

# Cytotoxic Necrotizing Factor-1 Toxin in Uropathogenic E. coli

*Is There a Role for Virulence Factors in Urinary Tract Infection?*

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# Acknowledgements

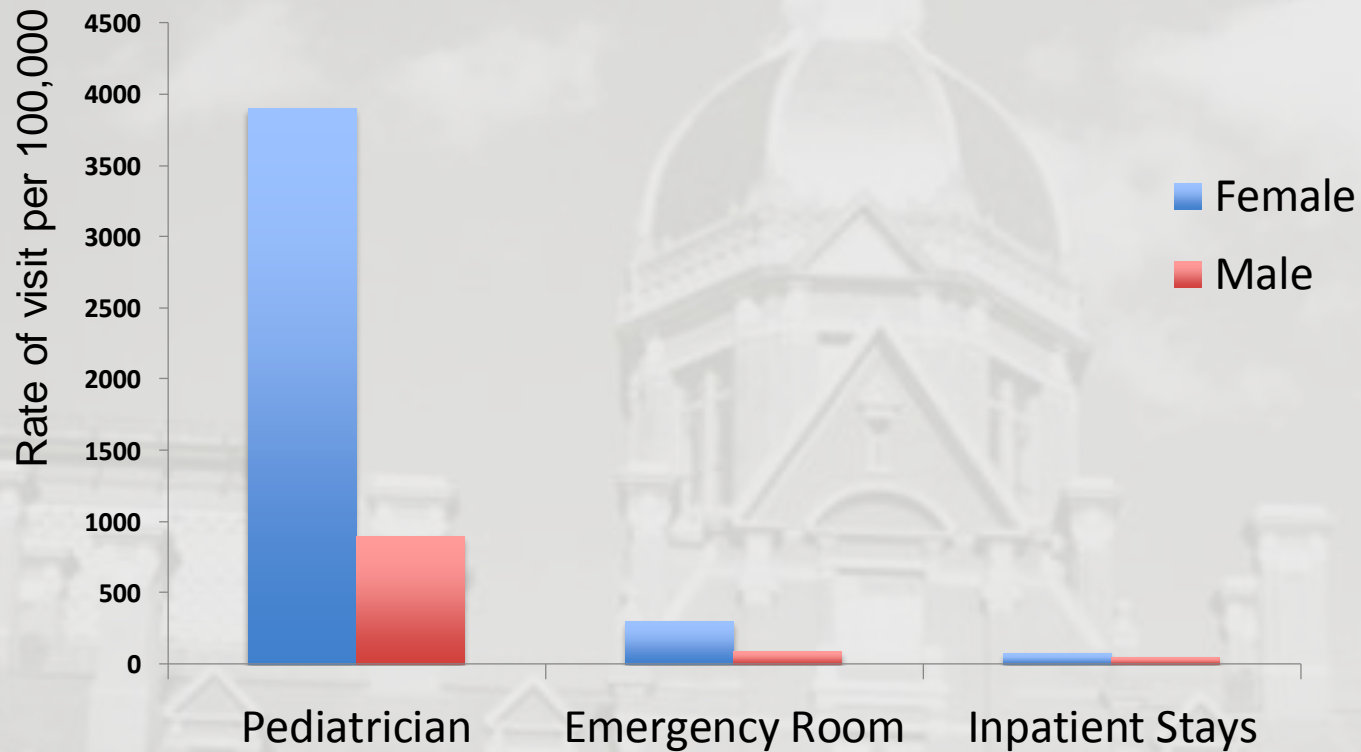
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# Background

- UTI is common in children, accounting for 1 million visits to the pediatrician yearly (NIH)
- Incidence of UTI in children <6 years old:
  - 3-7% in girls
  - 1-2% in boys
- Risk of recurrence is 40-60%
- Studies on pediatric UTIs are important because of risk of hypertension, chronic kidney disease

