

blocked the antimicrobial activity of RNase 7, resulting in increased bacterial growth. The open circles represent non-inoculated urine samples.

Figure 11. RI blocks RNase 7 binding to lipopolysaccharide.

(A) To determine if RI alters RNase 7 bacterial binding, *E. coli* were incubated with 2 μ M RNase 7 (R7), RNase 7 pre-incubated with RI (R7/RI), or 2 μ M RI alone. After centrifugation, the supernatant and pellet fraction were subjected to SDS-PAGE and visualized by Coomassie Blue staining. Supernatant represents the soluble fraction that contains unbound protein while the pellet fraction contains the *E. coli*-bound peptides. Results demonstrate that RNase 7 binds uropathogenic *E. coli* (E) and that binding is reduced in the presence of RI. (B) Displacement of LPS-bound Bodipy TR cadavarine with increasing concentrations of RNase 7, Polymyxin B, RNase A, and RI. Results confirm that RNase 7 binds LPS while RI does not. (C) Displacement of LPS-bound Bodipy TR cadavarine with increasing concentrations of RI pre-incubated with 1 μ M RNase 7. Results confirm that the addition of RI reduces RNase 7 binding to LPS. The addition of *p*-HMB to RI improves RNase 7 binding to LPS.

Supplemental Figure 1. RI and RNase 7 kidney peptide production during sterility and pyelonephritis. To confirm the decrease in *RNH1* message and increase in *RNASE7* message is accompanied by alterations in protein production, cationic peptides from the same non-infected kidney tissues (HK) and kidney tissue with pyelonephritis (P) were subjected to SDS-PAGE followed by Western immunoblot analysis. Results were normalized to kidney GAPDH production.

Supplemental Figure 2. RI is expressed by the renal tubules.

Immunohistochemistry demonstrates isolated cell specific expression (brown/arrows) in the collecting tubule of human kidney cortex (A, top panel) and medulla (A, bottom panel). RI was not expressed in the glomeruli or renal interstitium. Negative control renal cortex (B, top panel) and medulla (B, bottom panel) show no RI immunoreactivity. Magnification 20x.

Supplemental Figure 3. Urine silver stained gels demonstrate equal urine protein loading. (A) Silver stained PAGE gels confirmed equal urine protein loading from culture negative urine samples (NI) and from urine samples infected with *E. coli* (I) in Figure 6A. (B) Western immunoblot analysis confirmed that the secondary anti-mouse antibody did not recognize human immunoglobulin, which may be present during UTI. 90ng recombinant RI and 160 ng of human sera served as the control.

Supplemental Figure 4. Uropathogenic *E. coli* do not cause RI proteolysis.

Recombinant RI was incubated with clinical uropathogenic *E. coli* stains for 90 min (A-F represent patient samples outlined in Supplemental Table 2). Cationic peptides were separated by SDS-PAGE followed by Western immunoblot analysis using a monoclonal anti-RI antibody. 50ng recombinant RI served incubated without bacteria served as the control.

Supplemental Figure 5. RI inhibits the antimicrobial activity of RNase 7 against *E. faecalis*.

(A) Uropathogenic *E. faecalis* was exposed to 0.1 μ M RNase 7, equal concentrations of recombinant RNase 7 plus RI (RNase 7-RI), or 0.1 μ M of RI. The results are displayed as the percentage of remaining CFUs in relation to untreated controls. The antimicrobial activity of RNase 7 was significantly reduced in the presence of RI. Data represent the mean of triplicates \pm SEM. (B) *E. faecalis* were stained using a

1:1 mixture of SYTO9 and propidium iodide. The SYTO9-stained cells (green) represent live cells and the propidium iodide-stained cells (red) represent killed cells. Bacterial viability was visualized after exposure to RNase 7 with and without RI at 180 minutes. Magnification 63x. **(C)** *E. faecalis* were stained using a 1:1 mixture of SYTO9 and propidium iodide. Bacterial cultures were incubated with 0.1 μ M RNase 7, equal concentrations of recombinant RNase 7 plus RI (RNase 7-RI), or 0.1 μ M of RI. Bacterial viability over time was analyzed integrating fluorescent changes in SYTO9 and propidium iodide dye. Values are the average of three replicates.

Supplemental Figure 6. **RI neutralization of urinary RNase 7.**

The antimicrobial properties of urinary RNase 7 were measured as changes in turbidity of cultured human urine using the absorbance at 600 nm (OD_{600}). Three human urine samples **(A-C)** were inoculated with *E. coli* (PEDUTI-89 or CFT073) as shown by the dashed line. Addition of RI (solid black line) or RNase 7 monoclonal antibody (diamond studded line) blocked the antimicrobial activity of RNase 7, resulting in increased bacterial growth. The open circles represent non-inoculated urine samples.

