

## VARIATIONS IN URINE PH IN WOMEN WITH NO URINARY TRACT INFECTION DO NOT INFLUENCE BIOFILM FORMATION.

### Hypothesis / aims of study

There is an established link between urinary incontinence and urinary tract infections (UTI) in women. Prior study on biofilm formation using enteropathogenic *E.coli* strains suggested an effect of a more acid pH in lowering biofilm development, possibly explaining a protective effect of acid urine against UTI in women (1). This study tested this hypothesis in well established urinary strains of *E.coli* and *Pseudomonas* for biofilm formation using a range of urine pH as medium in women without on-going UTI.

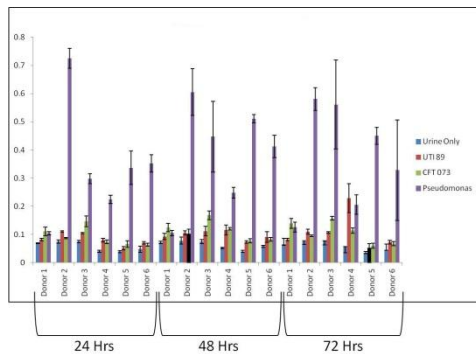
### Study design, materials and methods

Following IRB approval, urine samples of women with no UTI and with different pH levels were used as medium in an established biofilm formation assay (2). Urinary strains included UTI 89 and CFT 073 as well as *Pseudomonas aeruginosa* PA01. Urinalysis was negative in all urine samples. Bacterial suspensions were diluted 1:100 in 96-well plates containing urine as medium instead of the traditional LB broth with 1% glucose. "Urine only" served as a control. The plates were incubated at 37°C with agitated shaking (250 rpm) for 24, 48, and 72 hours with different pH environments (pH 5 to pH 7). Biofilm formation assay was done in triplicate using crystal violet staining; the attached bacterial biofilm was stained with 0.4% of crystal violet (Sigma-Aldrich) for 15 min at room temperature and washed excess dye with H<sub>2</sub>O then eluted with 100% ethanol. The OD550 (biofilm growth) and OD 600 (bacterial growth/count) were determined by spectrophotometry. To evaluate the variability of biofilm formation assay among women with no active UTI, several different donors were tested with a low pH of 5.0. Since biofilm formation can induce pH changes, pH measurement was repeated at each time point up to 72 hours to confirm whether the pH level had remained stable throughout the experiment. T-test values were obtained comparing 24hrs to 48 hrs, and again comparing 48hrs to 72hrs.

### Results

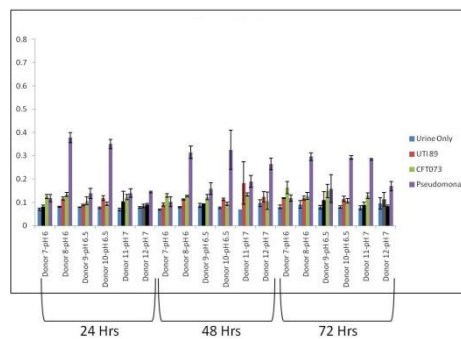
1. At an acid pH of 5.0, minimal variability was noted between 6 different donor samples for two *E.coli* strains. A much larger variability was observed for *Pseudomonas* (Fig. 1).
2. pH level remained stable over the course of the testing, up to 72 hours (data not shown).
3. Comparing the group with acid pH (5.0) (Fig. 1) to the group with higher urinary pH levels, no significant difference was noted in bacterial or biofilm growth for the *E.coli* strains (Fig. 2).
4. *Pseudomonas* formed the best biofilm, with a 3 fold increase on average compared to the urinary strains (Fig. 3). Biofilm formation by *Pseudomonas* appeared more favorable at low pH (Fig. 1 versus Fig. 2).
5. Maximal biofilm growth was established at 24 hours, and remained constant at 48 and 72 hours (no statistical difference observed) (Fig. 3).

Figure 1  
OD555 for pH 5 at 24, 48 & 72 hrs



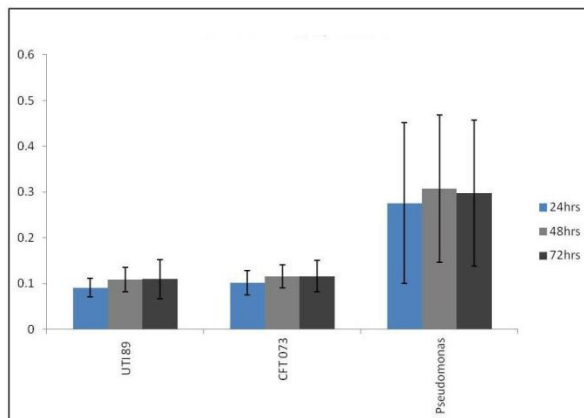
Black bars show no statistical significance

Figure 2  
OD555 for pH 6, pH 6.5 & pH 7 at 24,48 & 72 hrs



Black and gray bars show no statistical significance

Figure 3  
Mean OD555 at 24, 48 & 72hrs of all pH's averaged together to show pattern of biofilm production



Gray bars indicate no significance

### Interpretation of results

To our knowledge, this is the first study that uses human urine as a medium for biofilm formation assay. Strong biofilm formers, *E.coli* and *Pseudomonas* urinary strains, were selected since biofilms can provide an ideal habitat to protect bacteria from the effect of antibiotic therapies in women with UTI. Low urinary pH, believed to impair bacterial growth in human urine, did not appear to confer a protective effect against biofilm formation compared to higher urinary pH levels. *Pseudomonas* was a powerful biofilm producer early on, and remained such over time. An established biofilm was already noted at 24 hour with *E.coli* strains, thus theoretically favoring the clinical practice of a very early treatment of UTI.

### Concluding message

Using human urine as medium and comparing the effect of variable pH ranges during a biofilm formation assay, no protective mechanism of an acidic pH for biofilm-forming *E.coli* and *Pseudomonas* strains was observed. Furthermore, despite variable urinary pH levels, an established biofilm was noted as early as 24 hours, suggesting the need to implement rapid treatment of UTI to theoretically impair bacterial resistance from biofilm protectionism.

### References

1. Journal of Bacteriology, 188:3952-3961, 2006
2. Neurourology and Urodynamics 31(2): 234, 2012

### Disclosures

**Funding:** None **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Institutional Review Board of UT Southwestern Medical Center **Helsinki:** Yes **Informed Consent:** Yes