Journal of Bacteriology and Virology 2008. Vol. 38, No. 4 p.189 – 196

Detection of Virulence Genes of *Staphyloccus aureus* and *Staphylococcus epidermidis* Isolated from Suprapubic Urine from Infants with Fever

Hae-Kyung Park[1*], So-Youn Woo[1], Yun-Jae Jung[2], Eun Ok Lee[1], Je-Eun Cha[1], Hye-Sook Park[3] and Seung-Joo Lee[4]

[1]Department of Microbiology, School of Medicine, Ewha Womans University, Seoul, Republic of Korea; [2]Deprtment of Microbiology, Graduate School of Medicine, Gachon University of Medicine and Science, Incheon, Republic of Korea; [3]Department of Preventive Medicine, and [4]Department of Pediatrics, School of Medicine, Ewha Womans University, Seoul, Republic of Korea

Received : November 4, 2008
Revised : December 1, 2008
Accepted : December 10, 2008

While methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from urinary tract infection (UTI) has recently increased in elderly adult urology patients, it has been only rarely reported in infants. Therefore, in this study to understand whether MRSA may be involved in UTI of infants who run fever without other apparent causes, we identified and counted *S. aureus* and *S. epidermidis* in suprapubic urine from 750 febrile infants via microbiological methods, and confirmed the counts via multiplex PCR. And we also detected four virulence genes, *mecA*, PVL, *bbp* and *icaA* genes for *S. aureus* and *S. epidermidis* via multiplex PCR in the same specimens. *S. aureus* (28 cases) counts were as follows: $>10^4$ CFU/ml (3/28), $10^2 \sim 10^3$ CFU/ml (1/28) and $<10^2 \sim 10^3$ CFU/ml (24/28). *S. epidermidis* (26 cases) counts were as follows: $>10^4$ CFU/ml (2/26), $10^2 \sim 10^3$ CFU/ml (4/26) and $10^2 \sim 10^3$ CFU/ml (20/26). *S. aureus* virulence genes were detected in 26 cases as *mecA* (16/26, 59.3%), PVL (17/26, 63.0%), *bbp* (7/26, 26.9%) and *icaA* (20/26, 76.9%). *S. epidermidis* virulence genes were detected in 22 cases as *mecA* (17/22, 81.0%), PVL (15/22, 71.4%), *bbp* (3/22, 13.6%) and *icaA* (13/22, 50.1%). Therefore, *mec*A, PVL and *ica*A genes of MRSA and MRSE were detected with high positivity in urines from infants with fever. The results demonstrate that community-acquired MRSA or MRSE may be responsible for UTI incidence in febrile infants.

Key Words: Suprapubic urine, *Staphylococcus aureus*, *Staphyococcus epidermidis*, *mec*A, PVL, *bbp*, *ica*A

INTRODUCTION

Urinary tract infection (UTI) is a frequent and important problem in infants and young children with fevers (7,20). *Escherichia coli* is the most frequent responsible organism for UTI in all age groups, but community acquired methicillin-resistant *Staphylococcus aureus* (MRSA) has become a serious problem in hospitals for patients of all age groups worldwide (14,16,26). Recently, MRSA isolation frequency has increased in association with UTIs in patients over 70 years of age in Japan (23), the USA (9), and France (3), but has been reported rarely in infants.

To the best of our knowledge, there has been no report thus far regarding to the frequency of *S. aureus* and *S.*

*Corresponding author: Hae-Kyung Park, MD, PhD. Department of Microbiology, School of Medicine, Ewha Womans University 911-1 Mok-6-Dong, Yang-chun-gu, 158-710, Seoul, Republic of Korea.
Phone: +82-2-2650-5737, Fax: +82-2-2653-8891,
e-mail: parkhk@ewha.ac.kr

