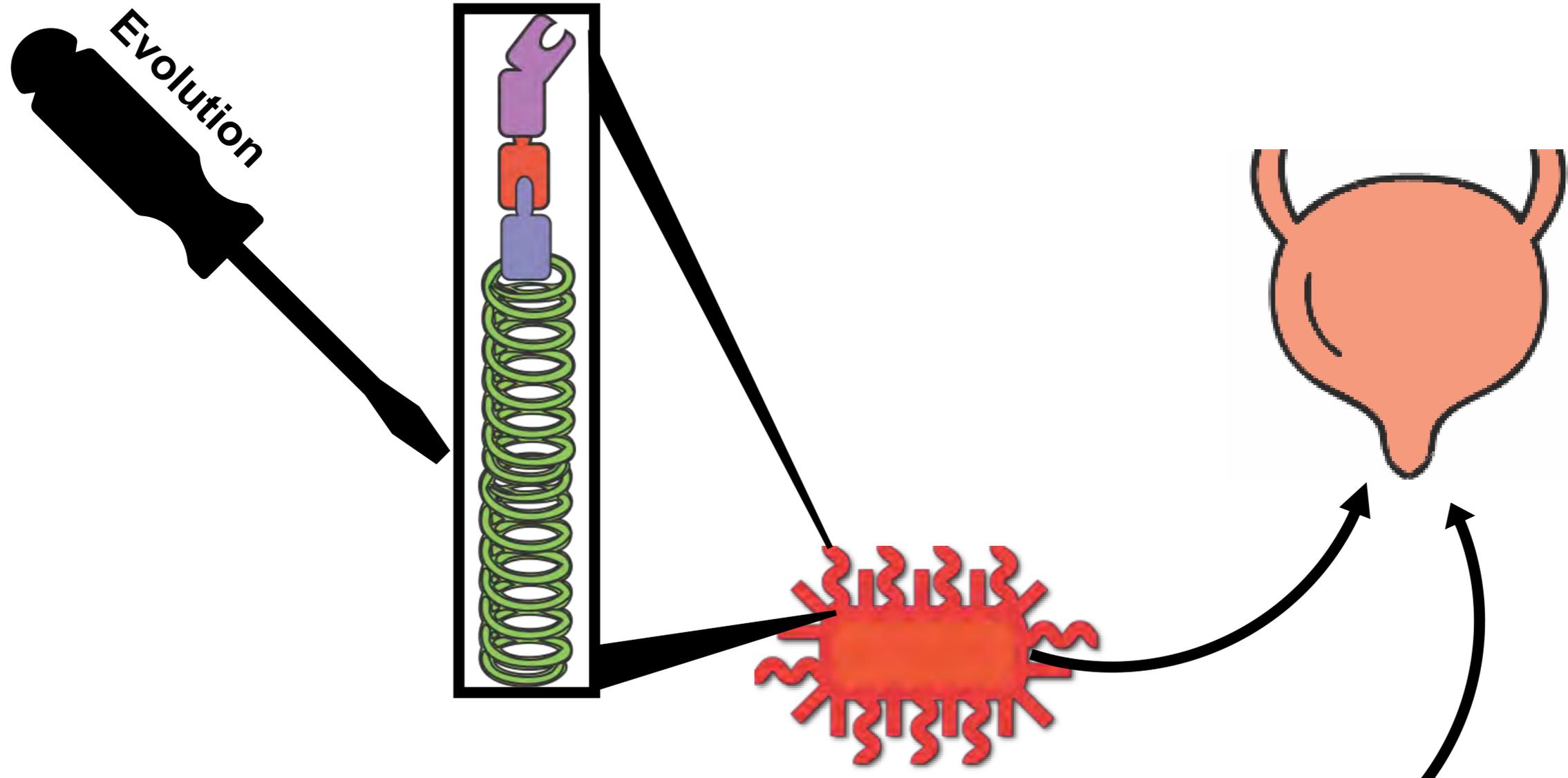
National Institute of Allergy and Infectious Diseases

NIDDK



OFFICE OF RESEARCH ON WOMEN'S HEALTH





The type 1 pilus has been “fine-tuned” through evolution to balance conservation of its “spring-like” function with diversification of its exterior surface.