Urease Activity is Enhanced During Co-culture of Common CAUTI Pathogens and Contributes to Severity of Disease in a Murine Infection Model

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INTRODUCTION

- Urinary catheters are common and utilized in over 60% of critically ill patients, 20% of patients in medical and surgical units, and 5-10% of residents in nursing homes[1,2].
- Up to 50% of individuals catheterized for ≥7 days develop a catheter-associated urinary tract infection (CAUTI)[1].
- CAUTI is often polymicrobial with long term catheterization.
- The Gram-negative urease-positive bacterium *Proteus mirabilis* is the most common cause of CAUTI in southeast Michigan nursing home residents, particularly in polymicrobial CAUTIs[3].
- CAUTI is the most common source of bacteremia in nursing homes, and *P. mirabilis* bacteremias are often polymicrobial.
- Polymicrobial bacteremia can be recapitulated in a murine UTI model[4].
- Determining the impact of polymicrobial colonization on disease progression may reveal new targets for reducing likelihood of bacteremia and severe consequences of CAUTI.

HYPOTHESIS STATEMENT

- Interactions with other urinary tract pathogens during polymicrobial colonization enhance the pathogenic potential of *P. mirabilis*.

OBJECTIVES

1. Establish dual-species colonization in a murine model of UTI and CAUTI.
2. Determine if dual-species colonization increases disease severity, as measured by bacterial burden, bacteremia, urolithiasis, and tissue damage.
3. Determine the contribution of bacterial urease to disease severity during dual-species colonization.

METHODS

Murine model of UTI and CAUTI:

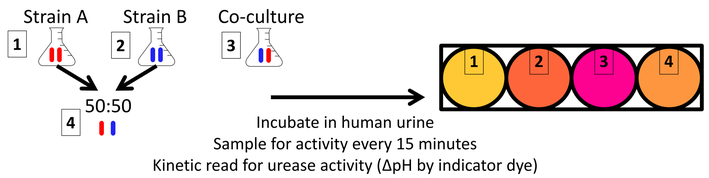
6 h | 24 h | 48 h | 72 h | 96 h

Transurethral Inoculation:
PBS
Proteus mirabilis
Providencia stuartii
Coinfection (50:50 mix of each)

Urine for pH
Urine for CFUs

Urine for pH
Urine, bladder, kidneys for CFUs
Bladder and kidneys for histology
Spleen for bacteremia

Urease activity:



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2. Dudeck, Edwards, Allen-Bridson, Gross, Malpiedi, Peterson, Pollock, Weiner, Sievert. *Am J Infect Control,* 2015. 43:206-221.
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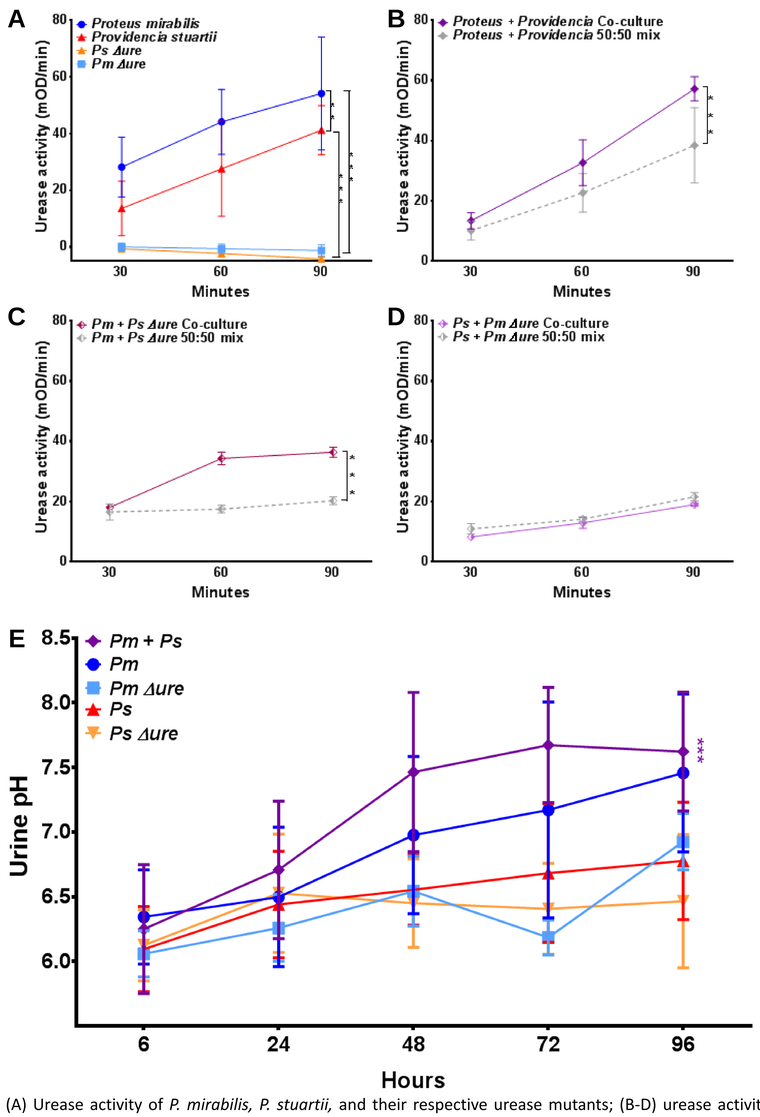
SUMMARY AND CONCLUSIONS

- *Providencia stuartii* enhances the pathogenic potential of *Proteus mirabilis*.
- Enhanced urease activity is associated with an increase in urine pH, urolithiasis, inflammation, tissue damage, and bacteremia in murine infection models.
- *Proteus mirabilis* urease activity is also enhanced by a variety of other Gram-negative and Gram-positive CAUTI pathogens.
- The underlying mechanism of enhanced urease activity may represent a widespread target for limiting detrimental consequences of polymicrobial catheter colonization.

