Bacterial virulence factors associated with the occurrence of acute pyelonephritis but not renal scarring

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ABSTRACT

Purpose: The goal of this study was to evaluate the influence of patient factors and virulence factors (VFs) of uropathogenic *Escherichia coli* (UPEC) on the occurrence of acute pyelonephritis (APN) and its sequela, renal parenchymal scarring.

Patients and Methods: In this study, we enrolled 80 boys and 45 girls (age 1 to 180 months) with febrile urinary tract infections (UTIs) who underwent an initial renal scan to diagnose APN, and follow-up DMSA scintigraphy at least 6 months later. Urinalysis, white blood cell (WBC) count, UPEC genotype, and vesicoureteral reflux (VUR) were measured. Voiding cystourethrogram (VCUG) was investigated after APN was confirmed by renal scanning and acute inflammation subsided, about 2-4 weeks later.

Results: Pediatric patients with UTIs and persistent fever before and after admission, elevated C-reactive protein (CRP), or positive renal ultrasound findings were significantly more likely to develop APN. *E. coli* strains with the *papG II* and *iha* genes were significantly more likely to occur in patients with APN. Patients with fevers for more than 3 days and CRP levels above 90.8 mg/L were significantly more likely to have renal scarring. Age was not an independent predictor of APN, but modified the effect of VFs on the development of APN.

Conclusions: Bacterial VFs and host factors are associated with the occurrence of APN. Host factors, such as patient age and VUR severity, modify the influence of VFs, but only host factors are associated with the occurrence of renal scarring.

INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) strains frequently cause urinary tract infections (UTIs),[1] especially in children. Beginning with periurethral colonization, UTIs can cause cystitis by ascending the urethra into the bladder, and acute pyelonephritis (APN) by ascending the ureters into the kidneys.[2] APN is a serious condition that can cause irreversible kidney damage, followed by renal failure and sepsis if left untreated.

Bacterial attachment and internalization are the initial stages of infection. Successful colonization of the urinary tract depends on the expression of fimbrial adhesion proteins, which facilitate attachment to the urothelium[3, 4] and on the presence of specific bacterial genes that encode virulence factors (VFs). The most extensively studied VFs include adhesins, among which components of the fimbriae or pili, S-fimbriae (*sfa*), afimbrial adhesin (*afa*), and P-fimbriae, have been identified.[5] A previous study showed that P-fimbrial adhesin proteins facilitate the establishment of bacteriuria in the mouse kidney.[6]

P-fimbriae (encoded by the pyelonephritis-associated [*pap*] genes) are composed of approximately 1000 subunits of the PapA protein, which assemble to form rigid stalks. Each stalk is attached to a flexible tip consisting of minor subunit proteins (PapE and PapF) and a receptor-binding adhesion (PapG) at the distal end[7, 8] that provides receptor-binding specificity.[9] Clinically, the class II papG allele is primarily associated with human pyelonephritis and bacteremia.[10] We have previously shown that of the three PapG adhesins (*papG I, II, III*), *papG II* is most significantly associated with the occurrence of upper UTIs.[11, 12]

Dimercaptosuccinic acid (DMSA) scintigraphy is currently the **gold standard for evaluation of renal parenchyma and renal cortical defects.[13,14]** When renal ultrasound (US) is used to detect APN and acute renal parenchymal changes, up to 25% of cortical

defects can be missed.[15] The objective of the present study was to evaluate the association between the VFs of UPEC strains and APN and its sequelae in pediatric patients with UTIs.

PATIENTS AND METHODS

Study population

All enrolled children were inpatients at the National Cheng Kung University Medical College and Hospital, a single tertiary referral medical center in Tainan. Each enrolled child had his/her first recorded incidence of febrile UTI and was given an initial Tc-99m DMSA renal single photon emission computed tomography (SPECT) scan between January 2005 and December 2006, and a follow-up scan at least six months later. All patients fulfilled the following inclusion criteria: (i) fever with a core temperature of 38°C or higher; (ii) positive urine culture (*E. coli* 10^5 colony-forming units/mL or greater); (iii) renal ultrasonography that was performed to exclude patients with any abnormal renal contour and patients with genitourinary abnormalities, except vesicoureteral reflux (VUR); and (iv) DMSA renal SPECT for APN diagnosis that was assessed by two experienced nuclear medicine physicians unaware of the clinical presentation. Patients with histories of recurrent UTI were excluded. The study protocol was approved by the institutional review board of our hospital.

