Community-associated *Staphylococcus aureus* infections and nasal carriage among children: molecular microbial data and clinical characteristics

G. Sdougkos¹, V. Chini², D. A. Papanastasiou³, G. Christodoulou¹, E. Stamatakis¹, A. Vris¹, I. Christodoulidi⁴, G. Protopapadakis¹ and I. Spiliopoulou²

¹Department of Orthopaedics, Karamandaneion Children’s Hospital, Patras, ²Department of Microbiology, School of Medicine, University of Patras, Patras, ³Department of Paediatrics, University Hospital of Patras and ⁴Department of Microbiology, Karamandaneion Children’s Hospital, Patras, Greece

**ABSTRACT**

An increasing number of infections caused by community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) carrying the Panton–Valentine leukocidin (PVL) genes was recently identified in Greece. In the present study, 170 patients with *S. aureus* infections and 123 uninfected children (<15 years old) who had been tested for nasal carriage were evaluated during a 2-year period. The MecA, PVL and superantigen family genes, and MRSA clones, were investigated by molecular methods. Sites of infection and laboratory findings for patients were recorded. The results were compared and statistically analysed. Among 123 uninfected children 73 (59%) carried *S. aureus*, including four MRSA strains. Of these, three MRSA and three methicillin-sensitive *S. aureus* (MSSA) strains were PVL-positive (p <0.0001). Ninety-six patients (96/170) exhibited skin and soft-tissue infections (SSTIs), and 74 exhibited invasive infections. The incidence of staphylococcal infections increased during July to September each year. In total, 110 *S. aureus* isolates were PVL-positive (81 from SSTIs and 29 from invasive infections, p <0.0001). Ninety-nine out of 106 MRSA (93%) isolates from 170 patients carried the PVL genes (p <0.0001); 97 belonged to the clonal complex CC80. Leukocytes and polymorphonuclear cell counts were higher among children with MRSA infections (p <0.005). MSSA predominated among patients with invasive infections (43/74), and carried mainly genes of the superantigen family. Children <5 years of age showed a higher risk of MRSA infection. The present study demonstrates that infections due to PVL-positive CA-MRSA spread easily among children, and SSTIs can lead to invasive infections. Nasal colonization may be an additional factor contributing to the emergence of CA-MRSA.

**Keywords** CA-MRSA, carriage, children, epidemiology, infections, *Staphylococcus aureus*

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**INTRODUCTION**

*Staphylococcus aureus* is the most frequent cause of skin and soft-tissue infections (SSTIs), as well as invasive infections, in adults and children [1]. Infections due to methicillin-resistant *S. aureus* (MRSA) in the absence of predisposing risk factors have been documented in the community, and referred to as community-associated MRSA (CA-MRSA) infections [2,3]. Reports have shown an increasing incidence of MRSA infections among children worldwide, including SSTIs, bacteraemias, and musculoskeletal infections [4–7].

Prevention of staphylococcal infections and reduction of the spread of CA-MRSA are of great importance. The association between *S. aureus* nasal carriage and infections has been established by identifying strains with the same genotype [8]. Genetic variations of strains reflect corresponding clinical manifestations. CA-MRSA isolates carry smaller staphylococcal cassette chromosome (SCCmec) elements (IV and V) than do hospital-associated strains, and therefore express a phenotype of greater antibiotic susceptibility [2,9,10].
In the majority of CA-MRSA isolates, the presence of the lukS-PV and lukF-PV genes encoding Panton–Valentine leukocidin (PVL), a pore-forming exotoxin, has been documented [2,9,11]. These strains usually cause SSTIs and necrotizing pneumonia [2,3,11–13].

The spread of infections caused by PVL-positive CA-MRSA in Greece is remarkable, similar to that in the USA [3,11,13,14]. Such strains are distributed in the community and hospital settings, and cause acute osteomyelitis among children [11,15].

In this report, CA-MRSA infections among children during a 2-year period are described. In parallel, _S. aureus_ nasal carriage was investigated, and the molecular characteristics of the isolated strains were set in relation to clinical and laboratory findings.

**MATERIALS AND METHODS**

**Description of studied cases**

In the present study, 170 children (<15 years of age) with _S. aureus_ infections proved by culture and available clinical and laboratory data (52% of total _S. aureus_ infections) during a 2-year period (2005–2006) were evaluated. Patients were admitted to the Department of Paediatrics of the University Hospital (95 patients) and Karamandaneion Children’s Hospital (75 patients) located in Patras, to which all children from western Greece are referred. These institutions have 65 and 90 paediatric beds, respectively, and receive c. 59 000 ambulatory visits and 9000 child admissions per year, covering an area of 1 200 000 inhabitants, roughly 12% of the total population of Greece.