**This study was supported by Youn Kyung Foundation (2007). June Seng Lee was acknowledged for his skilled cooperation.

epidermidis in UTIs in infant patients. We selected four virulence genes to detect by multiplex PCR and assessed their relationships to the pathogenicity of infant UTI. First, the methicillin resistance gene (*mecA*) gene was found in *S. aureus* or *S. epidermidis*, which were isolated from blood, urine, and soft skin infections in young children. Physicians must isolate such microorganisms early and select sensitive antibiotics and this is crucial to the treatment of infectious diseases (10). Second, owing to recent advances in PCR detection methods, allowing for the detection of the Panton-Valentine Leukocidin (PVL) gene, many countries can now use these methods to detect a variety of infections in patients, particularly skin and mucous membrane infections, pneumonia, endocarditis, and assorted other infections (4,5,8,27, 28). *S. aureus* harboring the PVL gene has been detected in urine, and this gene has been implicated in many different types of infections, including UTIs (12). Third, the intracellular adhesion (*ica*A) gene is associated with the adhesion of *S. aureus* or *S. epidermidis* in humans, and its association with adherence has been experimentally confirmed (17,19). The *ica*A genes may enhance the adherence of *S. aureus* to human cells and may also be involved in the induction of the inflammation process. Fourth, we selected the bone bound sialoprotein (*bbp*) gene of staphylococcus, which has been associated with osteomyelitis and arthritis (25), and compared it to the prevalence of *icaA* genes (2).

In this study, we collected 750 suprapubic urine samples from infants with fever and then isolated, counted and identified *S. aureus* and *S. epidermidis* in the samples. Identified *S. aureus* and *S. epidermidis* was confirmed via multiplex PCR. Two bacterial agents were counted and the prevalence of UTI was analyzed by these two bacteria. Four virulence genes--*mecA*, PVL, *bbp* and *ica*A--were all related to staphylococcal infection, although the simultaneous detection of four virulence genes from suprapubic urines from infants with fever has never previously been reported.

In this study, we also detected 4 virulence genes of *S. aureus* and *S. epidermidis* via two multiplex PCR assays, in order to evaluate the pathogenic role of UTI in febrile infants.

MATERIAL AND METHODS

1. Patients

Urine samples from 750 febrile infants and young children were collected by suprapubic tap for the diagnosis of UTI. The infants and young children examined in this study were all admitted to the emergency room of the Ewha Womans University, School of Medicine at Mok-dong Hospital, Seoul, Korea between 1 May and 31 October 2005, and between 1 February 2007 and 30 September 2008. These urine samples were taken for accurate diagnosis for UTI and we were received small volume (1 ml) for further study of virulence genes of *S. aureus* and *S. epidermidis*. Informed consents were taken from children's parents.

2. Identification and colony counting of *S. aureus* and *S. epidermidis* by microbiological culture methods

Ten microliters of suprabubic urine were inoculated on brain-heart infusion (BHI) agar plates, blood agar plates, and MacConkey agar plates, then incubated overnight at 37℃. The gram stain and coagulase test were followed by the identification of colonies using kits (bioMerieux, Inc., Hazelwood, MO, USA).

3. Multiplex polymerase chain reaction

We inoculated two colonies identified as *S. aureus* or *S. epidermidis* via the microbiological method described above into 200 μl of BHI broth and cultured them for 18 h at 37℃. Then, 60 μl of *S. aureus* or *S. epidermidis* suspension was added to 60 μl of sterile water, mixed, and incubated for 30 min at 85℃. The mixtures were centrifuged for 15 min at 14,000 rpm and the supernatants were collected for PCR analysis.

4. PCR

For the detection of *S. aureus*, we used the primer set (SA1, 5'-AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG-3'; SA2, 5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3') and obtained an amplified product of 108 bp (15). For the detection of *S. epidermidis*, we used