Tc-99m DMSA renal SPECT and Voiding cystourethrogram (VCUG)

The initial Tc-99m DMSA renal SPECT scan was performed within 1 week of admission to determine the status of renal parenchymal inflammation. Patients diagnosed with APN were encouraged to receive another scan in six months to check for scar formation. VCUG was investigated only after APN was confirmed by the SPECT and the acute inflammation had subsided, about 2-4 weeks later.

Clinical examination

Blood cultures were taken on admission and, for patients with bacteremia, repeated after 48 h of therapy. Urinalysis was performed to establish the diagnosis before initiation of antimicrobial therapy. White blood cell (WBC) counts and differentiation were routinely determined. Ultrasonography of the urinary tract was performed upon admission to exclude congenital uropathy and space-occupying lesions.

Bacterial isolates

All *E. coli* strains were identified using standard methods[16] and stored in 20% glycerol at –70°C after isolation. Genetic determinants of isolated *E. coli* strains were detected using a polymerase chain reaction (PCR).

PCR primers

Primer pairs discriminating for the 3 PapG adhesin (*papG*) genes (I, II, III) of P-fimbriae and genes for type l fimbrial adhesins (*fimH*), S-/F1C-fimbriae (*sfa/foc*), afimbrial adhesins (*afa*), hemolysin (*hlyA*), cytotoxic necrotizing factor 1 (*cnf1*), aerobactin receptor (*iutA*), catecholate siderophore receptor (*iroN*), iron regulated gene A homologue adhesin (*iha*), uropathogenic-specific protein (*usp*), and outer membrane protease T (*ompT*) have been previously described.[6, 17-20]

Amplification procedures

Bacteria were harvested from Luria-Bertani agar, suspended in 200 μL of sterile water, incubated at 100°C for 10 minutes, and centrifuged at 13,000*g*. The supernatant was used as the DNA template. Amplification was performed in 50-μL reaction mixtures that contained 5 μL of prepared template DNA, 50 pmol of each primer, 0.2 mmol of each deoxyribonucleoside triphosphate (dNTP), and 1 unit of DNA polymerase (DynaZyme II; Finnzymes, Oy, Finland) in 1 × PCR buffer II. PCR was performed in a thermal cycler (Gene Amp, PCR System 9600; Perkin-Elmer, Foster City, CA), using established protocols.[11,17-20] PCR products were electrophoresed on agarose gels, stained with ethidium bromide, and

photographed using UV transillumination. All assays were performed in duplicate, and positive and negative control strains for traits of interest were included in each assay. Product sequences for each virulence gene were determined with an autosequencing system (3100 Genetic Analyzer; ABI Prism, Foster City, CA) to confirm the identity of amplification products.

Statistical methods

For all study participants, we recorded basic demographic information, specific clinical features of the UTI upon admission, laboratory measurements, and *E. coli* genotype by use of a mean and standard deviation (SD) for continuous variables, and numbers for categorical factors. If a patient had bilateral VUR, the renal unit with the higher VUR grade was used for analysis. Univariate and multiple logistic regression analyses were used to determine the association between variables and acute pyelonephritis.

Univariate logistic regression analysis was also used to assess the risk of renal scarring in patients with acute pyelonephritis. Then, age, gender, duration of fever before admission, and CRP levels were considered as potential confounders for multivariate analysis that assessed the risk of specific *E. coli* genotypes on the incidence of acute pyelonephritis. Odds ratios with 95% confidence intervals for each variable were used to estimate the relative risk of APN, and renal scarring. Independent associations between *E. coli* genotypes and the occurrence of normal VUR, **mild VUR (grade I and II), and severe VUR (grade III to V)** were assessed by multinomial logistic regression models. A *p* value less than 0.05 was considered statistically significant. All statistical analyses were two-sided and performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