Supplemental Figure 7. **RI blocks RNase 7 binding to peptidoglycan.**

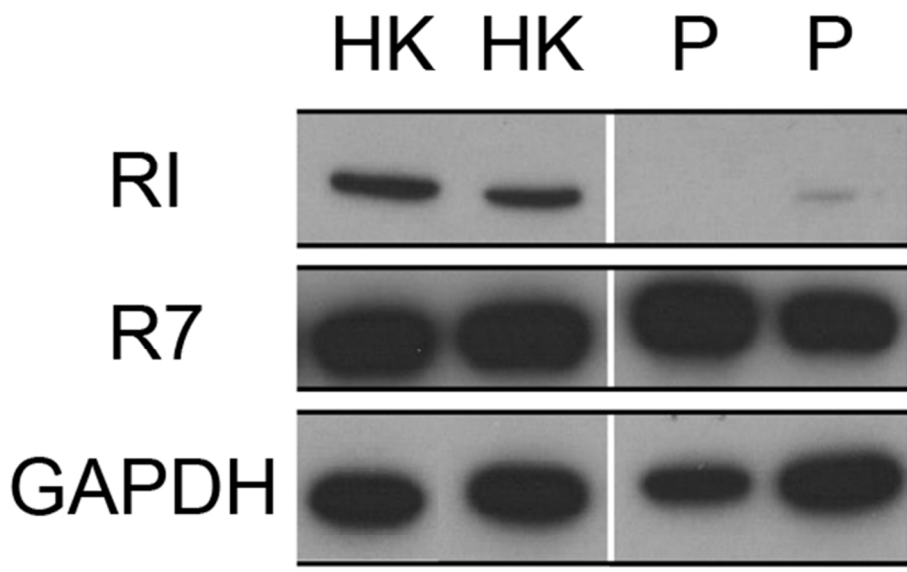
To determine if RI alters RNase 7 bacterial binding, *E. faecalis* were incubated with RNase 7 (R7), RNase 7 pre-incubated with RI (R7/RI), or RI alone. After centrifugation, the supernatant and pellet fraction were subjected to SDS-PAGE and visualized by Coomassie Blue staining. Supernatant represents the soluble fraction that contains unbound protein while the pellet fraction contains the *E. faecalis*-bound peptides. Results demonstrate that RNase 7 binds uropathogenic *E. faecalis* (Ent) and that binding is reduced in the presence of RI. **(B)** To assess if RI affects RNase 7 binding to the Gram-positive cell wall, peptidoglycan was incubated with RNase 7, equal concentrations of recombinant RNase 7 plus RI (R7/RI), or RI. Lysozyme (L) and BSA (B) served as the positive and negative controls, respectively. For each reaction, the supernatant and pellet fractions were included. The supernatant represents the soluble fraction containing unbound protein, while the pellet represents the insoluble fraction containing PGN-bound peptides. Samples were analyzed by SDS-PAGE and visualized by Coomassie Blue staining. Results indicate that RNase 7 binds PGN, and that RI blocks RNase 7 binding to PGN.