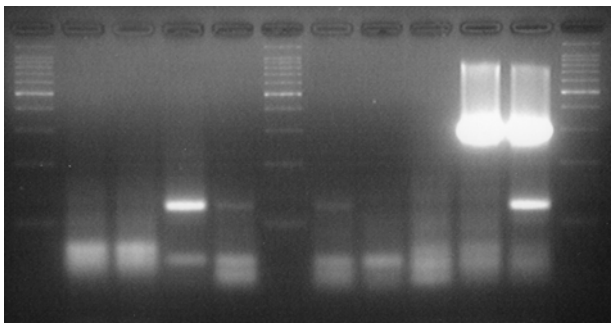

Figure 1. *S. aureus* and *S. epidermidis* isolated from the suprapubic urines of infants were confirmed via multiplex PCR. Lane 1 = DNA marker (100 bp ladder); lane 2 = positive control for *S. aureus* (108 bp); lane 3 = *S. aureus* positive sample; lane 4 = positive control for *S. epidermidis* (125 bp); lane 5 = *S. epidermidis* positive sample; lane 6 = DNA marker; lane 7 = *S. epidermidis* positive; lane 8 and 9 = *S. aureus* positive; lane 10 = *S. aureus* and *mecA* positive; lane 11 = *S. epidermidis* and *mec*A positive; lane 12 = DNA marker.

the primer set (SE1, 5'-ATC AAA AAG TTG GCG AAC CTT TTC A-3'; SE2, 5'-CAA AAG AGC GTC GAG AAA AGT ATC A-3') and obtained 125 bp of amplified product (15). For the detection of the *mec*A and PVL gene, we employed the following primer set (mecA1, 5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3'; mecA2, 5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3') and obtained 310 bp of amplified product (6). The PVL primer set (PVL1, 5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3'; PVL2, 5'-GCA TCA AST GTA TTG GAT AGC AAA AGC-3') and 433 bp of amplified product (13). For the detection of the *bbp* gene and *ica*A gene, we used the *bbp* primer set (bbp1, 5'-AAC TAC ATC TAG TAC TCA ACA ACA G-3'; bbp2, 5'-ATG TGC TTG AAT AAC ACC ATC ATC T-3') and obtained 575 bp of amplified product (25). The *ica*A primer set (icaA1, 5'-ACA GTC GCT ACG AAA AGA AA-3'; icaA2, 5'-GGA AAT GCC ATA ATG ACA AC-3') yielded an amplified product of 103 bp (2). The PCR reaction was conducted with 10× PCR buffer, 2 mM $MgCl_2$, 200 μM dNTP, 25 pmole or 35 pmole or 45 pmole for each primer, and 2 U of *Taq* polymerase in a final volume of 50 μl. A thermocycler (MJ Research, Ramsey, MN, USA) was utilized for amplification at 95℃, for 1 min at 51℃ or 60℃ for 1 min, and 72℃ for 1 min at 40 cycles, followed by a final 8 min extension step at 72℃. The PCR products were stored at 4℃ and resolved via electrophoresis with 3.3% or 3.57% NuSieve agar gel and analyzed.

Table 1. Bacterial counts of *S. aureus* and *S. epidermidis* in 54 suprapubic urine samples from infants and young children with fever

Group (CFU/ml)	*S. aureus*		*S. epidermidis*	
	N	%	N	%
Non-UTI ($<10^2$)	24	85.7	20	76.9
Suspicious UTI ($10^{2\sim3}$)	1	3.6	4	15.4
UTI ($\geq10^4$)	3	10.7	2	7.7
Total	28	100.0	26	100.0

$P_{M\text{-}H} = 0.74$

RESULTS

A total of 750 suprapubic urine samples were analyzed for the detection of *S. aureus* and *S. epidermidis* via culture on MacConkey and BHI agar plates. We determined that, in 28 out of 750 cases, *S. aureus* was grown (28/750, 3.7%), and in 26 cases, *S. epidermidis* was grown (26/750, 3.5%) after inoculating the urine samples on BHI, MacConkey, and blood agar plates. Among the 28 samples in which *S. aureus* was detected, the colony numbers were as follows: $>10^4$ CFU/ml (3/28), $10^2\sim10^3$ CFU/ml (1/28) and $<10^2\sim10^3$ CFU/ml (24/28). Among the 26 samples in which *S. epidermidis* was detected, the colony counts were as follows: $>10^4$ CFU/ml (2/26), $10^2\sim10^3$ CFU/ml (4/26) and $10^2\sim10^3$ CFU/ml (20/26).

