Changing epidemiology of methicillin-resistant *Staphylococcus aureus* colonization in paediatric intensive-care units

C. R. HERMOS1*, T. J. SANDORA2,3, L. E. WILLIAMS2, N. MOSAMMAPARAST4 AND A. J. MCDADAM2

1 Division of Paediatric Immunology and Infectious Diseases, UMass Memorial Children’s Medical Center, Worcester, MA, USA
2 Department of Laboratory Medicine, Children’s Hospital Boston, Boston, MA, USA
3 Division of Infectious Diseases, Children’s Hospital Boston, Boston, MA, USA
4 Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, USA

Received 26 July 2012; Final revision 11 October 2012; Accepted 15 October 2012; first published online 29 November 2012

SUMMARY

Community-associated methicillin-resistant *S. aureus* (CA-MRSA) accounts for a growing proportion of hospital-onset infections, and colonization is a risk factor. This study aimed to determine changes in the prevalence of CA-MRSA colonization in paediatric intensive-care units (ICUs). A total of 495 paediatric patients colonized with MRSA from neonatal, medical, surgical, and cardiac ICUs between 2001 and 2009 were identified. Isolates were characterized by *spa* type, staphylococcal cassette chromosome (*SCC*) *mec* type and the presence of the genes encoding Panton–Valentine leukocidin (PVL). The proportion of patients colonized with MRSA remained stable (average 3.2%). The proportion of isolates with *spa* type 1, *SCCmec* type IV and PVL increased over time to maximums in 2009 of 36.1% (*P* < 0.001), 54.2% (*P* = 0.03) and 28.9% (*P* = 0.003), respectively. Antibiotic susceptibility patterns showed increasing proportions susceptible to clindamycin, gentamicin, tetracycline and trimethoprim-sulfamethoxazole (*P* values <0.001). In conclusion, the proportion of MRSA-colonized children in ICUs with CA-MRSA increased significantly over time.

Key words: Colonization, infection control, intensive-care units, methicillin-resistant *Staphylococcus aureus*, paediatrics, staphylococcus.

INTRODUCTION

*Staphylococcus aureus* is a leading cause of hospital-onset infections, and the proportion of such infections caused by methicillin-resistant *S. aureus* (MRSA) is increasing in the USA and internationally [1, 2]. Colonization with MRSA is a risk factor for the development of hospital-onset MRSA infections [3, 4]. The Society for Healthcare Epidemiology of America recommends that hospitals implement routine active surveillance cultures to identify MRSA colonization for patients at high risk for colonization and hospital-onset infections [5], which often includes patients in intensive-care units (ICUs). One goal of identifying MRSA colonization is to ensure that colonized patients are cared for using contact precautions to...
minimize the transmission of MRSA, as recommended by the Centers for Disease Control and Prevention [6].

Although MRSA was once mainly a cause of nosocomial infections, it is now an important cause of community-associated infections. Community-associated MRSA (CA-MRSA) causes severe skin and soft tissue infections [7], necrotizing pneumonias [8], and other invasive infections in patients without healthcare exposures. Colonization is likely to be an important risk factor for MRSA infections, and in the USA, MRSA nasal carriage rates in children in the community range from 0.8% to 2.5% [9–12], although a marked rise from 0.8% to 9.2% over 3 years was found in children in Nashville, Tennessee [13]. The relationship between CA-MRSA and hospital-associated (HA)-MRSA infections and specific strains of MRSA is complex, but CA-MRSA infections were initially associated with strains that are genetically distinct from HA-MRSA [14]. However, recent reports show that genetically typed CA-MRSA strains are increasingly responsible for hospital-onset infections [15–20].