RESULTS

Figure 1: *P. stuartii* enhances *P. mirabilis* urease activity *in vitro* and *in vivo*



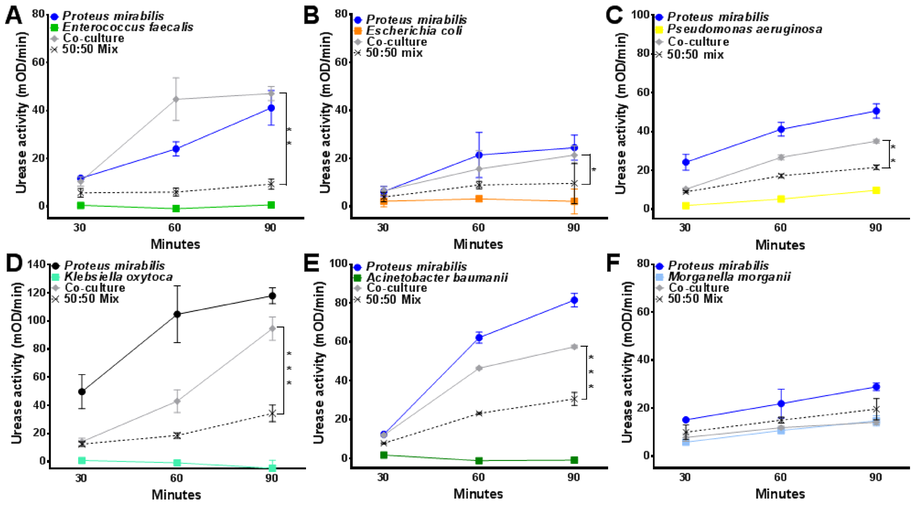
- (A) Urease activity of *P. mirabilis, P. stuartii,* and their respective urease mutants; (B-D) urease activity resulting from continuous co-culture compared to a 50:50 mixture of single species cultures. Error bars represent mean ± SD for at least three technical replicates.
- (E) Urine pH following transurethral inoculation with *P. mirabilis, P. stuartii,* their respective urease mutants, or coinfection with *P. mirabilis* and *P. stuartii* (total N of 11-23 per infection group).
- ***P<0.001 by two-way ANOVA.

Table 1: Incidence of severe disease following single species or polymicrobial infection in murine models of ascending UTI and CAUTI.

	Single Species Infections					Coinfections					All
	Pm	*Pm Δure*	*Ps*	*Ps Δure*	Total	*Pm + Ps*	*Pm + Ps Δure*	*Ps + Pm Δure*	*Pm Δure + Ps Δure*	Total	*Total*
Ascending UTI											
N of mice	20	11	21	17	**69**	23	17	12	12	**64**	***133***
Urine pH [a]	7 (35)	0 (0)	0 (0)	0 (0)	**7 (10)**	11 (48)	3 (18)	0 (0)	0 (0)	**14 (22)**	***21 (16)***
Urolithiasis [b]	1 (5)	0 (0)	1 (5)	0 (0)	**2 (3)**	8 (35)	3 (18)	0 (0)	0 (0)	**11 (17)**	***13 (10)***
Tissue Damage [c]	8 (40)	0 (0)	6 (29)	2 (12)	**16 (23)**	16 (70)***	5 (29)***	3 (25)	0 (0)	**24 (38)**	***40 (30)***
Bacteremia [d]	6 (30)	2 (18)	4 (19)	2 (12)	**14 (20)**	12 (52)**	10 (59)**	3 (25)	4 (33)	**29 (45)***	***43 (32)***
CAUTI											
N of mice	17	12	19	12	**60**	20	12	11	12	**55**	***115***
Urolithiasis [b]	3 (18)	0 (0)	0 (0)	0 (0)	**3 (5)**	5 (25)	3 (25)	0 (0)	0 (0)	**8 (14)**	***11 (10)***
Tissue Damage [c]	7 (35)	2 (18)	5 (24)	2 (12)	**16 (23)**	12 (52)###	8 (47)###	3 (25)	2 (17)	**25 (39)**	***41 (36)***
Bacteremia [d]	11 (65)	5 (42)	13 (68)	7 (58)	**36 (60)**	19 (95)##	12 (100)##	6 (55)	5 (42)	**42 (76)#**	***78 (68)***

- [a]At any time post-inoculation; [b]assessed by gross macroscopic examination of bladder and kidneys; [c]a combined score of 4 or greater for cystitis and pyelonephritis (each scored on a scale of 0-4); [d]assessed by spleen colonization.
- *OR=2.5 [1.2-5.4] P<0.015; **OR=3.3 [1.2-9.2] P<0.024; ***OR=8.3 [2.3-30.2] P<0.001
- #OR=2.3 [1.0-5.0];P<0.048; ##OR=17.4 [3.4-90.5] P<0.001; ###OR=15.5 [3.3-72.9] P<0.001

Figure 2: *P. mirabilis* urease activity is enhanced by other CAUTI pathogens



- Graphs are representative of at least two independent experiments. Error bars represent mean ± SD for at least three technical replicates.
- (A) *Enterococcus faecalis*, N=3 isolates tested; B) *Escherichia coli*, N=4 isolates tested; C) *Pseudomonas aeruginosa*, N=3 isolate tested; (D) *Klebsiella pneumoniae,* N=4 isolate tested; (E) *Acinetobacter baumanii*, N=2 isolates tested; and (F) *Morganella morganii*, N=4 isolate tested.
- *P<0.05, **P<0.01, ***P<0.001 by two-way ANOVA.