A total of 125 patients (80 boys, 45 girls; age range: 1 to 180 months; mean age: 14.02 ± 27.43 months; median age: 5 months) were enrolled. On average, follow-up Tc-99m DMSA renal SPECT scans were performed 10.83 ± 6.68 months (median: 8 months; range: 6-33 months) after acute infection. The initial scan identified 89 (71.2%) children with APN, with no significant gender differences. Follow-up scans in 67 (75.3%) children indicated that lesions completely resolved in 24 children, but become permanent scar foci in the other 43 children. For the 89 patients who were scan positive, 75 (84.3%) had VCUG, 57 did not have VUR, and 18 (24%) had VUR (nine males, nine females). Thirteen of the VUR cases were unilateral (7 left, 6 right; VUR-grade I/II: 2 patients, grade III: 4 patients, grade V: 5 patients) and five were bilateral (VUR-grade I: 3 renal units, grade II: 1 renal unit, grade III: 5 renal units, grade IV: 1 renal unit).

Statistical analysis indicated an elevated risk of APN with higher maximal body temperature, longer duration of fever before admission, increased duration of fever after admission, higher levels of CRP, positive renal echo finding, and *E. coli* genotype *papG II*. After adjustment for age and gender, the adjusted ORs indicated that risk factors for acute pyelonephritis were in the 2nd tertile of duration of fever before admission (OR = 5.60; 95% CI: 1.11-28.17), positive renal echo finding (OR = 32.83; 95% CI: 6.09-177.08), *E. coli* genotype *papG II* (OR = 5.95; 95% CI: 1.20-29.47), and *E. coli* genotype *iha* (OR = 9.37; 95% CI: 1.28-68.56) (Supplementary Table 1).

Then, clinical features, laboratory measurements, and *E. coli* genotype were used to determine the association between variables and renal scar formation. Only the 3rd tertile of duration of fever after admission (relative to the 1st tertile) (OR = 7.69; 95% CI: 1.85-31.91) and higher level of CRP were significant predictors of renal scarring (OR = 9.56; 95% CI: 1.67-54.89). In the present study, we confirmed APN in 89 children (71.2%). Follow-up

SPECT scans indicated VUR in 20.8% of the children without renal scarring and in 28.9% of the children with renal scarring (p = 0.48) (Supplementary Table 2).

After adjustment for age, gender, duration of fever before admission, and CRP level, we performed multivariate analysis to estimate the adjusted ORs for *E. coli* genotypes in APN (Table 1). The effects of *papG II* and *iha* on the incidence of APN were dramatically modified by age: children older than 5 months were more likely to have *papG II* (OR = 11.56; 95% CI: 1.40-95.59) and children 5 months or younger were more likely to have *iha* (OR = 3.32; 95% CI: 1.03-10.64) (Table 1).

We also performed multivariate analysis to estimate the adjusted ORs for *E. coli* genotype on renal scarring based on the occurrence of VUR. Although not statistically significant, there was an elevated risk of renal scarring for the *E. coli* genotypes *aer* (OR = 15.52; 95% CI: 0.47-508.43) and *afa* (OR = 13.11; 95% CI: 0.51-336.08) in VUR-positive (+) patients with APN (data not shown). **Based on the severity of VUR, we found a significantly higher incidence of *papG II* (54/57, 94.74%) strains in patients with normal VUR, and a lower incidence of *papG II* (9/13, 69.23%) strains in patients with severe grade VUR (grade III-V). After adjustment for age, gender, duration of fever before admission, and level of CRP level, we found a negative association between the *papG II* genotype and severe VUR (-7.29 ±2.76; p=0.012) And a marginally significant association between the *iha* genotype and severe VUR (-5.89 ±3.07, p=0.055) (Table 2).**

DISCUSSION

We previously showed that P-fimbriae are crucial for the establishment and persistence of UPEC in mouse kidneys, but do not influence bladder colonization.[6] In the present study, we used DMSA scintigraphy of children with UTI and showed that adhesion

proteins encoded by the *papG* II and *iha* genes are important in the establishment of APN, but not renal parenchymal scarring.