White blood cell (WBC) counts, polymorphonuclear neutrophil (PMN) counts, erythrocyte sedimentation rates and C-reactive protein (CRP) were determined in the studied patients upon admission. Depending on the site, cultures were performed for all children with suspected ongoing infection accordingly. Invasive infections included osteomyelitis, septic arthritis, deep-seated wound infections, lymphadenitis, otitis, pyomyositis, deep abscesses involving organs and structures other than skin and subcutaneous tissues, urinary tract infections, pneumonia, and bacteremia; SSTIs included cellulitis, pyoderma, superficial wound infections, boils, conjunctivitis, paronychia, impetigo and neonatal omphalitis. A case was considered as community-associated or hospital-associated according to CDC definitions [16]. The presence of known risk factors for MRSA was assessed from a questionnaire completed by the child’s accompanying care-giver.

In parallel, _S. aureus_ nasal carriage was investigated among 123 healthy uninfected children of similar gender and age attending primary-care outpatients’ facilities for a regular checkup (with permission of the accompanying care-giver). Nasal swabs from both anterior nares were placed in Stuart transport medium, inoculated into Tryptic Soy Broth (TSB, BBL, Becton Dickinson, Le Pont de Claix, France) and incubated at 37°C for 18 h. Samples were subcultured onto mannitol-salt agar (BBL, Becton Dickinson) and further incubated at 37°C for 48 h [17]. Yellow colonies were tested for coagulase production by the Slidex Staph Plus agglutination test (bioMérieux, RCS Lyon, France) and other standard methods [18].

**Phenotypes of bacterial isolates**

_S. aureus_ isolates originated from different patients with signs of infection (one isolate per patient). Antibiotic susceptibility testing was performed by the disk diffusion method with cefoxitin, vancomycin, kanamycin, gentamicin, tobramycin, netilmicin, erythromycin, clindamycin, ciprofloxacin, sulphamethoxazole–trimethoprim, linezolid and fusidic acid (BBL, Becton Dickinson) [19,20]. β-Lactamase production was tested in all isolates (including those from uninfected children) with nitrocefin disks (BBL, Becton Dickinson), and inducible resistance to clindamycin with the D-test [19]. The MIC of oxacillin was determined with the Etest (AB Biodisk, Solna, Sweden) [21].

**Molecular typing**

The presence of the _mecA_ gene encoding resistance to methicillin, and the identity of the PVL genes _lukS-PV_ and _lukF-PV_, of _tst_ (encoding toxic shock syndrome toxin-1), _eta_ and _etb_ (encoding exfoliative toxins A and B) and of _seg_ and _sem_ of the enterotoxin gene cluster, were studied using PCR with specific primers [9,10,22,23]. MRSA clones were defined on the basis of pulsed-field gel electrophoresis (PFGE) patterns of _Smal_-digested DNA [11,24] and _agr_ typing [25]. Representative strains based on PFGE/ _agr_ type and toxin gene profile were selected, further characterized by multilocus sequence typing [24,26] and compared to previously identified clones [11,13,15]. Methicillin-sensitive _S. aureus_ (MSSA) clones were identified by PFGE of _Smal_ DNA digests.

**Statistical analysis**

Statistical analyses were conducted using the _spss_ v.12.0 software package for Windows (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as percentages and quantitative data as mean value (± standard deviation or ± standard error of the mean). Age was expressed as median (intraquartile range (IQR)) because values were skewed. Patients were stratified into two age groups, younger or older than 60 months. Differences between groups were calculated by the unpaired _t_-test or Mann–Whitney _U_-test (non-parametric) as appropriate for quantitative data, and the chi-squared test was used for categorical data. In addition, ORs and 95% CIs were calculated. A probability value <0.05 was considered to be significant.