In the UTI group, we noted no differences in the detection rate between *S. aureus* and *S. epidermidis* (Table 1). For the further confirmation of *S. aureus* and *S. epidermidis* colonies obtained from total 54 positive samples, we conducted multiplex PCR, the results of which were consistent with those of the microbiological culturing methods (Fig. 1).

The demographic features of the 28 cases in which *S. aureus* was detected showed that sex and age differences were statistically significant. In the UTI (3 cases) and suspicious UTI (1 case) cases in which *S. aureus* was isolated, the patients were all female. By way of contrast, males were predominant among the non-UTI group ($P_{M\text{-}H} = 0.0108$). When the UTI group was compared with the non-UTI

Table 2. Demographic features of the 28 cases in which *S. aureus* was detected

	UTI (n=3)	Suspicious UTI (n=1)	Non-UTI (n=24)
Gender (male : female)*	0:3	0:1	17:7
Median age (month)	12	3	2
Age group**			
<1 month	0 (0.0)	0 (0.0)	1 (100.0)
1~6 month	0 (0.0)	1 (5.3)	18 (94.7)
6 month ~ 1 years	1 (33.3)	0 (0.0)	2 (66.7)
≥ 1 years	2 (40.0)	0 (0.0)	3 (60.0)

* $P_{M\text{-}H} = 0.0108$, ** $P_{M\text{-}H} = 0.0125$

Table 3. Demographic features of the 26 cases in which *S. epidermidis* was detected

	UTI (n=2)	Suspicious UTI (n=4)	Non-UTI (n=19)
Gender (male : female)	1:1	2:2	10:8
Median age (month)	2	2	4
Age group			
<1 month	0 (0.0)	0 (0.0)	2 (100.0)
1~ 6 month	2 (13.3)	4 (26.7)	9 (60.0)
6 month ~ 1 years	0 (0.0)	0 (0.0)	3 (100.0)
≥ 1 years	0 (0.0)	0 (0.0)	5 (100.0)

group for *S. aureus*, as shown in Table 2, the average age of the UTI group (2/3, 40%, ≥ 1 yr) was older than in the non-UTI group (18/24, 94.7%, 1~6 mo) ($P_{M\text{-}H} = 0.0125$). The demographic features of the 26 cases in which *S. epidermidis* were detected showed that when the UTI group was compared to the non-UTI group, there is no differences in the sex and age distributions (Table 3).

The prevalence of virulence genes detected in *S. aureus* from 26 infants and young children with fever was assessed via multiplex PCR. Among the 26 cases that were positive for *S. aureus*, the *mec*A (59.3%), and PVL (63.0%) genes were detected at a higher rate. We noted no differences between the UTI and non-UTI groups in the detection of *mec*A and PVL genes (Fig. 2, 3 and Table 4). However, the number of UTI samples in which *S. aureus* was identified was relatively low (3/26 cases), thus rendering comparison difficult. The prevalence of virulence genes detected in *S. epidermidis* from 22 infants and young children with fever was determined via multiplex PCR. Among the 22 cases in

Table 4. Prevalence of virulent genes detected in *S. aureus* from 26 infants and young children with fever by multiplex PCR

	UTI (n=3)	Suspicious UTI (n=1)	Non-UTI (n=19)	Total (n=26)
mecA (presence)	2 (66.7)	0 (0.0)	14 (60.9)	16 (59.3)
PVL (presence)	2 (66.7)	0 (0.0)	15 (65.2)	17 (63.0)
bbp (presence)	1 (33.3)	0 (0.0)	6 (27.3)	7 (26.9)
*ica*A (presence)	2 (66.7)	1 (100.0)	17 (77.3)	20 (76.9)