One of our primary objectives was to investigate the effect of patient age on the occurrence of APN. Our findings indicated that age was not an independent predictor of APN, but age was different among children infected with the *papG II* and *iha* genotypes. However, *E. coli* genotype had no influence on renal scarring in children with APN. UPEC PapG adhesins are the most extensively investigated VFs of these *E. coli* stains, and have been shown to increase resistance to antimicrobials and to promote establishment and persistence of UPEC.[21] Less is known about the role of Iha (iron-regulated gene homologue adhesin) in the pathogenesis of APN and UTIs. One study[22] reported that two different *E. coli* strains (*iha* CFT073 deletion mutants) had impaired ability to colonize mouse urinary tracts, suggesting that *iha* may be a virulence factor. On the other hand, two other studies that assayed the association of *iha* and urinary tract infections found no effect.[20,23] In the present study, we found that *iha* strains were significantly associated with APN in children with UTI, especially in children younger than 5 months. Based on these results, we preliminarily suggest that *iha* may have a stronger effect in infants who are younger, have physiologic anemia, or have limited iron storage. Additional studies are clearly needed to better understand the role of the *iha* gene.

In the present study, we found no association of VFs and renal scarring, possibly because of the small number of enrolled patients. Nevertheless, this observation is consistent with the hypothesis that the renal damage which occurs in conjunction with UTI is caused by the inflammatory response, rather than the bacteria *per se*.[24] Our results also indicated an elevated risk of renal scarring was correlated with an increased duration of fever after admission and higher level of CRP. These findings indicate that clinicians should consider

anti-inflammatory treatment in conjunction with antibiotics to treat APN to reduce the risk of scarring.

The role of bacterial VFs is also associated with host factors, such as the inflammatory response of the immune system. We found a significantly higher incidence of *papGII* in patients with normal or **mild** VUR, but a low incidence of *papG II* strains in patients with severe VUR. It was recently reported that *E. coli* strains lacking *papG II* or adhesin were more common in children with urinary tract abnormalities.[25] In the present study, we found that *E. coli* strains with *papG II* were significantly associated with the occurrence of APN and infrequently associated with severe VUR in children with APN (β=0.29, SE:2.76).

Our study has certain limitations. First, due to the observational design, we can only comment on the association of different factors with outcome, not on cause and effect relationships. Second, not knowing the VUR status of all febrile UTI patients limited our ability to determine the relationship of VUR with APN and renal scarring, and of how this may be modified by VFs and host factors (HFs). Third, our reported incidence of renal scarring is higher than that of many other reports. This may be due to geographic factors, an insufficient interval between initial and subsequent DMSA scans, high VUR incidence, high incidence of phimosis in Taiwan (where circumcision is rare), and/or because our study was performed at a referral tertiary medical center. **Finally, although we only enrolled children who were febrile and had positive *E. coli* urine cultures, we cannot exclude the possibility that some of the renal defects that we observed were due to congenital reflux nephropathy, and that infection was secondary.**

CONCLUSIONS

Our results indicate that VFs and HFs are associated with the occurrence of APN, that host factors (age, severity of VUR) modify the influence of VFs, and that HFs, but not VFs, are associated with the occurrence of renal scarring.

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Table 1. Logistic regression analysis of the effect of patient age (≤ 5 months or > 5 months) on the occurrence of acute pyelonephritis by different *E. coli* genotypes for children with urinary tract infections.