Supplemental Table 1. **Patient demographics from infected urine samples.**

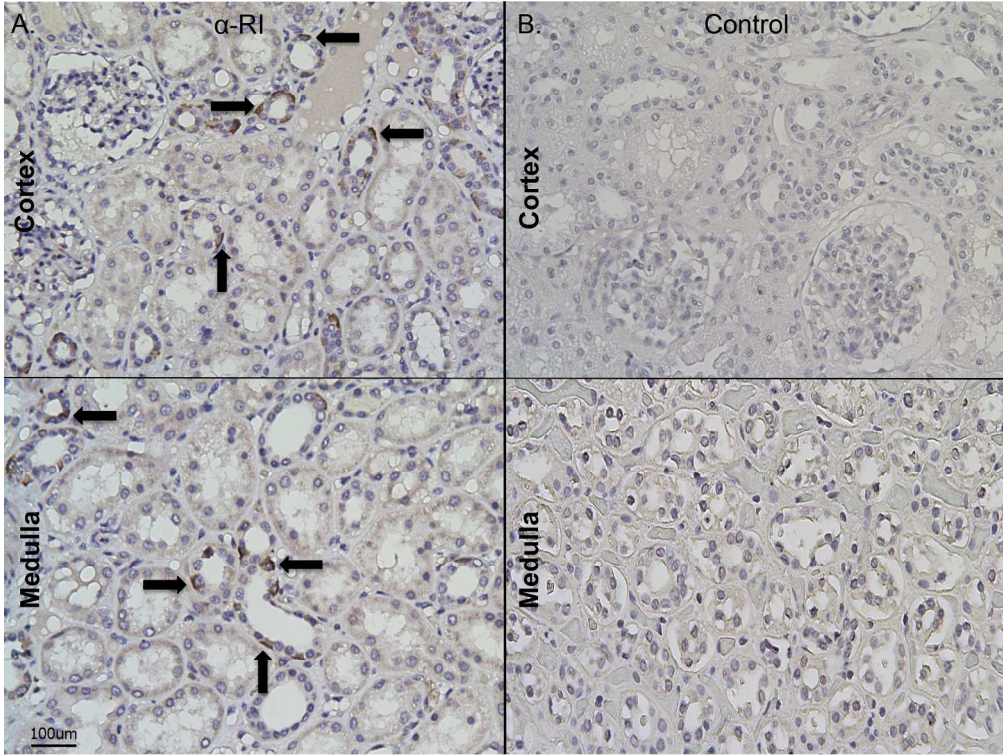
Patient age, gender, and clinical symptoms, and imaging are outlined. To confirm the identification of uropathogenic *E. coli*, clinical urine isolates were subjected to common culture techniques, serotyped for the O-antigen, and genes for common virulence factors were identified using previously published methods.^{46, 47}

Supplemental Table 2. **Patient demographics from kidney specimens**

Patient age, gender, and underlying diagnosis of patients undergoing nephrectomy. **(A-G)** Non-infected tissue samples were free of microscopic signs of disease or inflammation. **(H-N)** Infected kidney tissue was obtained from patients undergoing nephrectomy for the clinical history of pyelonephritis. Tissue was provided by the Cooperative Human Tissue Network.

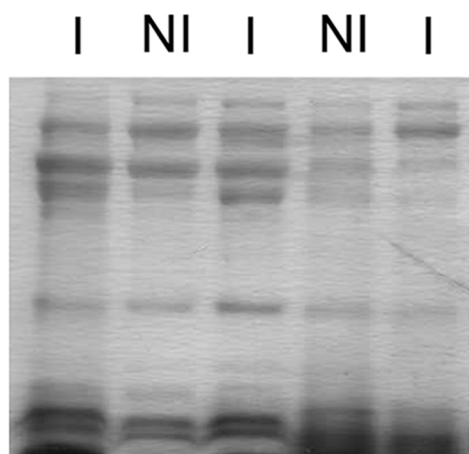


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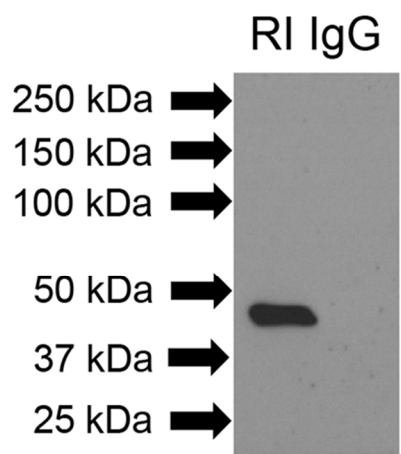