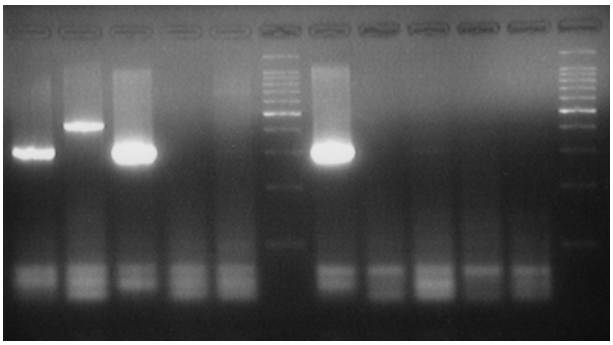

Figure 2. *mec*A gene and *PVL* gene were detected via multiplex PCR from *S. aureus* and *S. epidermidis* isolated from the suprapubic urines of infants. Lane 1 = positive control for *mecA* gene (310 bp); lane 2 = positive control for *PVL* gene (433 bp); lane 3 = *mecA* positive sample; lane 4 = *PVL* positive samlple; lane 5 = *mec*A and *PVL* positive; lane 6 = DNA marker (100 bp ladder); lane 7 = *mec*A and *PVL* positive; lane 8 = *PVL* positive; lane 9 = *mec*A and *PVL* positive; lane 10 = negative control for *mec*A and *PVL* gene; lane 11 = *mec*A and *PVL* negative sample; lane 12 = DNA marker.

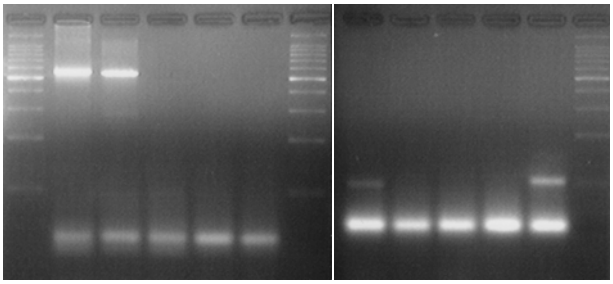

Figure 3. *bbp* gene and *ica*A gene were detected via multiplex PCR from *S. aureus* and *S. epidermidis* isolated from the suprapubic urines of infants. Lane 1 = DNA marker (100 bp ladder); lane 2 = positive control for *bbp* gene (575 bp); lane 3 = *bbp* and *ica*A positive sample; lane 4, 5, and 6 = *ica*A positive; lane 7 = DNA marker (100 bp ladder); lane 8 = positive control for *ica*A (103 bp); lane 9 = negative control for *ica*A; lane 10 = negative control for *bbp*; lane 11 and 12 = *ica*A positive; lane 13 = DNA marker.

Table 5. Prevalence of virulent genes detected in *S. epidermidis* from 22 infants and young children with fever by multiplex PCR

	UTI (n=3)	Suspicious UTI (n=1)	Non-UTI (n=22)	Total (n=26)
mecA[*] (presence)[1]	0 (0.0)	1 (50.0)	16 (94.1)	17 (81.0)
PVL (presence)[1]	1 (50.0)	2 (100.0)	12 (70.6)	15 (71.4)
bbp (presence)[2]	0 (0.0)	0 (0.0)	3 (16.7)	3 (13.6)
*ica*A (presence)[2]	1 (100.0)	3 (100.0)	9 (50.0)	13 (50.1)

[*] $P_{M\text{-}H} = 0.0009$

which *S. epidermidis* was detected, in the non-UTI group the *mec*A gene was quite highly (94.1%) detected ($P_{M\text{-}H}$ = 0.0009), but *mec*A was not detected in the UTI group (Fig. 2, 3 and Table 5).

DISCUSSION

Although the virulence factors from *S. aureus* have rarely been described in cases of UTI (3), the principal objective of this study was to determine the prevalence of *mec*A, PVL, *bbp* and *ica*A obtained from *S. aureus* and *S. epidermidis* isolated from presumed UTI specimens of infants in Seoul, Korea.

In this study, UTI was diagnosed with clinical and laboratory findings of ≥ 5 white blood cells/mm^3 and ≥ 10^4 CFU/ml pure bacterial growth in the culture (12) of infants' suprapubic urine. We identified 28 positive cases of *S. aureus* and 26 cases of *S. epidermidis* infection from 750 urine samples of suspected UTI cases. However, the prevalence of diagnosed UTI containing *S. aureus* (3/28) and *S. epidermidis* (2/26) was relatively low (Table 1). In this study, *S. aureus* was identified in 5/28 urine specimens, and *S. epidermidis* was identified in the urine of 1/26 cases, in which weak hematuria appeared. In addition, one of our tested patients (7 years, male) was in a terminal state of urinary tract infection.