Various molecular typing methods can be used to identify genetically defined CA-MRSA strains. Pulsed-field gel electrophoresis (PFGE) classifies S. aureus into pulsed field types, including USA300, the most common CA-MRSA strain in the USA [7, 21]. Sequencing of the short sequence repeat region of the protein A gene (spa) provides a high level of discrimination between MRSA strains, comparable to PFGE [22]. Spa typing also has the advantages of speed, ease and objective interpretation, making it a useful tool for hospital investigations [23, 24]. Importantly, the USA300 strain is spa type 1 (Ridom type t008), thus allowing the use of spa typing to detect the dominant CA-MRSA genotype [14, 25]. Other phenotypic and genotypic characteristics of CA-MRSA strains include susceptibility to non-β-lactam antibiotics and carriage of genes for staphylococcal cassette chromosome (SCC) mec type IV and Panton–Valentine leukocidin (PVL) [26].

Recent reports describe an increased prevalence of CA-MRSA colonization in neonatal ICUs (NICUs) [25, 27–30]. The purpose of this study was to determine if the prevalence of MRSA colonization has increased in all paediatric ICUs at Children’s Hospital Boston (CHB), and whether the proportion of colonizing MRSA that are CA-MRSA strains has increased. Routine active MRSA surveillance cultures were collected from patients in the ICUs at CHB since 2001. We report the prevalence of MRSA colonization, as well as the distribution of spa types, antibiotic susceptibilities, SCCmec types and proportion of PVL-positive isolates in colonizing MRSA strains cultured from children admitted to our paediatric ICUs from 2001 to 2009.

METHODS
Setting
CHB is a 396-bed quaternary care paediatric hospital with four ICUs, including a NICU, medical/surgical ICU (MSICU), and cardiac ICU (CICU), as well as a medical ICU (MICU) that opened in 2008. In 2001, the Infection Prevention and Control Program recommended that all patients be screened by culture of the nares for MRSA colonization upon admission to any ICU, and weekly thereafter during the remainder of their ICU stay. Patients colonized with MRSA were placed on contact precautions; no decolonization procedures were undertaken routinely. The CHB Committee on Clinical Investigation approved this study.

Study design and subjects
We performed a retrospective cohort study of all patients found to be colonized with MRSA during an admission to a CHB ICU between 1 January 2001 and 31 December 2009. The prevalence of MRSA colonization and antibiotic susceptibilities and molecular profiles of isolates were determined. Colonization was defined as an isolate obtained from a MRSA surveillance culture (nares, skin, throat) or bone marrow transplant surveillance culture (nares, throat, rectum). While MRSA surveillance cultures from the nares of ICU patients were obtained according to infection control policy, other surveillance cultures were ordered at the discretion of a treating physician.

Data collection
Microbiology laboratory records were reviewed to find MRSA surveillance and bone marrow transplant surveillance culture results from patients in ICUs. Chart reviews were performed to ascertain antibiotic susceptibilities and patient demographics. To analyse trends in antibiotic susceptibilities and molecular characteristics, we included the first colonizing MRSA isolate from each patient cultured during an ICU admission within the study period.
Microbiological methods

Cultures were processed using standard microbiological methods [31]. From January 2001 to May 2006, samples were plated on mannitol-salt agar and 5% sheep’s blood agar. From May 2006 onwards, Chromagar MRSA agar (BD Diagnostic Systems, USA) was used. Colonies with morphology consistent with S. aureus were identified by Gram stain, catalase test and coagulase test. Isolates were tested for antibiotic susceptibilities using the Vitek system (bioMérieux, USA), except for susceptibilities for trimethoprim-sulfamethoxazole (TMP/SMX), which were performed using the disk diffusion method until this test became available for Vitek. Between October 2002 and November 2003, TMP/SMX susceptibilities were not performed. D-tests were performed to detect erythromycin-inducible clindamycin resistance on 252/255 clindamycin-susceptible, erythromycin-resistant isolates using standard methods [32], and isolates with inducible clindamycin resistance were reported as clindamycin resistant.