	≤ 5 months			> 5 months		
	Acute Pyelonephritis			Acute Pyelonephritis		
	No (n=25)	Yes (n=43)	Adjust Odds Ratio (95% CI)[†]	No (n=11)	Yes (n=46)	Adjust Odds Ratio (95% CI)[†]
papG II (Positive / Negative)	17/8	37/6	1.92 (0.50-7.34)	5/6	42/4	11.56 (1.40-95.59)[*]
papG III (Positive/ Negative)	3/22	8/35	1.49 (0.31-7.21)	2/9	5/41	1.35 (0.11-16.32)
aer (Positive/ Negative)	20/5	34/9	0.56 (0.13-2.40)	6/5	37/9	1.69 (0.26-11.04)
cnf1(Positive/ Negative)	5/20	8/35	1.10 (0.26-4.68)	3/8	9/37	1.87 (0.21-16.80)
fimH (Positive /Negative)	25/0	42/1	NA	10/1	45/1	2.43 (0.07-86.87)
hly‡ (Positive /Negative)	13/12	26/17	2.03 (0.65-6.30)	6/5	23/22	1.54 (0.24-9.74)
afa (Positive /Negative)	11/14	20/23	1.23 (0.42-3.59)	6/5	19/27	0.25 (0.03-1.86)
foc (Positive /Negative)	4/21	10/33	3.29 (0.70-15.51)	1/10	4/42	0.93 (0.05-18.86)
sfa (Positive/ Negative)	0/25	0/43	NA	0/11	1/45	NA
iha (Positive /Negative)	7/18	23/20	3.32 (1.03-10.64)[*]	4/7	21/25	0.81 (0.14-4.81)
usp (Positive /Negative)	16/9	35/8	2.08 (0.62-7.04)	8/3	30/16	0.28 (0.04-2.11)
iroN (Positive /Negative)	14/11	31/12	1.86 (0.59-5.83)	3/8	25/21	1.83 (0.29-11.49)
ompT (Positive/ Negative)	19/6	37/6	1.47 (0.39-5.54)	9/2	36/10	0.48 (0.05-4.81)

NA: not applicable. CI: 95% confidence interval.

[*]$p<0.05$.

[†]Multiple logistic regression analysis was used.

[‡]Incomplete data set.

Table 2. Multinomial logistic regression analysis of the occurrence of **no vesicoureteral reflux (No VUR), grade I-II VUR (Mild VUR), and grade III-V VUR (Severe VUR)** in children with acute pyelonephritis (n = 75).

	No VUR† (n=57)	**Mild** VUR Grade I-II (n=5)		Severe VUR Grade III-V(n=13)	
	n	n	β (SE)	n	β (SE)
papG II (Positive / Negative)	54/3	3/2	-7.95 (59.01)	9/4	-7.29 (2.76)*
papG III **(**Positive/ Negative)	9/48	1/4	3.71 (122.83)	0/13	NA
aer (Positive/ Negative)	47/10	2/3	1.01 (7.31)	11/2	4.66 (2.81)
cnf1(Positive/ Negative)	13/44	1/4	-15.81 (40.15)	1/12	-5.72 (3.31)
fimH (Positive /Negative)	56/1	5/0	NA	13/0	NA
hly‡ (Positive /Negative)	34/22	3/2	11.48 (11.30)	3/10	2.62 (2.02)
afa (Positive /Negative)	25/32	2/3	16.82 (45.31)	7/6	2.28 (1.55)
foc (Positive /Negative)	12/45	0/5	NA	1/12	NA
sfa (Positive/ Negative)	0/57	1/4	NA	0/13	NA
iha (Positive /Negative)	32/25	1/4	-3.83 (58.66)	4/9	-5.89 (3.07)
usp (Positive /Negative)	45/12	2/3	-14.08 (50.63)	9/4	-2.37 (1.77)
iroN (Positive /Negative)	38/19	2/3	-2.88 (21.43)	6/7	-2.44 (1.81)
ompT (Positive/ Negative)	49/8	1/4	-11.62 (58.62)	12/1	4.94 (2.95)

VUR: vesicoureteral reflux. NA: not applicable. SE: standard error. *$p<0.05$.

† Reference group.

‡Incomplete data set.

Key of Definitions for Abbreviations

APN—acute pyelonephritis

CRP—C-reactive protein

DMSA—dimercaptosuccinic acid

HF---host factor

OR—odds ratio

PCR—polymerase chain reaction

SPECT—single photon emission computed tomography

SD—standard deviation

US—ultrasound

UTI—urinary tract infection

UPEC—uropathogenic *Escherichia coli*

VUR—vesicoureteral reflux

VF—virulence factor

VCUG—voiding cystourethrogram

WBC—white blood cell

Supplementary Table 1. Basic statistics and logistic regressions analyses for the occurrence of acute pyelonephritis among children with urinary tract infection (N=125)