**RESULTS**

**Characteristics of patients**

The increased incidence of _S. aureus_ infections accounted for the majority of admissions in both the Department of Paediatrics of the University Hospital and the Karamandaneion Children’s Hospital during recent years. More specifically,
the percentages of S. aureus infections among the total number of hospitalized children were 0.9% in 2004, 1.03% in 2005, and 1.4% in 2006, respectively. The total numbers of patients with MRSA among all those with S. aureus infections, including patients from both institutions, were 96/181 (53%) in 2005 and 126/178 (71%) in 2006. Community-onset MRSA infections were attributed to 62% of the studied cases (105/170), whereas, only one MRSA case was hospital-associated. The monthly distribution of both MRSA-infected and MSSA-infected children, as well as the cases studied during 2005 and 2006, are shown in Fig. 1. There was a distinct seasonal distribution of staphylococcal infections, which occurred mainly between July and September during both years of the study. Among the 170 studied patients (90 in 2005 and 80 in 2006), 114 occurred mainly between July and September. The monthly distribution of both MRSA-infected and MSSA-infected cases during both years of the study, as well as the cases studied during 2005 and 2006, is shown in Fig. 1. There was a distinct seasonal distribution of staphylococcal infections, which occurred mainly between July and September during both years of the study. Among the 170 studied patients (90 in 2005 and 80 in 2006), 114 occurred mainly between July and September.

Ninety-six patients had SSTIs, including cellulitis (25), pyodermia (23), superficial wound infections (18), boils (eight), conjunctivitis (eight), paronychia (six), impetigo (five) and neonatal omphalitis (three). Laboratory blood examination of patients with invasive infections, when compared to SSTIs, revealed that only CRP values (mean value ± standard error of the mean) were statistically significant (4.0 ± 0.74 vs. 2.0 ± 0.32, p 0.0071).

Whereas the median age of MRSA-infected children was 30 months (IQR 15–60 months), that of the MSSA-infected group was 27 months (IQR 5–96 months, p 0.61). The incidence of MRSA infections in children younger than 60 months was 72%, and among older patients it was 52% (p 0.027; OR = 2.26; 95% CI = 1.09–4.71). The majority of MRSA infections (75/106 or 71%) were SSTIs, whereas MSSA predominated in invasive infections (43/64, 67%) (Table 1). A statistically significant difference was calculated for WBC and PMN counts among MRSA-infected patients as compared to the MSSA-infected group (Table 1). The differences in erythrocyte sedimentation rates and CRP levels were not statistically significant (p >0.05).

Characteristics of S. aureus strains

MRSA isolates were classified into four clones according to their PFGE/agr and MLST types (Table 2). The majority of PVL-positive isolates

Table 1. Clinical and laboratory findings for the 170 studied patients with Staphylococcus aureus infections

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with MRSA infection (n = 106)</th>
<th>Patients with MSSA infection (n = 64)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep-seated infections (%)</td>
<td>31 (29%)</td>
<td>43 (67%)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>SSTIs (%)</td>
<td>75 (71%)</td>
<td>21 (33%)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>WBC count (mean ± SD)</td>
<td>1453 ± 5427</td>
<td>11430 ± 3981</td>
<td>0.0017*</td>
</tr>
<tr>
<td>PMN count (mean ± SD)</td>
<td>843 ± 453</td>
<td>6055 ± 3541</td>
<td>0.0042*</td>
</tr>
</tbody>
</table>

*Chi-squared test.

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Table 2. Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from the studied patients and nasal carriers

<table>
<thead>
<tr>
<th>MRSA</th>
<th>PFGE/age</th>
<th>MLST (CC): number of strains</th>
<th>PVL</th>
<th>tst</th>
<th>sem</th>
<th>seg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studied patients (n = 106)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSTIs (n = 75)</td>
<td>C/3</td>
<td>ST80 (CC80): 71</td>
<td>71</td>
<td>–</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>A/3</td>
<td>ST30 (CC30): 2</td>
<td>–</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>G/1</td>
<td>ST377: 1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Deep-seated infections (n = 31)</td>
<td>C/3</td>
<td>ST239 (CC8): 1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>A/3</td>
<td>ST30 (CC30): 26</td>
<td>26</td>
<td>–</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>G/1</td>
<td>ST30 (CC30): 4</td>
<td>–</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total patients</td>
<td>C/3</td>
<td>ST80 (CC80): 3</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nasal carriers (n = 4)</td>
<td>A/3</td>
<td>ST30 (CC30): 1</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

SSTIs, skin and soft-tissue infections; PFGE, pulsed-field gel electrophoresis; MLST (CC), multilocus sequence types and clonal complexes.

*p-value*: Clones defined by PFGE and *agr* types.