# Prevalence of UTI in the Pediatric Population

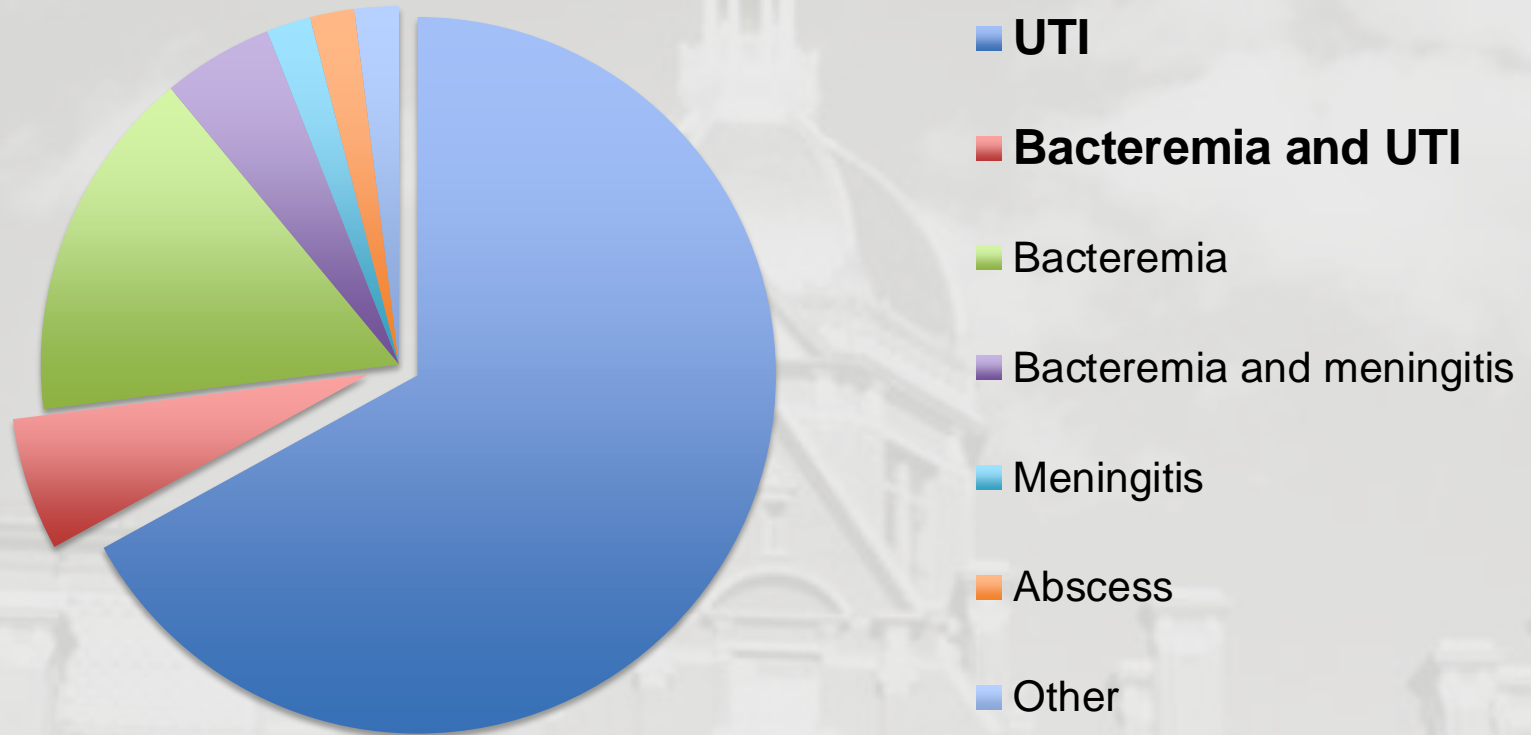
## Utilization of Care for UTIs in the U.S.



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# Prevalence of UTI in the Pediatric Population