	Acute Pyelonephritis			
	No (n=36)	Yes (n=89)	Odds Ratio (95% CI)[†]	Adjust Odds Ratio (95% CI)[‡]
Age (months) (mean±SD)	8.50±16.92	16.26±30.47	1.02 (0.99-1.04)	1.00 (0.97-1.03)
Gender (Male/ Female)	27/9	53/36	0.49 (0.21-1.17)	1.39 (0.33-3.82)
Highest body temperature				
1st tertile (<39.0)	22	19	1.00	-
2nd tertile (39.0-39.5)	8	29	4.20 (1.55-11.35)*	-
3rd tertile (>39.5)	6	41	7.91 (2.76-22.70) *	-
Duration of fever before admission (days)				
1st tertile (<1.5)	30	41	1.00	1.00
2nd tertile (1.5-3.0)	4	32	5.85 (1.87-18.32)*	5.60 (1.11-28.17)*
3rd tertile (>3.0)	2	16	5.85(1.25-27.40)*	8.78 (0.84-91.6)
Duration of fever after admission (days)				
1st tertile (<1.5)	30	40	1.00	-
2nd tertile (1.5-3.0)	6	24	3.00 (1.09-8.25)*	-
3rd tertile (>3.0)	0	25	NA	-
CRP level (mg/L)				
1st tertile (<39.4)	24	18	1.00	-
2nd tertile (39.4-90.8)	7	35	6.67 (2.41-18.41)*	-
3rd tertile (>90.8)	5	36	9.60 (3.14-29.35)*	-
White cell counts (10^3/ml)				
1st tertile (<14.0)	16	25	1.00	-
2nd tertile (14.0-19.4)	10	32	2.05 (0.79-5.28)	-
3rd tertile (>19.4)	10	32	2.05 (0.79-5.28)	-
Percentage if WBC shifting to left				

1st tertile (<51.0)	14	26	1.00	-
2nd tertile (51.0-65.0)	14	29	1.12 (0.45-2.77)	-
3rd tertile (>65.0)	8	34	2.29 (0.84-6.27)	-
Renal echo (Positive/Negative)	3/33	55/34	17.79 (5.06-62.54)*	32.83 (6.09-177.08) *
papG II (Positive/ Negative)	22/14	79/10	5.03 (1.97-12.86)*	5.95 (1.20-29.47)*
papG III (Positive /Negative)	5/31	13/76	1.06 (0.35-3.23)	0.65 (0.07-5.86)
aer (Positive/ Negative)	26/10	71/18	1.52 (0.62-3.71)	0.22 (0.03-1.90)
cnf1 (Positive /Negative)	8/28	17/72	0.83 (0.32-2.13)	1.01 (0.15-6.74)
fimH (Positive /Negative)	35/1	87/2	1.24 (0.11-14.15)	0.97 (0.04-27.28)
hly§ (Positive /Negative	19/17	49/39	1.12 (0.52-2.45)	0.76 (0.14-3.98)
afa (Positive / Negative)	17/19	39/50	0.87 (0.40-1.90)	0.76 (0.19-2.97)
foc (Positive /Negative)	5/31	14/75	1.16 (0.38-3.49)	3.16 (0.41-24.52)
sfa (Positive /Negative)	0/36	1/88	NA	NA
iha (Positive/ Negative)	11/25	44/45	/2.22 (0.98-5.06)	9.37 (1.28-68.56)*
usp (Positive/ Negative)	24/12	65/24	1.35 (0.59-3.13)	0.48 (0.06-3.60)
iroN (Positive/ Negative)	17/19	56/33	1.90 (0.87-4.15)	3.17 (0.64-15.73)
ompT (Positive /Negative)	28/8	73/16	1.30 (0.50-3.38)	0.26 (0.03-2.20)

NA: not applicable. CI: confidence interval. CRP: C-reactive protein. WBC: white blood cell.

Dash indicates the variable was not included in the multiple logistic regression model.

*$p<0.05$.

†Univariate logistic regression analysis was used.

‡Multiple logistic regression analysis was used.

§Incomplete data set.