(97) and three additional PVL-positive MRSA isolates from nasal carriers belonged to clonal complex CC80 (ST80). Two additional isolates were of the ST377 type. Six *tst*-positive MRSA isolates from patients and one *tst*-negative isolate from an uninfected child belonged to ST30 (CC30), whereas the unique hospital-associated strain belonged to ST239 (CC8). The antibiotic resistance profiles of 110 MRSA isolates were clone-related. All CC80 isolates (100) were resistant to kanamycin and fusidic acid, six were also resistant to erythromycin, and three of the latter were additionally resistant to clindamycin. ST377 strains (two) were resistant to kanamycin, tobramycin and gentamicin. CC30 isolates (seven) were resistant only to β-lactams, whereas the single CC8 isolate was resistant to kanamycin, gentamicin, erythromycin, clindamycin, ciprofloxacin, fusidic acid and sulphonamethoxazole–trimethoprim. Among MSSA isolates (64), 17 PFGE types were identified, including type A, which was also detected among MRSA. Eleven MSSA isolates were resistant to erythromycin, of which six were also resistant to clindamycin, two were also resistant to gentamicin, and three were also resistant to fusidic acid. Six of ten clindamycin-resistant isolates showed inducible resistance. Fifty-four of 64 MSSA isolates (84%) and 102 of 106 MRSA isolates (96%) produced β-lactamase (p 0.0149).

Ninety-nine of 106 MRSA isolates from the studied patients carried the PVL genes, exhibiting a statistically significant difference as compared to MSSA isolates (93% vs. 17%, p <0.0001) (Table 3). However, MSSA isolates carried, at higher frequencies, genes of the superantigen family (*tst*, *seg*, *sem*) (Table 3). The presence of PVL was associated with six MSSA clones, whereas *tst* was associated with seven clones, including PFGE type A (CC30), which was also detected among *tst*-positive MRSA isolates. *S. aureus* isolates from uninfected children carried *seg* at a higher frequency (Table 3). No *S. aureus* isolates carried exfoliative toxin genes.

The presence of PVL in clinical isolates was strongly correlated with SSTIs (81/96 or 84.4% vs. 39.2%, p <0.001), whereas the presence of superantigen genes, mainly *tst*, was correlated with invasive infections (13/74 or 17.6% vs. 4.2%, p 0.0085). The combined presence of *sem*/*seg*, as

Table 3. Comparative gene content in *Staphylococcus aureus* isolates from patients and nasal carriers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patients</th>
<th>Total patients (n = 170), n (%)</th>
<th>S. aureus nasal carriers (n = 73), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA (total n = 106), n (%)</td>
<td>MSSA (total n = 64), n (%)</td>
<td>p-value*</td>
</tr>
<tr>
<td>mzaA</td>
<td>106 (93.4)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PVL</td>
<td>99 (93.4)</td>
<td>11 (17.2)</td>
<td>–</td>
</tr>
<tr>
<td>tst</td>
<td>5 (7.3)</td>
<td>37 (57.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sem</td>
<td>5 (4.7)</td>
<td>29 (45.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>seg</td>
<td>11 (10.4)</td>
<td>41 (64.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PVL and sem</td>
<td>1 (0.94)</td>
<td>6 (9.4)</td>
<td>0.0225</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; PVL, Panton–Valentine leukocidin.

*p-value*: Chi-squared test.
well as PVL/sem/seg, between the groups was statistically insignificant. Treatment was performed according to antibiotic susceptibility tests. Patients with invasive infections received intravenous therapy, whereas most SSTI patients were treated by mouth. Surgical intervention was undertaken for all patients with abscesses, septic arthritis, deep-seated wound infections, pyomyositis, and boils, as well as in 12 cases of osteomyelitis. All patients recovered after treatment.

### Characteristics of uninfected children

Among the 123 uninfected children (71 boys and 52 girls, median age 54 months, IQR 14–120 months), 73 were nasal *S. aureus* carriers (59%). Four isolates were MRSA (5.5%), of which three carried PVL genes. The same genes were found in only three of the remaining 69 MSSA isolates (p <0.0001). The presence of superantigen genes was statistically insignificant (p >0.05).

### DISCUSSION

A marked increase in *S. aureus* infections among children, especially those due to CA-MRSA, is demonstrated in the present study. With the exception of one case of hospital-associated MRSA, all cases in the study were community-associated. Identification of MRSA is no longer limited to previous hospitalization or predisposing risk factors [2,3,9]. In western Greece, total MRSA infections involving both children and adults have increased, and were calculated to be 45% and 49% during 2005 and 2006, respectively. Among children, MRSA constituted 53% and 71% of total *S. aureus* infections during 2005 and 2006, respectively. This phenomenon may be due to an increase in MRSA colonization among children, and is in agreement with findings of other investigators, who have identified higher rates of CA-MRSA among children than among adults (69.8% vs. 58.5%) [27]. In this study, children <5 years of age have more than a two-fold increased risk of MRSA infection as compared to older children (OR = 2.26). The predominance of staphylococcal infections in boys has not as yet been explained. The monthly distribution of *S. aureus* infections during two consecutive years showed a pronounced increase during the hot season. MRSA nasal carriage and cases of osteomyelitis involving increased rates of hospital admission in late summer and autumn have been reported among Japanese children [28,29].