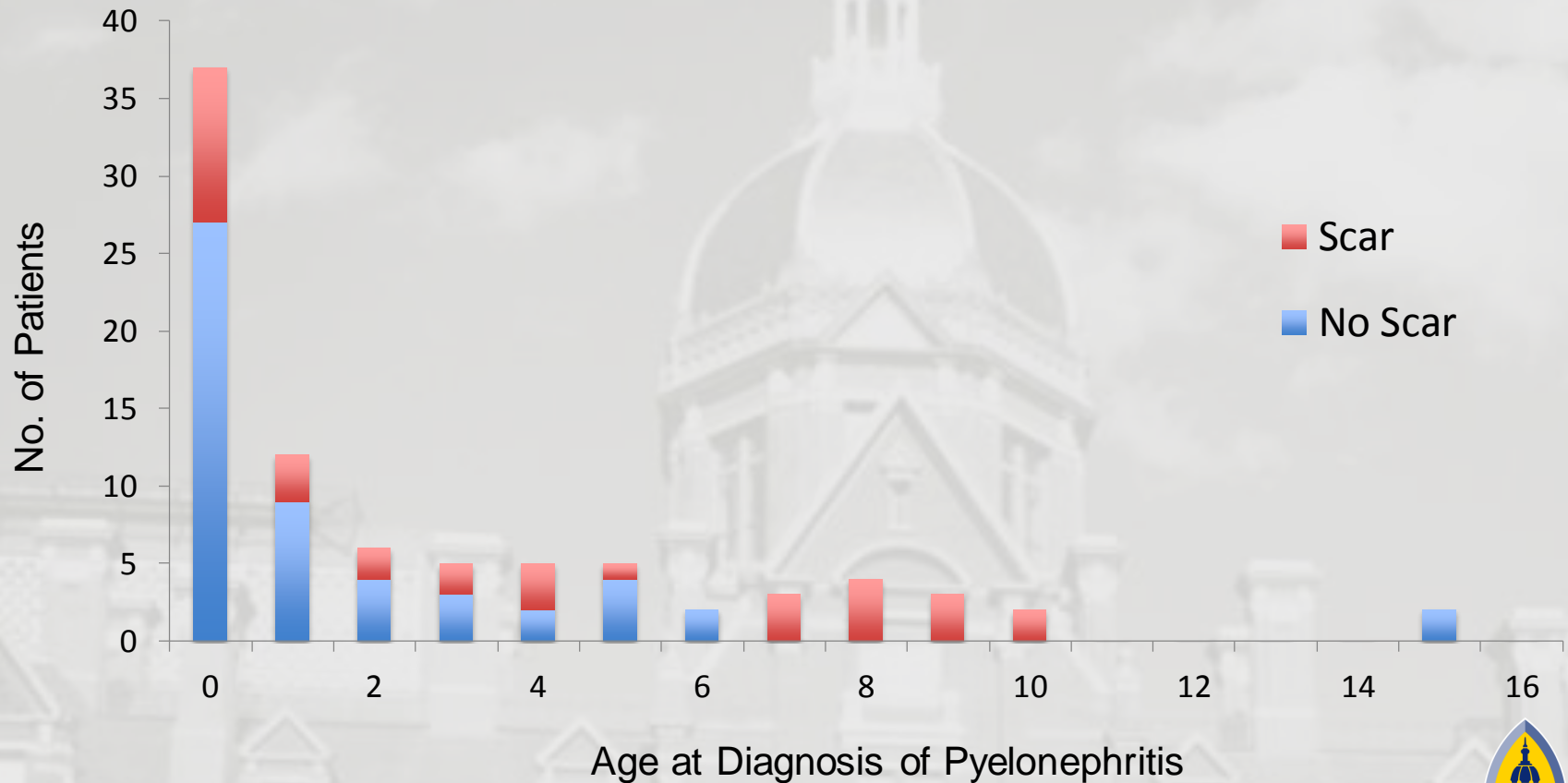
## Etiology of Neonates Admitted for Serious Bacterial Infection





