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

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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), 10^2~10^3 CFU/ml (1/28) and <10^2~10^3 CFU/ml (24/28). *S. epidermidis* (26 cases) counts were as follows: >10^4 CFU/ml (2/26), 10^2~10^3 CFU/ml (4/26) and 10^2~10^3 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, 77.3%), *PVL* (15/22, 71.4%), *bbp* (3/22, 13.6%) and *icaA* (13/22, 59.1%). Therefore, *mecA*, *PVL* and *icaA* 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*, *Staphylococcus epidermidis*, *mecA*, *PVL*, *bbp*, *icaA*

INTRODUCTION

Urinary tract infection (UTI) is a frequent and important problem in infants and young children with fevers (7,20).

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*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.
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 (icaA) 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 icaA 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 icaA—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°C. 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°C. 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°C. 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
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 mecA 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 bpb gene and icaA gene, we used the bpb primer set (bpb1, 5'-AAC TAC ATC TAG TAC TCA ACA ACA G-3'; bpb2, 5'-ATG TGC TTG AAT AAC ACC ATC ATC T-3') and obtained 575 bp of amplified product (25). The icaA primer set (icaA1, 5'-ACA GTC GCT ACG AAA AGA AA-3'; icaA2, 5'-GGA AAT GCC GTA ATG ACA AC-3') yielded an amplified product of 103 bp (2). The PCR products were stored at 4 °C and resolved via electrophoresis with 3.3% or 3.57% NuSieve agar gel and analyzed.

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 ~ 10^3 CFU/ml (1/28) and <10^2 ~ 10^3 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 ~ 10^3 CFU/ml (4/26) and 10^2 ~ 10^3 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 (PMH = 0.0108). When the UTI group was compared with the non-UTI group, the differences were not statistically significant (PMH = 0.74).

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

<table>
<thead>
<tr>
<th>Group (CFU/ml)</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-UTI (&lt;10^2)</td>
<td>24</td>
<td>85.7</td>
</tr>
<tr>
<td>Suspicious UTI (10^2~10^3)</td>
<td>1</td>
<td>3.6</td>
</tr>
<tr>
<td>UTI (≥10^4)</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100.0</td>
</tr>
</tbody>
</table>

PMH = 0.74
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) \( (P_{MH} = 0.0125) \).

The demographic features of the 26 cases in which *S. epidermidis* were detected showed that there were 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 *mecA* (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 *mecA* 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.
The objective of this study was to determine the prevalence of S. epidermidis isolated from presumed UTI specimens of infants in Seoul, Korea. 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 mecA, PVL, bbp and icaA 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/mm³ and ≥ 10⁴ 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, 10² 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 mecA 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 mecA (16/26, 59.3%) and icaA (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 mecA 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 icaA and mecA genes were detected significantly more in the infected cases than in the contaminated cases (p<0.02), 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 mecA 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

| 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) |
| icaA (presence)² | 1 (100.0) | 3 (100.0) | 9 (50.0) | 13 (50.1) |

¹ P<0.0009

which S. epidermidis was detected, in the non-UTI group the mecA gene was quite highly (94.1%) detected (P<0.0009), but mecA 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 mecA, PVL, bbp and icaA obtained from S. aureus and S. epidermidis isolated from presumed UTI specimens of infants in Seoul, Korea.

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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 _mecA_ 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 hematogenous 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 _icaA_ 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 _icaA_ genes as a virulent marker in the pathogenesis of implant-associated orthopedic infections (2). Our results showed that _S. aureus icaA_ genes (20/26, 76.9%) and _S. epidermidis icaA_ genes (13/22, 50.1%) were detected at a high rate (Table 4 and 5). We believe that the _icaA_ 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 _mecA_, PVL and _icaA_ may be involved in UTI incidence in febrile infants.

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