The most frequent site of *S. aureus* colonization is the anterior nares, although it can be found elsewhere on the body [8]. Usually, nasal carriage is high in newborns, showing a gradual reduction with age [8]. A relationship between *S. aureus* nasal carriage and infection was proved by genotypic analyses of isolated strains [8]. The high percentage (59%) of *S. aureus* nasal carriers in the present study, including 5.5% of MRSA carriers, correlates with the younger age of uninfected children. In a study performed among healthy Japanese children, 28.2% were nasal *S. aureus* carriers, of whom 4.3% were colonized by PVL-negative MRSA [28].

SSTIs constituted the majority of MRSA cases among studied patients. However, pneumonia, bacteraemia, osteomyelitis, deep-seated wound infections and abscesses were also identified to a lesser extent, as similarly reported elsewhere [2,3,12]. Invasive infections were mainly associated with MSSA isolates. This is in agreement with a study among children in Taiwan, where CA-MSSA isolates were three times more likely to be associated with invasive infections [6]. MRSA infections do not differ in severity or outcome from those caused by MSSA, and it has not yet been proved unequivocally that they are more virulent than those caused by MSSA [30]. In the present study, MSSA isolates mostly carried genes of the superantigen family (*tst, sem, seg*) that were not clone-related. A high percentage of superantigen gene carriage was observed in previous studies, even among nasal *S. aureus* isolates (57% for *seg* and 11% for *tst*) [23]. Even though no case of toxic shock syndrome was diagnosed among the studied patients, carriage of superantigen genes, combined with predominance of invasive infections, poses the likelihood of more severe clinical manifestations among MSSA-infected children.

Molecular analysis revealed a relatively high percentage of PVL-positive MSSA isolates (17%) distributed among different clones, as compared to other studies (6.7% of consecutive MSSA isolates) [14]. Taken together, these results may lead to the conclusion that horizontal transfer of lukS-PV and lukF-PV is continuous in the *S. aureus* population, as they are phage-borne [2,3]. On the other hand, a predominance of PVL-positive isolates was observed among MRSA (93%).
specifically, 97 of 99 isolates belonged to the European clone CC80, which has already spread in Greece and other European countries [2,3,11,15]. The three PVL-positive MRSA isolates from carriers also belonged to CC80, providing a possible source for future endogenous infections or intrafamilial spread of such strains. Clonal spread was also shown in a study performed among 446 children in Philadelphia, with a notable increase in CA-MRSA, the majority of isolates belonging to a common PVL gene-positive clone [31]. Classification of the remaining two PVL gene-positive MRSA isolates as members of ST377, a clone recently identified in France and Greece [12,13], reinforces the possibility of parallel horizontal gene transfer. The statistically significant differences concerning WBCs and PMNs among MRSA-infected patients, as compared to those infected with MSSA, may be due to the predominance of PVL-producing isolates in this bacterial population. We have recently shown that osteomyelitis cases were caused by PVL gene-positive S. aureus strains [15].

The paediatric population studied was treated by mouth or intravenously, with or without surgical intervention, according to the severity of infection and the antibiotic susceptibility pattern of the isolated strain. CA-MRSA isolates express heterogeneous resistance to oxacillin, with lower MICs of the antibiotic, which accounts for improvement after treatment with β-lactams [6]. Since the lately encountered spread of CA-MRSA in Greece, most physicians re-adjust treatment according to antibiotic susceptibility tests, despite the low MICs of oxacillin. Vancomycin is reserved for severe hospital-acquired infections, whereas erythromycin, clindamycin and sulphonamethoxazole–trimethoprim are suitable oral antibiotics for MRSA infections, as reported elsewhere [27,32].

In the present study, a seasonal distribution of staphylococcal infections was shown, with an increasing incidence of CA-MRSA among younger children, implying that this may be due mainly to clonal spread of PVL-positive isolates. The genotypes of MRSA clones identified in S. aureus from nasal carriers correlated well with those of patient isolates. The majority of CA-MSSA isolates were associated with invasive infections, and carried predominantly genes of the superantigen family. Successful treatment of S. aureus infections in children requires epidemiological data for the country, in combination with information on genetic profiles and antibiotic susceptibility patterns.

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TRANSPARENCY DECLARATION

The authors have no conflict of interest to declare.

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