# Renal Consequences of Pyelonephritis

## Age Distribution and Renal Scarring in Patients with Pyelonephritis



# Challenges

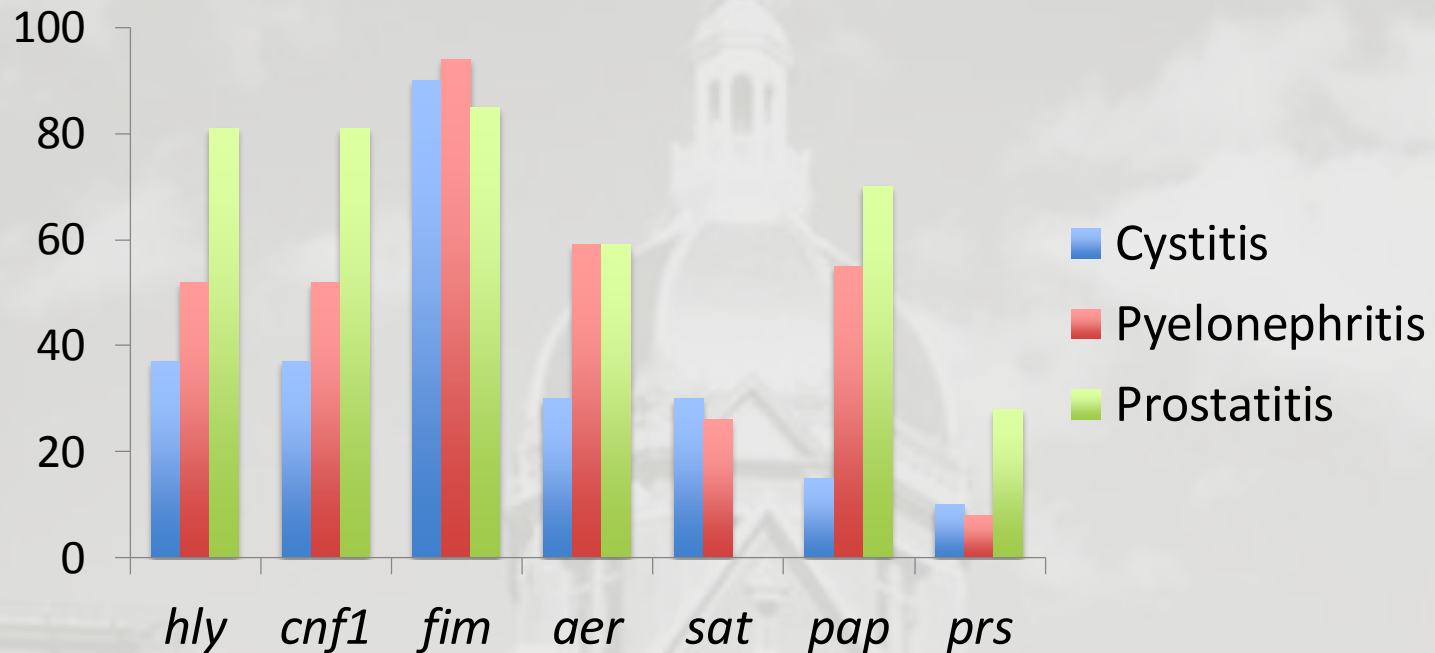
- Rising incidence of antibiotic-resistant bacteria
  - Widespread use of antibiotics
  - Pathogenesis of UTI is multifactorial
  - Host and bacterial phenotypes play critical roles
- *Therefore, it is important to be able to examine bacterial pathogenicity in a variety of ways, including in vivo models*

# *E. coli*

- Most common pathogen in UTI
  - 50-80% of all culture-proven UTIs
- UTI-associated pathogenic *E. coli* (UPEC) isolates express cytotoxic necrotizing factor 1 (CNF-1)
  - CNF-1 expressing *E. coli* have been shown *in vitro* to release proinflammatory cytokines and to exhibit resistance to host neutrophil and monocyte phagocytosis



# Virulence factors in Uropathogenic *E. coli* isolates



*cnf-1* and *hly* are inherited as a pathogenicity island and are part of the *prs* operon

# CNF-1

- Found in strains of *E. coli* implicated in UTI and neonatal meningitis
- CNF-1 belongs to a group of cytotoxins that cause activation of Rho guanosine triphosphatases (GTPases)
  - Alters host cell actin cytoskeleton
  - Promotes bacterial invasion of endothelium that comprises blood-brain barrier

# CNF-1

- However, there have been conflicting reports on the exact role of the *E. coli* cytotoxic necrotizing factor-1 (CNF-1) in the pathogenesis of UTI

# Goals

- Establish a murine model for examination of uropathogenic *E. coli* in the urinary tract
- Elucidate the role of CNF-1 in UTIs

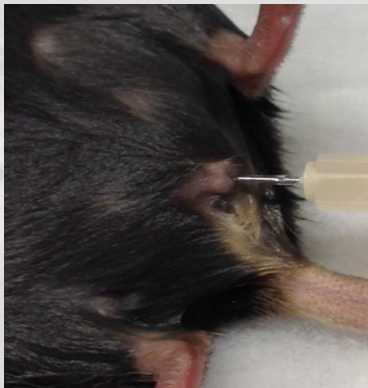
# Materials/Methods

- Targeted deletion of *cnf-1* in the isolate of UPEC (*cnf-1*-null strain)
- Comparative studies in several mouse strains, with transurethral inoculations of wild-type or *cnf-1*-null strain UPEC
  - $1-2 \times 10^7$  CFU/ mouse in 50  $\mu$ l of PBS
  - Urine samples were collected and plated to verify pre-inoculation sterility
- At various time points, kidney and bladder tissues were harvested and analyzed for inflammation and bacterial burden