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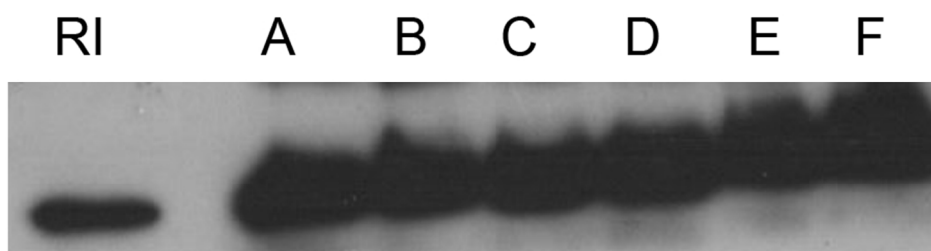
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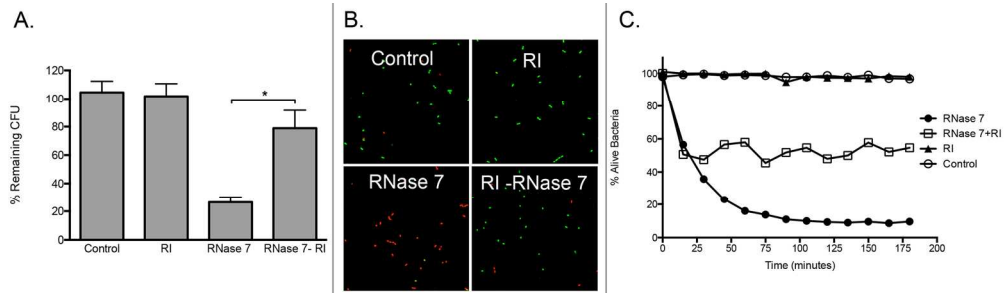
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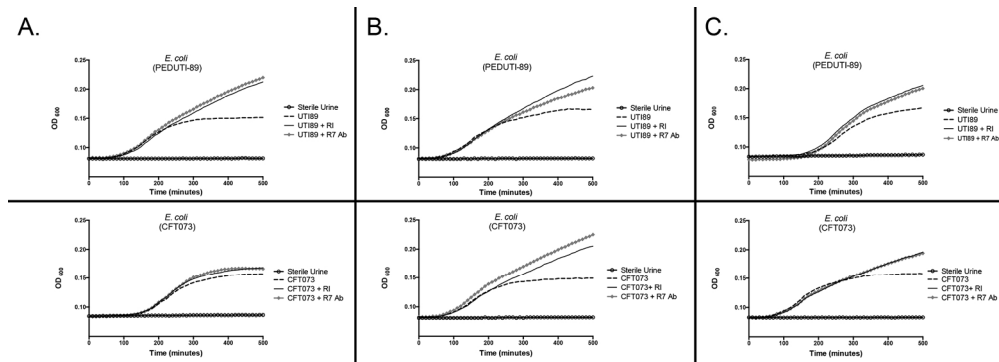
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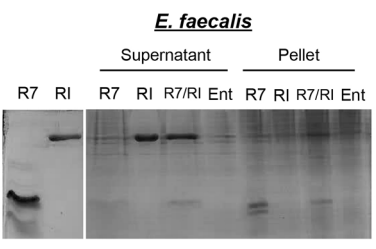


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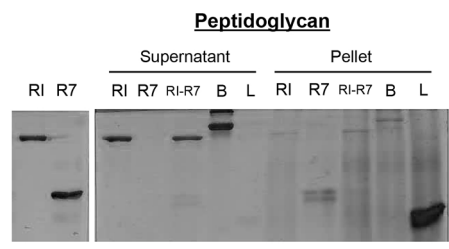


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B.



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Patient	Age	Gender	Bacteria	Serotype	Virulence Factor(s)	Symptoms	Imaging
A	6y	Female	<i>E. coli</i>	O18	<i>papAH, papC, fimH, hlyA</i>	Afebrile, Vomiting	None
B	12y	Female	<i>E. coli</i>	O2	<i>papAH, papC, fimH</i>	Febrile, Abdominal Pain, Vomiting, Dysuria	Abdominal X-ray
C	16y	Female	<i>E. coli</i>	O18	<i>papAH, papC, fimH, hlyA, cnf1</i>	Afebrile, Dysuria, Frequency	None
D	11y	Female	<i>E. coli</i>	O18	<i>papAH, papC, fimH, hlyA, cnf1</i>	Febrile, Vomiting, Dysuria	Abdominal X-ray
E	6y	Female	<i>E. coli</i>	O18	<i>papAH, papC, fimH, hlyA</i>	Febrile, Abdominal Pain, Vomiting	None
F	14y	Female	<i>E. coli</i>	O16	<i>papAH, papC, fimH</i>	Afebrile, Abdominal Pain, Hematuria	None
G	3y	Female	<i>E. coli</i>	O6	<i>papAH, papC, fimH, cnf1</i>	Febrile, Abdominal Pain, Frequency	Abdominal X-ray
H	11y	Female	<i>E. coli</i>	O18	<i>papAH, papC, fimH, hlyA</i>	Afebrile, Dysuria, Frequency, Hematuria	Renal Ultrasound

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Patient	Age	Gender	Diagnosis
A	32y	Male	Renal Mass
B	72y	Male	Hydronephrosis, Hypertension
C	58y	Female	Renal Mass
D	72y	Female	Renal Mass
E	38y	Male	Renal Mass
F	43y	Female	Renal Mass
G	18y	Female	Hydronephrosis
H	42y	Female	Acute + Chronic Pyelonephritis
I	75y	Male	Chronic Pyelonephritis
J	37y	Male	Acute + Chronic Pyelonephritis
K	42y	Male	Chronic Pyelonephritis
L	60y	Female	Acute + Chronic Pyelonephritis
M	55y	Female	Chronic Pyelonephritis
N	78y	Female	Acute + Chronic Pyelonephritis

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