Although the rate of detection of UTI by *S. aureus* and *S. epidermidis* was quite low in Korean infants, as was observed in this study, the frequency of *S. aureus* increased over time, corresponding to an increase in the occurrence of MRSA during 20 years of study (23). The rate of isolation of *S. aureus* in each of the years between 1998~2002 in Japan ranged from 6.6% and evidenced an increasing trend over the most recent 5 years ($p<0.05$) over 70 years of patient studies. Our results showed a 3 year-follow-up trend of prevalence of *S. aureus* (28/750, 3.7%), *S. epidermidis* (26/750, 3.5%) in febrile infants. Therefore, the isolated uropathogens evidenced greater diversification and a marked overall increase in isolated gram-positive bacteria, including MRSA, was observed. In this study, 10^2 CFU/ml colonies were counted, which corresponds to a high prevalence, but in UTI cases, the detection rate was low.

Our results demonstrated that the detection rate via multiplex PCR of *mec*A and PVL in *S. aureus* and *S. epidermidis* was significantly higher than that of the *bbp* and *icaA* genes (Tables 4 and 5). Our results showed *mec*A (16/26, 59.3%) and *ica*A (20/26, 76.9%) in *S. aureus*. In addition, *S. epidermidis* was found to contain *mecA* (17/22, 81.0%) and *icaA* (13/22, 50.1%). Those results were comparable to those of previous studies: Martineau *et al*. previously reported that 30.6% of *S. aureus* and 79.3% of *S. epidermidis* strains harbored the *mec*A gene (15). Our results showed a higher percentage of *mecA*, with a 59.3% rate in *S. aureus* and an 81.0% rate in *S. epidermidis*. The *ica*A and *mec*A genes were detected significantly more in the infected cases than in the contaminated cases ($p\leq0.02$), and this appeared to be associated with the potential virulence of *S. epidermidis* (21).

Nowadays MRSA has also emerged worldwide as a community acquired pathogen among postpartum women (22). The spread of PVL-positive *S. aureus* has also been described within families (18) and among healthcare staff. In our study, the high percentage of *mec*A and PVL genes detected herein was suspected to have been transmitted from family members and healthcare staffs to infants. The suspected reasons for this included that the chance for contact may have been limited because they were very young (less than 4 months) (Table 2 and 3).

PVL belongs to the recently described family of synergohymenotropic toxins (24). These toxins damage the membranes of host defense cells and two erythrocytes by the synergistic action of a non-associated class of secretory proteins designated S and F. Keneko *et al*. reported that PVL genes have been recently localized on a phage particle

that contains, in addition to Luk S-PV and Luk F, more than 60 potential open reading frames, some of which may play a participatory role in virulence (11). Owing to the presence of the PVL positive strains in hospitals, it is crucial to establish appropriate infection control measures to prevent their spread in the community and in hospitals (12). PVL damages the membranes of human polymorphonuclear cells and macrophages by forming pores in the membranes of leukocytes, resulting in an increase in membrane permeability and cell lysis (21).

The majority of the PVL positive isolates (96%) were MRSA, and all PVL positive MRSA isolates harbored the type IV SCC *mec* element (10), but we did not test the SCC *mec* element in this study. PVL-positive MRSA strains were associated with skin and soft tissue infections. Our results demonstrated that the *mec*A-positive cases of *S. aureus* and *S. epidermidis* were also PVL-positive (Table 4 and 5). PVL carriage is more common in MRSA than MRSE, particularly among community-acquired strains (1). Karahan *et al*. proposed that PVL positive *S. aureus* strains can cause infection in nearly every system without the need for additional risk factors (12).

Only genes encoding *bbp* were significantly associated with hematogenous osteomyelitis, arthritis, and endocarditis, thereby indicating their possible involvement in hematogeneous tissue infections (25). Our results showed that the rates of detection of the *bbp* gene in *S. aureus* (7/26, 26.9%) and *S. epidermidis* (3/22, 13.6%) were low. In this study, the lower *bbp* detection rate may be attributed to the fact that urine samples were used (Table 4 and 5). Otsuka *et al*. reported that the presence of *bbp* could confer to MRSA with a strong ability of adherence (colonization), reflecting a pandemic spread via skin-to-skin contact (19).