# Murine Model of Cystitis and Pyelonephritis

Transurethral *E. coli*  
Inoculation



Outcomes

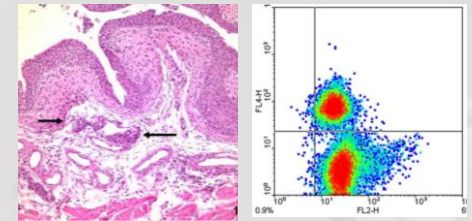
Bacterial  
Burden

Inflammatory  
Response



Kidney and bladder  
bacterial cultures

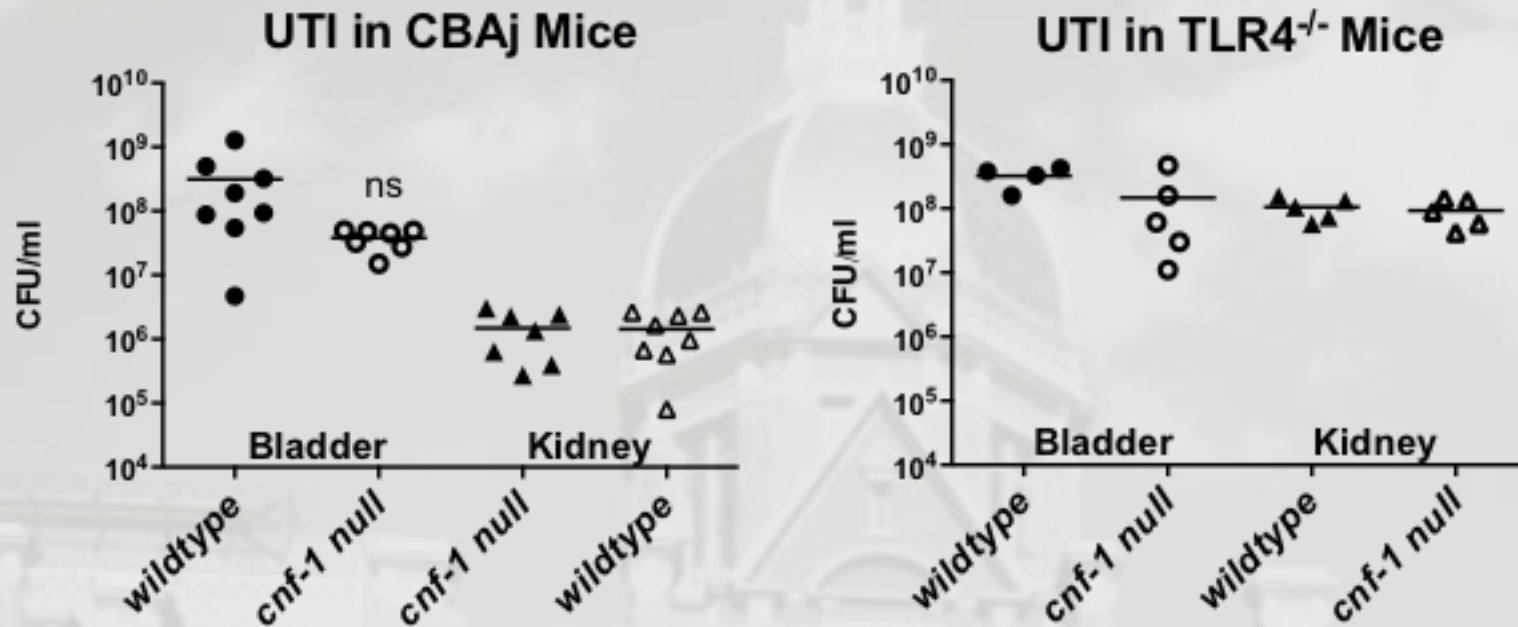
Pathology and  
Flow cytometry



# Results

- Bacterial counts were consistently present in infected mice, and absent from control mice (PBS)
- Both the wild-type UPEC strain and the *cnf-1* null strain caused robust and reproducible bladder and kidney infections

# Examination of *cnf-1* in Murine UTI

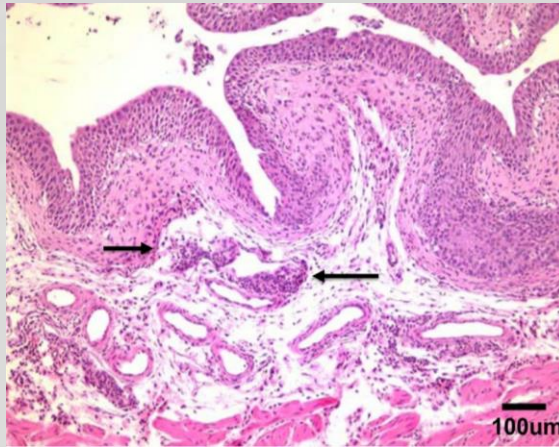


Bacterial burdens of mice infected with wild-type or *cnf-1*-null *E. coli* were not significantly different