Beginning 1 January 2001, the microbiology laboratory began storing the first MRSA isolate cultured from each patient (whether from surveillance or clinical cultures). If the patient’s stored MRSA isolate was different from his/her ICU MRSA surveillance culture, we included the stored isolate for molecular analysis if it was collected ≤1 month prior to the positive surveillance culture.

Genomic DNA was purified from cultures using a QIAamp DNA Mini kit (Qiagen, USA). The presence of the mecA gene was confirmed by polymerase chain reaction (PCR) on each isolate. SCCmec typing was performed by multiplex PCR [33]; the presence of PVL-encoding genes (lukS/lukF) was determined by PCR [34]; and spa typing was performed by PCR followed by sequencing of the spa gene with previously described protocols [24]. The spa gene PCR product was purified using a QIAquick PCR Purification kit (Qiagen). Sequences were analysed on the eGenomics software platform (www.egenomics.com), which provided a Kreiswirth repeat pattern and spa type. The Kreiswirth repeat pattern was then analysed by the Ridom software (spaserver, ridom.de) to determine the Ridom spa type [23]. All spa types with a Kreiswirth repeat pattern reported as ‘new’, and Ridom spa types reported as ‘unknown’, were re-sequenced for confirmation. MRSA strains with spa type 1 were determined as representing CA-MRSA.

Statistical analysis

Total and annual prevalences of MRSA colonization in the ICUs were calculated as the number of patients with at least one positive result in the given year divided by the total number of patients screened that year. Trends in the prevalence of MRSA colonization and the prevalence of spa types, SCCmec types II and IV, PVL-encoding genes and specific antibiotic susceptibilities of MRSA isolates over time were determined using the Cochran–Armitage trend test. Analyses were performed using SAS version 9.1 (SAS Institute Inc., USA).

RESULTS

Prevalence and demographics of MRSA carriage

From 1 January 2001 to 31 December 2009, 15295 patients were screened for colonization with MRSA, where each patient was counted once per year, and 495 (3.2%) were found to be colonized. The total number of surveillance cultures performed and yielding MRSA increased each year (Fig. 1). Total yearly ICU admissions increased by 27% over the study period, from 4033 to 5104. The proportion of screened patients who were MRSA colonized did not change significantly over time (P = 0.82). Males accounted for 53.1% of all MRSA-colonized children, and the proportion of males did not change significantly over time. The median age of MRSA-colonized patients was 2 years. Patients aged <1 year, 2–4 years, 5–12 years, 13–18 years and >18 years accounted for 42%, 18%, 19%, 13%, and 8% of MRSA-colonized patients, respectively. Of colonized children, 116 (23.4%) were NICU patients. The proportion of patients found to be colonized with MRSA each year who were aged <1 year decreased over time (P < 0.001), while the proportions in age groups 2–4, 5–12 and 13–18 years, increased over time (P = 0.02, 0.03, 0.04 respectively); the proportion aged >18 years did not change significantly over time.

Antibiotic susceptibility of colonizing MRSA

For susceptibility and molecular analysis, 42 cultures obtained in subsequent years from previously positive patients were excluded. Of the remaining 453 MRSA cultures, two susceptibility panels were not available, so we reviewed antibiotic susceptibilities of 451 MRSA cultures.
The susceptibility of colonizing MRSA isolates to several antibiotics changed significantly over time (Table 1). The proportion of isolates susceptible to gentamicin, tetracycline and TMP/SMX each increased between 2001 and 2003 and then remained stable, while susceptibility to clindamycin increased over the study period. The proportion of isolates resistant to clindamycin with inducible resistance by D-test increased significantly over the study period. The proportion of isolates susceptible to erythromycin did not change significantly. All isolates were susceptible to vancomycin.