Synthesis of the capsular polysaccharide is mediated by the *ica*A operon. Upon the activation of this operon, a polysaccharide intracellular adhesion (PIA) is synthesized. This supports the notion that cell-to-cell bacterial contacts occur by means of a multilayered biofilm (2). Arciola *et al*. reported the role of *ica*A genes as a virulent marker in the pathogenesis of implant-associated orthopedic infections (2). Our results showed that *S. aureus ica*A genes (20/26, 76.9%) and *S. epidermidis ica*A genes (13/22, 50.1%) were detected at a high rate (Table 4 and 5). We believe that the *ica*A genes may enhance the adherence of *S. aureus* and *S. epidermidis* to host cells of the urinary tract, and may play a pathogenic role of UTI in febrile infants.

Our results demonstrated that community-acquired MRSA or MRSE virulence genes, especially *mec*A, PVL and *ica*A may be involved in UTI incidence in febrile infants.

REFERENCES

1) **Aires-de-Sousa M, Conceição T, de Lencastre H:** Unusually high prevalence of nosocomial Panton-Valentine leukocidin-positive *Staphylococcus aureus* isolates in Cape Verde Islands. *J Clin Microbiol* **44:** 3790-3793, 2006.

2) **Arciola CR, Campoccia D, Gamberini S, Donati ME, Baldassarri L, Montanaro L:** Occurrence of ica genes for slime synthesis in a collection of *Staphylococcus epidermidis* strains from orthopedic prosthesis infections. *Acta Orthop Scand* **74:** 617-621, 2003.

3) **Baba-Moussa L, Anani L, Scheftel JM, Couturier M, Riegel P, Haikou N, Hounsou F, Monteil H, Sanni A, Prévost G:** Virulence factors produced by strains of *Staphylococcus aureus* isolated from urinary tract infections. *J Hosp Infect* **68:** 32-38, 2008.

4) **Bahrain M, Vasiliades M, Wolff M, Younus F:** Five cases of bacterial endocarditis after furunculosis and the ongoing saga of community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Scand J Infect Dis* **38:** 702-707, 2006.

5) **Diep BA, Sensabaugh GF, Somboona NS, Carleton HA, Perdreau-Remington F:** Widespread skin and soft-tissue infections due to two methicillin-resistant *Staphylococcus aureus* strains harboring the genes for Panton-Valentine leucocidin. *J Clin Microbiol* **42:** 2080 -2084, 2004.

6) **Frebourg NB, Lefebvre S, Baert S, Lemeland JF:** PCR-based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *J Clin Microbiol* **38:** 877-880, 2000.

7) **Hoberman A, Chao HP, Keller DM, Hickey R, Davis HW, Ellis D:** Prevalence of urinary tract infection in

febrile infants. *J Pediatr* **123:** 17-23, 1993.

8) **Issartel B, Tristan A, Lechevallier S, Bruyere F, Lina G, Garin B, Lacassin F, Bes M, Vandenesch F, Etienne J:** Frequent carriage of Panton-Valentine leucocidin genes by *Staphylococcus aureus* isolates from surgically drained abscesses. *J Clin Microbiol* **43:** 3203-3207, 2005.

9) **Johnson LB, Venugopal AA, Pawlak J, Saravolatz LD:** Emergence of community-associated methicillin-resistant *Staphylococcus aureus* infection among patients with end-stage renal disease. *Infect Control Hosp Epidemiol* **27:** 1057-1062, 2006.

10) **Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, Bradley JS:** Rates of antimicrobial resistance among common bacterial pathogens causing respiratory, blood, urine, and skin and soft tissue infections in pediatric patients. *Eur J Clin Microbiol Infect Dis* **23:** 445-455, 2004.