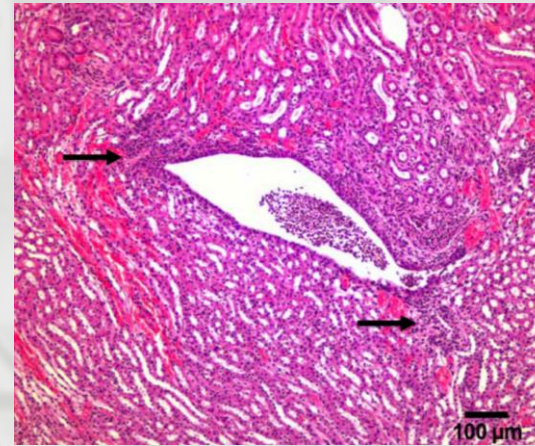


# Examination of Inflammation in Murine UTI

Bladder



Kidney



Preliminary data show no alteration in inflammation with *cnf-1*-null bacteria





# Conclusions

- A role for CNF-1 in the pathogenesis of UTI is often inferred from the prevalence of CNF-1 expression in clinical isolates of UPEC
- Our preliminary data casts doubt on the clinical virulence of CNF-1
- Additional studies are in place to study potential genetic linkage of *cnf-1* with other factors that might increase UPEC clinical virulence



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# References

1. Byington C, Rittichier K, et. al. *Pediatrics* 2003;111;964
2. Freedman A. *Journal of Urology*. Vol. 173, 949–954, March 2005
3. Jakobsson B, Berg J, et. al. *Archives of Disease in Childhood* 1994; 70: 111-115
4. Krieger JN, Nyberg L Jr, Nickel JA. NIH consensus definition and classification of UTI. *JAMA* 282:235-237, 1999.
5. National Collaborating Centre for Women's and Children's Health Clinical Guideline, Urinary tract infection in children diagnosis, treatment and long-term management, Clinical Guideline, August 2007.
6. Bergstrdm, T. Sex differences in childhood urinary tract infection. *Archives of Disease in Childhood*, 47, 227, 1972.
7. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 365 (9459): 579–87, 2005.
8. Mears EM. UTI – a review. *Urol Clin North Am* 2:3-27, 1975.
9. Lipsky BA, Byren I, Hoey CT. Treatment of bacterial UTI *Clin Infect Dis* 50:1641-1652, 2010.
10. Andreu A, Stapleton AE, Fennell C, Lockman HA, Xercavins M, Fernandez F, Stamm WE. Urovirulence determinants in *Escherichia coli* strains causing UTI. *J Infect Dis* 176:464-469, 1997.
11. Mitsumori K, Terai A, Yamamoto S, Ishitoya S, Yoshida O. Virulence characteristics of *Escherichia coli* in acute bacterial UTI. *J Infect Dis* 180:1378-1381, 1999.

1. Ruiz J, Simon K, Horcajada JP, Velasco M, Barranco M, Roig G, Moreno-Martinez A, Martinez JA, Jimenez de Anta T, Mensa J, Vila J. Differences in virulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and UTI in men. *J Clin Microbiol* 40:4445-4449, 2002.
2. Terai A, Yamamoto S, Mitsumori K, Okada Y, Kurazono H, Takeda Y, Yoshida O. *Escherichia coli* virulence factors and serotypes in acute bacterial UTI. *Int J Urol* 4:289-294, 1997.
3. Rippere-Lampe K, Lang M, Ceri H, Olson M, Lockman HA, O'Brien AD. Cytotoxic necrotizing factor 1-positive *Escherichia coli* causes increased inflammation and tissue damage to the prostate in a rat UTI model. *Infect Immun* 69:6515-6519, 2001.
4. Rippere-Lampe K, O'Brien AD, Conran R, Lockman HA. Mutation of the gene encoding cytotoxic necrotizing factor 1 (*cnf1*) attenuates the virulence of uropathogenic *Escherichia coli*. *Infect Immun* 69:3954-3964, 2001.
5. Boquet P. The cytotoxic necrotizing factor 1 (CNF1) from *Escherichia coli*. *Toxicon* 39:1673-1680, 2001.
6. Falzano L, Quaranta MG, Travaglione S, Filippini P, Fabbri A, Viora M, Donelli G, Fiorentini. Cytotoxic necrotizing factor 1 enhances reactive oxygen species-dependent transcription and secretion of proinflammatory cytokines in human uroepithelial cells. *Infect Immun* 71:4178-4181, 2003.