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 27 days develop a catheter-associated urinary tract infection (CAUTI).3

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.4

CAUTI is the most common source of bacteremia in nursing homes, and P. mirabilis bacteremias are often polymicrobial.5

Polymicrobial bacteremia can be recapitulated in a murine UTI model6

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:

- Transurethral inoculation
- PBS
- Proteus mirabilis
- Providencia stuartii
- colonization (5x10^6 CFU each)

Urease activity:

- Strain A
- Strain B
- Co-culture

Incubate in human urine

Sample for activity every 15 minutes

Kinetic read for urease activity (half-life by indicator dye)

REFERENCES


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

<table>
<thead>
<tr>
<th>Single Species Infections</th>
<th>Co-infections</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pm</td>
<td>Ps</td>
<td>Pm + Ps</td>
</tr>
<tr>
<td>Ascending UTI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N of mice</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Urine pH</td>
<td>7 (15)</td>
<td>7 (15)</td>
</tr>
<tr>
<td>Urease activity</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (4)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>CAUTI</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>N of mice</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Urine pH</td>
<td>7 (15)</td>
<td>7 (15)</td>
</tr>
<tr>
<td>Urease activity</td>
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</tr>
<tr>
<td>Total</td>
<td>3 (4)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

• At any time post-inoculation; *assessed by gross macroscopic examination of bladder and kidneys; &bole's score of 4 or greater for cystitis and pyelonephritis (each scored on a scale of 0-4); **assessed by splenic colonization.

• OR=2.5 (1.2-5.4) P=0.024; **OR=8.3 (2.3-30.3) P=0.001

• OR=x.3 (1.05-1.0) P=0.046; OR=17.4 (3.4-90.5) P=0.001; **OR=15.6 (3.3-72.9) P=0.001

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

- (A) Urease activity of P. mirabilis, P. stuartii, and their respective urease mutants; (B-D) urease activity resulting from co-culture compared to a 50:50 mixture of single species cultures. Error bars represent mean ± SD for at least three technical replicates.

- (E) Urease activity 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.

- (F) 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 isolates tested; (D) Klebsiella pneumoniae, N=4 isolate tested; (E) Acinetobacter baumannii, N=2 isolates tested; and (F) Morganella morganii, N=1 isolate tested.

- *P<0.05, **P<0.01, ***P<0.001 by two-way ANOVA.