Molecular analysis and spa typing of colonizing MRSA

Of the 453 unique isolates, 394 (87.4%) isolates were available for molecular testing because 59 isolates were not stored. Six of the 394 isolates tested were non-surveillance cultures obtained within 1 month of the surveillance MRSA isolate (three sputum, one blood, two wound). As expected, all isolates were positive for the meca gene. The majority of the MRSA isolates contained SCCmec type II (54.3%) or type IV (42.6%). Other SCCmec types identified included type I (n = 4, 1.0%) and type VI (n = 3, 0.8%); five (1.3%) isolates had an indeterminate SCCmec type. Over the 9-year period, the proportion of MRSA isolates carrying SCCmec type II decreased (P = 0.06), and the proportion carrying SCCmec type IV increased (P = 0.03), but these trends appear to begin in 2003, following 2 years in which only a small number of isolates were available for testing (Fig. 2).

The proportion of MRSA isolates carrying the PVL-encoding genes increased significantly over the study period (P = 0.003) (Fig. 3). The proportion carrying PVL genes was 21.1% overall and ranged from 0% in 2003 to 28.9% in 2009.

There were 70 different spa types in the 394 isolates analysed (see Supplementary Table S1 which lists all the spa types identified in this cohort). Forty-seven spa types were each found in a single isolate and 23 spa types were identified in more than one isolate. Spa types 2 and 1 were the most common, accounting for 182 (46.2%) and 85 (21.6%) isolates, respectively (Table 2). Of all MRSA isolates, the proportion with spa type 1 increased significantly (P < 0.001), while the proportion with spa type 2 remained stable (P = 0.21) and the proportion with other spa types decreased over the study period (P = 0.03). The proportion of MRSA-colonized patients with spa type 1 increased significantly in the NICU (P = 0.03) and MSICU (P = 0.02), and trended towards an increase in the CICU (P = 0.12, data not shown). In 2009 the proportion of MRSA isolates with spa type 1 in the NICU, MSICU, CICU and MICU were 27.3%, 33.3%, 31.8% and 52.9%, respectively.

An analysis of the SCCmec type and presence of the genes for PVL was performed for the specific spa types (Table 2). Nearly all spa type 1 isolates were SCCmec type IV, and most were PVL positive. In contrast, most spa type 2 isolates had SCCmec type II and nearly all were PVL negative. The majority of isolates with spa types other than 1 or 2 were SCCmec II or IV in roughly equal proportions, and most were PVL negative. Isolates of the same spa type, other
DISCUSSION

The increasing prevalence of MRSA spa type 1 in patients colonized with CA-MRSA in paediatric ICUs at CHB indicates that CA-MRSA colonization increased in our study population, and the increased prevalence of SCCmec type IV and PVL is seen most clearly from 2003 on, preceded by an apparent fall in these numbers from 2001 and 2002. It is difficult to interpret the data from 2001 and 2002, as only a small number of isolates (f15) were available from each of these years. Compared to a recent survey of adult ICUs, our paediatric cohort had a lower rate of MRSA colonization overall (3.2% vs 8.11%), but a much higher proportion of colonizing MRSA were CA-MRSA strains (36.2% in 2009 vs. 12.2%). The increasing prevalence of MRSA spa type 1 is consistent with other reports that CA-MRSA infection, and in particular USA300, has become more common in healthcare settings [17–19, 22, 27–30]. These reports describe an increasing number of hospital-onset bloodstream infections [17], surgical site infections [19], and infections in NICU patients with CA-MRSA strains [25, 28–30]. MRSA colonization is a precursor for hospital-onset infection, especially in patients with central venous catheters and endotracheal tubes, devices that are often necessary for ICU patients [3–4]. The increased prevalence of CA-MRSA infection in the paediatric ICU population might therefore increase the risk for hospital-onset CA-MRSA colonization in hospital settings [18–30].

CA-MRSA colonization in paediatric ICUs 1987–2009

than 1 or 2, carried the same SCCmec type with the exception of isolates with spa types 12, 14, 17 and 19 which included isolates with SCCmec type II and IV.
that the programme resulted in the identification of a larger number of colonized patients, and a growing number of patients colonized with CA-MRSA who very likely would not have been identified in the absence of a universal screening programme.