11) **Kaneko J, Kimura T, Narita S, Tomita T, Kamio Y:** Complete nucleotide sequence and molecular characterization of the temperate staphylococcal bacteriophage phiPVL carrying Panton-Valentine leukocidin genes. *Gene* **215:** 57-67, 1998.

12) **Karahan ZC, Tekeli A, Adaleti R, Koyuncu E, Dolapci I, Akan OA:** Investigation of Panton-Valentine leukocidin genes and SCC*mec* types in clinical *Staphylococcus aureus* isolates from Turkey. *Microb Drug Resist* **14:** 203-210, 2008.

13) **Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J:** Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* **29:** 1128-1132, 1999.

14) **Manzur A, Dominguez AM, Pujol M, González MP, Limon E, Hornero A, Martín R, Gudiol F, Ariza J:** Community-acquired methicillin-resistant *Staphylococcus aureus* infections: an emerging threat in Spain. *Clin Microbiol Infect* **14:** 377-380, 2008.

15) **Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, Ouellette M, Bergeron MG:** Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* **44:** 231-238, 2000.

16) **Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA:** Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med* **355:** 666-674, 2006.

17) **O'Gara JP:** ica and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. *FEMS Microbiol Lett* **270:** 179-188, 2007.

18) **Osterlund A, Kahlmeter G, Bieber L, Runehagen A, Breider JM:** Intrafamilial spread of highly virulent *Staphylococcus aureus* strains carrying the gene for Panton-Valentine leukocidin. *Scand J Infect Dis* **34:** 763-764, 2002.

19) **Otsuka T, Saito K, Dohmae S, Takano T, Higuchi W, Takizawa Y, Okubo T, Iwakura N, Yamamoto T:** Key adhesin gene in community-acquired methicillin-resistant *Staphylococcus aureus*. *Biochem Biophys Res Commun* **346:** 1234-1244, 2006.

20) **Papanastasiou DA, Dimitracopoulos G, Drakou A, Haliotis F, Spiliopoulou I:** Significant bacteriuria in infants and young children and relation to bacterial species and pyuria. *Clin Microbiol Infect* **4:** 284-287, 1998.

21) **Prévost G, Mourey L, Colin DA, Menestrina G:** Staphylococcal pore-forming toxins. *Curr Top Microbiol Immunol* **257:** 53-83, 2001.

22) **Saiman L, O'Keefe M, Graham PL 3rd, Wu F, Saïd-Salim B, Kreiswirth B, LaSala A, Schlievert PM, Della-Latta P:** Hospital transmission of community-acquired methicillin-resistant *Staphylococcus aureus* among postpartum women. *Clin Infect Dis* **37:** 1313-1319, 2003.

23) **Shigemura K, Tanaka K, Okada H, Nakano Y, Kinoshita S, Gotoh A, Arakawa S, Fujisawa M:** Pathogen occurrence and antimicrobial susceptibility of urinary tract infection cases during a 20-year period (1983~2002) at a single institution in Japan. *Jpn J Infect Dis* **58:** 303-308, 2005.

24) **Supersac G, Prévost G, Piémont Y:** Sequencing of leucocidin R from *Staphylococcus aureus* P83 suggests that staphylococcal leucocidins and gamma-hemolysin are members of a single, two-component family of toxins. *Infect Immun* **61:** 580-587, 1993.

25) **Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G:** Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. *J Clin Microbiol* **41:** 4465-4467, 2003.

26) **Wang CC, Lo WT, Chu ML, Siu LK:** Epidemiological typing of community-acquired methicillin-resistant *Staphylococcus aureus* isolates from children in Taiwan. *Clin Infect Dis* **39:** 481-487, 2004.

27) **Wannet WJ, Spalburg E, Heck ME, Pluister GN, Tiemersma E, Willems RJ, Huijsdens XW, de Neeling AJ, Etienne J:** Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in The Netherlands. *J Clin Microbiol* **43:** 3341-3345, 2005.

28) **Witte W, Strommenger B, Cuny C, Heuck D, Nuebel U:** Methicillin-resistant *Staphylococcus aureus* containing the Panton-Valentine leucocidin gene in Germany in 2005 and 2006. *J Antimicrob Chemother* **60:** 1258-1263, 2007.