Supplementary Table 2. Basic statistics and univariate analyses for the occurrence of renal scar among children with acute pyelonephritis by logistic regressions (N=67)

Variables	Renal Scar		Odds Ratio	
	No (n=24)	Yes (n=43)	(95% CI)†	P value
Age (months) (mean±SD)	26.71±50.52	14.70±19.09	0.99 (0.98-1.01)	0.19
Gender (Male /Female)	17/7	20/23	0.36 (0.12-1.04)/	0.06
Highest body temperature				
1st tertile (<39.0)	6	5	1.00	
2nd tertile (39.0-39.5)	7	16	2.74 (0.62-12.08)	0.18
3rd tertile (>39.5)	11	22	2.40 (0.60-9.64)	0.22
Duration of fever before admission				
1st tertile (<1.5)	13	18	1.00	
2nd tertile (1.5-3.0)	7	15	1.55 (0.49-4.87)	0.46
3rd tertile (>3.0)	4	10	1.81 (0.46-7.05)	0.40
Duration of fever after admission				
1st tertile (<1.5)	15	13	1.00	
2nd tertile (1.5-3.0)	6	10	1.92 (0.55-6.75)	0.31
3rd tertile (>3.0)	3	20	7.69 (1.85-31.91)	<0.01*
CRP level (mg/L)				
1st tertile (<39.4)	9	2	1.00	
2nd tertile (39.4-90.8)	8	17	9.56 (1.67-54.89)	<0.05*
3rd tertile (>90.8)	7	24	15.43 (2.69-88.63)	<0.01*
White cell counts (10^3/ml)				
1st tertile (<14.0)	8	10	1.00	
2nd tertile (14.0-19.4)	7	15	1.71 (0.47-6.24)	0.41
3rd tertile (>19.4)	9	18	1.60 (0.47-5.46)	0.45
Percentage if WBC shifting to left				
1st tertile (<51.0)	5	10	1.00	
2nd tertile (51.0-65.0)	7	15	1.07 (0.26-4.34)	0.92
3rd tertile (>65.0)	12	18	0.75 (0.21-2.75)	0.66

VUR‡ (Positive/ Negative)	5/19	11/27	1.55 (0.46-5.19)	0.48
VUR status‡				
Non-VUR	19	27	1.00	
Unilateral	3	10	2.35 (0.57-9.68)	0.24
Bilateral	2	1	0.35 (0.03-4.17)	0.41
Severity VUR‡				
No VUR	19	27	1.00	
Grade I-II VUR (Mild VUR)	2	3	1.06 (0.16, 6.94)	0.96
Grade III-V VUR (Severe VUR)	3	8	1.88 (0.44, 8.01)	0.40
papG II (Positive/Negative)	22/2	36/7	0.47 (0.09-2.46)	0.37
papG III (Positive/ Negative)	2/22	7/36	2.14 (0.41-11.23)	0.37
aer (Positive/ Negative)	18/6	35/8	1.46 (0.44-4.85)	0.54
cnf1 (Positive/ Negative)	4/20	7/36	0.97 (0.25-3.73)	0.97
fimH (Positive /Negative)	23/1	42/1	1.83 (0.11-30.58)	0.68
hly‡ (Positive /Negative)	10/14	25/17	2.06 (0.74-5.70)/	0.17
afa (Positive /Negative)	8/16	21/22	1.91 (0.68-5.39)	0.22
foc (Positive /Negative)	5/19	4/39	0.39 (0.09-1.62)	0.20
sfa (Positive /Negative)	0/24	1/42	NA	
iha (Positive/ Negative)	12/12	23/20	1.15 (0.42-3.13)	0.78
usp (Positive/ Negative)	16/8	32/11	1.46 (0.49-4.33)	0.50
iroN (Positive/ Negative)	13/11	28/15	1.58 (0.57-4.38)	0.38
ompT (Positive/ Negative)	17/7	37/6	2.54 (0.74-8.71)	0.14

NA: not applicable. CI: confidence interval. CRP: C-reactive protein. WBC: white blood cell. VUR: vesicoureteral reflux.

*$p<0.05$.

†Univariate logistic regression analysis was used.

‡Incomplete data set.