Previous studies have reported that CA-MRSA is commonly found to colonize and infect infants in NICUs [25, 27–30]. Our study adds to these findings by documenting colonization trends in children admitted to paediatric medical/surgical and cardiac ICUs over a 9-year timeframe. Two studies of NICU settings also described increasing prevalence of CA-MRSA colonization over time, but these studies differ from ours in several important ways [25, 30]. First, these studies included MRSA isolates obtained primarily during outbreak investigations, while our isolates were obtained from routine active surveillance cultures. Second, while both of these studies reported total proportions of isolates with PVL and specific SCC\textit{mec} types, we have also documented increasing prevalence of PVL and SCC\textit{mec} type IV in colonizing MRSA isolates over time.

Recently, Milstone \textit{et al.} reported a prevalence of MRSA colonization of 6\% in children admitted to

---

**Fig. 2.** Proportion of methicillin-resistant \textit{Staphylococcus aureus} isolates from colonized children in intensive-care units between 2001 and 2009 carrying SCC\textit{mec} types II and IV.

![Graph showing SCC\textit{mec} types II and IV](image)

**Fig. 3.** Proportion of methicillin-resistant \textit{Staphylococcus aureus} isolates from colonized children in intensive-care units between 2001 and 2009 carrying genes that encode Panton–Valentine leukocidin.
Table 2. Panton-Valentine leukocidin status and SCCmec types of methicillin-resistant Staphylococcus aureus isolates with spa types 1, 2 and other spa types from colonized patients in paediatric intensive-care units

<table>
<thead>
<tr>
<th>Year</th>
<th>Total no. typed</th>
<th>Spa type 1</th>
<th>Spa type 2</th>
<th>Other spa types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. typed (%) of total</td>
<td>No. spa type 1 (%) of total</td>
<td>PVL + (%) of spa type 1</td>
<td>SCCmec II (%) of spa type 1</td>
</tr>
<tr>
<td>2001</td>
<td>15</td>
<td>1 (6.7)</td>
<td>1 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2002</td>
<td>13</td>
<td>2 (15.4)</td>
<td>1 (50.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2003</td>
<td>25</td>
<td>2 (8.0)</td>
<td>0 (0)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>2004</td>
<td>35</td>
<td>4† (11.4)</td>
<td>4 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2005</td>
<td>25</td>
<td>3 (12.0)</td>
<td>3 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2006</td>
<td>55</td>
<td>10 (18.2)</td>
<td>10 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2007</td>
<td>67</td>
<td>16 (23.9)</td>
<td>15 (93.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2008</td>
<td>76</td>
<td>17 (22.4)</td>
<td>18 (82.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2009</td>
<td>83</td>
<td>30 (36.1)</td>
<td>23 (76.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>394</td>
<td>85 (21.4)</td>
<td>83 (83.5)</td>
<td>1 (1.2)</td>
</tr>
</tbody>
</table>

* One isolate, SCCmec type indeterminate.
† One isolate, SCCmec type 6.
‡ One isolate, SCCmec type 1.
§ One isolate, SCCmec type 1 and one SCCmec type indeterminate.
|| Two isolates, SCCmec type indeterminate.
the paediatric ICU at Johns Hopkins Hospital in Baltimore, Maryland over a 15-month period [36]. Forty (61%) out of 66 MRSA colonizing isolates were CA-MRSA strains, and healthcare-associated transmission of CA-MRSA resulted in cases of both colonization and infection. These authors described an ICU population with a higher prevalence of MRSA and CA-MRSA colonization than our cohort, probably due to high rates in the community [20], and their study offered several important observations. First, a substantial reservoir of MRSA-colonized patients would not have been identified without routine active surveillance cultures. Second, paediatric ICU patients colonized with CA-MRSA were similar epidemiologically to those harbouring HA-MRSA, apart from those with HA-MRSA being more likely to have had a prior ICU admission. While the clinical impact of CA-MRSA colonization in ICUs remains unclear, our data and those of Milstone et al. highlight the importance of both routine surveillance in paediatric ICUs and an understanding of the changing epidemiology of MRSA colonization on a local level.

