

Genus-level Identification of Bladder-Resident Bacteria Associated with Recurrent Urinary Tract infection in Post-Menopausal

BACKGROUND

- Antibiotic-recalcitrant recurrent urinary tract infection (rUTI) is common in postmenopausal women¹.
- Cystoscopy with Electrofulguration (EF) of areas of visible chronic cystitis is implemented when antibiotic therapies fail.
- Chronic cystitis may be staged by degree of bladder tissue surface areas involved (Stage 1-4).
- Hypothesized benefit due to physical destruction of tissue-resident bacterial communities^{2,3}
- Genera-specific identification of urothelial tissue-resident bacteria by FISH has not yet been reported in the literature²

STUDY GOALS

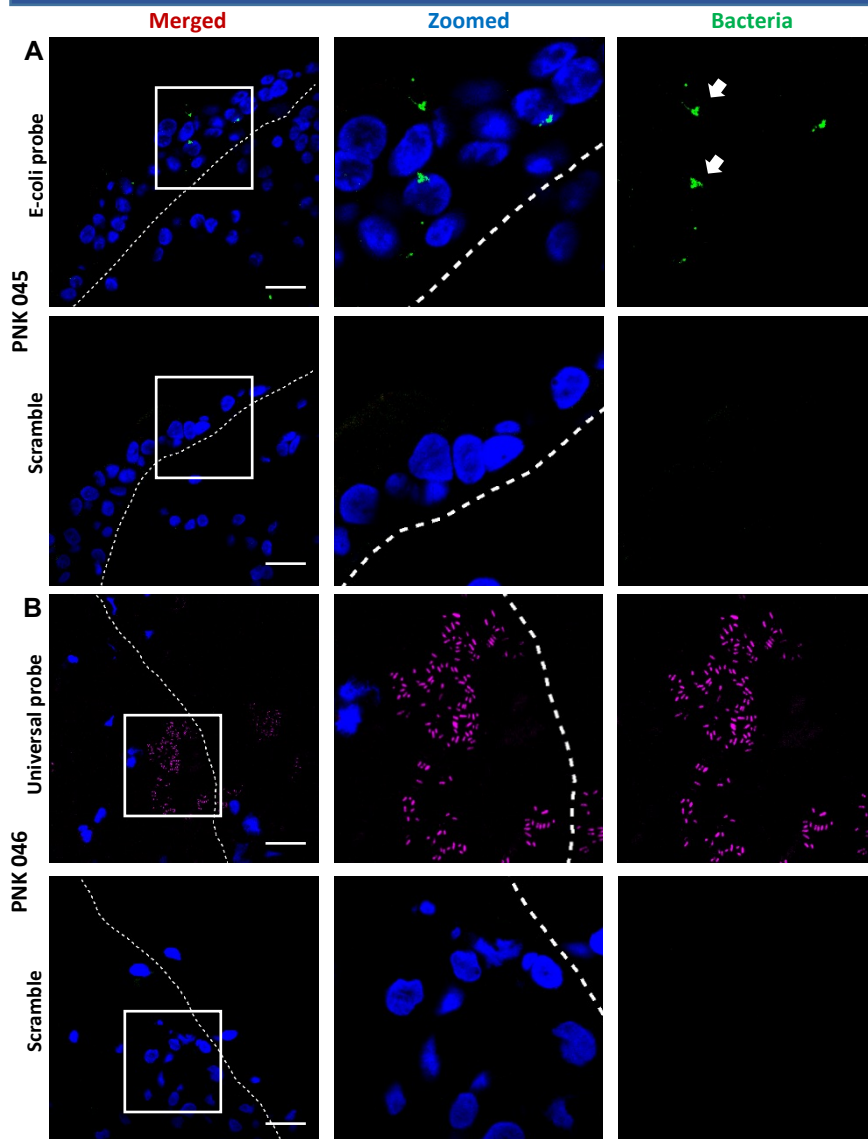
Identification and quantification of tissue-resident *Escherichia spp.* using genus-specific FISH probes on bladder biopsies taken from postmenopausal women with rUTI undergoing electrofulguration, in order to:

1. Prove intra-tissue *Escherichia spp.* presence and
2. Investigate the relationship between detected bacterial community sizes and stage of cystitis.

METHODS

- IRB approval
- Bladder biopsies obtained from consenting postmenopausal women who elected EF for the advanced management of rUTI.
- Biopsies were immediately fixed in paraformaldehyde and then paraffin-embedded and sectioned (5 μ m)
- FISH protocol adopted from Vaishnav et al.⁴ and Neugent et al.⁵, using the following probe sets on series of 3 adjacent tissue sections:
 - 1. Scramble-AlexaFluor488/647 (control),
 - 2. Universal 16S rRNA-AlexaFluor647 (all bacteria)
 - 3. *Escherichia* 16S rRNA-AlexaFluor488.
- Slides imaged using a Zeiss LSM880 with a 63x objective
- 10 randomly sampled images collected for each biopsy section (1 μ m²)
- Least mean squares statistical analysis to generate an average number of bacterial organisms per 10x1 μ m² for each cystitis stage.

RESULTS



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- Genera-specific FISH and imaging performed on bladder biopsies from **23 women**
- Universal 16S rRNA probe detected tissue-resident bacteria in the biopsies of 95.7% (22/23) women
- Tissue-resident *Escherichia spp.* were detected in the bladder biopsies of 52% (12/23) women**
- Highest average bacterial community sizes observed in Stage 1 cystitis bladder biopsies (8.4 per 10x1 μ m², 95% C.I. 6.6 – 10.1)

Least Square Means of FISH Scores Based on an Interaction Model

	Stage 1 (6)	Stage 2 (7)	Stage 4 (9)
Scramble	0 (0, 1.8)	0.06 (0, 1.7)	0 (0, 1.5)
E. Coli	0.4 (0, 2.1)	0.6 (0, 2.3)	0.2 (0, 1.7)
Universal + E. coli	8.4 (6.6, 10.1)*	0.9 (0, 2.6)	2.1 (0.6, 3.5) *p <0.0001

Figure 1. Detection of Intratissue bacteria in rUTI patient bladder urothelium. Representative micrographs from Patient PNK045 (A) E-coli probe and Scramble probe), and PNK046 (B) Universal probe and Scramble. Inflamed regions of the bladder are stained with Alexa 647-labeled 16S rRNA universal (magenta) and Genus specific *Escherichia coli* Alexa 488-labeled 16S rRNA (green). The nuclei are counterstained with Hoechst-33342(blue). The scale bar represents 20 μ m

CONCLUSIONS

- For the first time, genera-specific 16S rRNA FISH was used to detect tissue-resident *Escherichia spp.* in the urothelium of postmenopausal women electing to undergo cystoscopy with EF for the management of antibiotic-refractory rUTI.
- Bladder tissue-resident bacterial community sizes were highest in the urothelium biopsied from women with Stage 1 chronic cystitis.

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