There are multiple potential sources from which to acquire MRSA. Colonized patients in our study may have carried MRSA upon admission or, despite infection control precautions, may have acquired MRSA from other patients, healthcare providers or family members [37]. We did not distinguish patients who were admitted for MRSA infections. Therefore, some of the increase in CA-MRSA colonization may have been due to an increasing number of children hospitalized with serious or invasive CA-MRSA infections over the study period [38]. However, given that we observed the increased proportion of CA-MRSA in the CICU and NICU, where fewer admissions for community-onset CA-MRSA infections are expected, we suspect that CA-MRSA infections alone cannot explain the significant increase in colonization that we observed. Additionally, reliable data on recent hospitalizations or transfers were not available for this retrospective study, so we could not address any associations between colonization and recent healthcare exposures.

Our study has other potential limitations. First, although we did not perform PFGE of MRSA isolates, spa type 1 has been shown to correlate with PFGE type USA300 [14, 25, 39], the genotype responsible for most CA-MRSA in the USA. In fact, it has been reported that spa typing more accurately identifies USA300 strains causing community-associated infections, and that other USA300 strains with a different spa type (type 24) more often cause healthcare-associated infections [39]. Second, this is an observational study from a single quaternary care hospital, and therefore may not be generalizable to all facilities. Other reports suggest that CA-MRSA colonization in hospitalized patients is prevalent in other urban areas [16, 17, 19, 25], although prevalence is likely to vary, and it is important for healthcare providers to be aware of local epidemiological trends. Third, in most cases, we only had molecular data on the first MRSA strain isolated from each patient. Many patients had repeat admissions and positive surveillance cultures on subsequent ICU admissions, and we could not determine if these patients retained the same MRSA strain or acquired new strains, because only the first isolate of MRSA from each patient was stored.

Since the implementation of active surveillance for MRSA colonization in 2001, we have not experienced an outbreak of MRSA infection in our paediatric ICUs. Between 2001 and 2009 there was an increase in the number of colonized patients identified, but no increase in the overall prevalence of MRSA colonization. Current evidence about the efficacy of routine surveillance for MRSA and the institution of contact precautions in reducing the frequency of hospital-onset MRSA colonization and infection in adult institutions is not conclusive [40, 41]. However, Geva et al. found that in a NICU setting, identification of colonized infants and subsequent cohorting and contact precautions reduced the risk of MRSA colonization in other infants by 35% [37]. Our data suggest that while community-associated strains of MRSA are becoming more prevalent in paediatric ICUs, these strains are replacing other strains rather than increasing the overall prevalence of MRSA colonization. As CA-MRSA has been shown to be easily transmissible between contacts, it is possible that enhanced surveillance for and identification of MRSA-colonized patients has helped limit the further spread of these strains in the ICU setting [42].

The proportion of screened children found to be MRSA colonized remained stable over 9 years of routine MRSA surveillance in our paediatric ICUs. Importantly, the proportions of children colonized with spa type 1, SCCmec type IV, or PVL-positive strains increased significantly over the 9-year study period. The increasing prevalence of CA-MRSA colonization in paediatric ICUs has the potential to increase the risk of hospital-onset CA-MRSA infections.
in this vulnerable population, and further research should be performed to assess whether the frequency of such infections is increasing.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268812002476.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Program for Patient Safety and Quality at Children’s Hospital Boston. We thank eGenomics for the complimentary use of their software. We thank Leslie Kalish, Sc.D. for statistical support.

DECLARATION OF INTEREST

None.

REFERENCES


