2012

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Epidemiol. Infect. (2013), **141**, 1983–1992. © Cambridge University Press 2012
doi:10.1017/S0950268812002476

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

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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, SCC*mec* 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

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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, SCC*mec* 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 $\leqslant 1$ 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. SCC*mec* 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, SCC*mec* 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 ($P=0{\cdot}82$). 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 ($P<0{\cdot}001$), while the proportions in age groups 2–4, 5–12 and 13–18 years, increased over time ($P=0{\cdot}02$, 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.

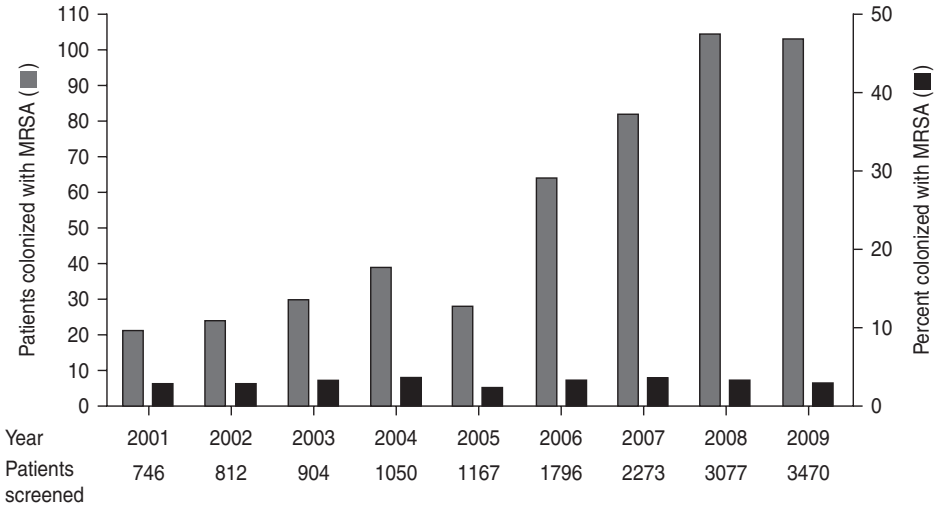

Fig. 1. Annual number and proportion of intensive-care unit patients colonized with methicillin-resistant *Staphylococcus aureus* between 2001 and 2009.

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 SCC*mec* type II (54·3%) or type IV (42·6%). Other SCC*mec* types identified included type I ($n=4$, 1·0%) and type VI ($n=3$, 0·8%); five (1·3%) isolates had an indeterminate SCC*mec* type. Over the 9-year period, the proportion of MRSA isolates carrying SCC*mec* 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 SCC*mec* type and presence of the genes for PVL was performed for the specific *spa* types (Table 2). Nearly all *spa* type 1 isolates were SCC*mec* type IV, and most were PVL positive. In contrast, most *spa* type 2 isolates had SCC*mec* type II and nearly all were PVL negative. The majority of isolates with *spa* types other than 1 or 2 were SCC*mec* II or IV in roughly equal proportions, and most were PVL negative. Isolates of the same *spa* type, other

Table 1. *Antibiotic susceptibilities of methicillin-resistant* Staphylococcus aureus *isolates from colonized patients in paediatric intensive-care units*

Year	No. of MRSA isolates	Erythromycin (% susceptible)	Gentamicin (% susceptible)	Tetracycline (% susceptible)	TMP/SMX (% susceptible)	Clindamycin (% susceptible)	Inducible clindamycin resistance (%)*
2001	21	9·5	52·4	52·4	57·1	4·8	10·0
2002	21	9·5	76·2	66·7	91·7†	25	13·3
2003	28	3·6	100·0	96·4	100·0†	11·5	26·1
2004	38	13·2	92·1	92·1	97·2†	29·7	61·5
2005	26	11·5	96·2	92·3	96·0†	30·8	38·9
2006	60	11·7	100·0	96·7	100·0	35	46·2
2007	73	8·2	98·6	91·8	95·9	28·8	48·1
2008	93	20·4	95·7	91·4	96·8	44·1	44·2
2009	91	8·8	98·9	97·8	98·9	50·6	57·8
Total	451	11·8	94·5	90·9	95·4	35·1	43·0
P value‡		0·33	<0·001	<0·001	<0·001	<0·001	<0·001

* Proportion of clindamycin-resistant isolates with inducible resistance. Denominators for each year are 20, 16, 23, 26, 18, 39, 52, 52 and 45, respectively.
† Number of MRSA isolates tested for susceptibility to TMP/SMX was 12 in 2002, four in 2003, 36 in 2004 and 25 in 2005.
‡ *P* value for trends calculated using the Cochran–Armitage trend test.

than 1 or 2, carried the same SCC*mec* type with the exception of isolates with *spa* types 12, 14, 17 and 139 which included isolates with SCC*mec* type II and IV.

DISCUSSION

The increasing prevalence of MRSA *spa* type 1 in patients colonized with MRSA in paediatric ICUs at CHB indicates that CA-MRSA colonization increased in our study population, and the increased prevalence of SCC*mec* type IV, PVL and susceptibility to non-β-lactam antibiotics support this conclusion. The increase in prevalence of isolates with SCC*mec* 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 ($\leqslant$15) 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.* 11%), but a much higher proportion of colonizing MRSA were CA-MRSA strains (36·2% in 2009 *vs.* 12%) [35]. 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 [15–19, 25, 27–30]. These reports describe an increasing number of hospital-onset bloodstream infections [17], surgical site infections [16], other skin and soft tissue 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 colonization might therefore increase the risk for hospital-onset CA-MRSA infection in the paediatric ICU population.

The initiation of a MRSA active surveillance programme for all ICU patients in our hospital in 2001 led to a marked increase in the total number of patients screened. The number of patients screened for MRSA increased each year, which is explained not only by the increase in ICU admissions over the study period, but also improved adherence to the active surveillance programme. It is possible that as adherence improved, children without healthcare exposures were more likely to be screened for MRSA colonization, which may contribute the larger proportion of CA-MRSA-colonized patients. However, it is clear

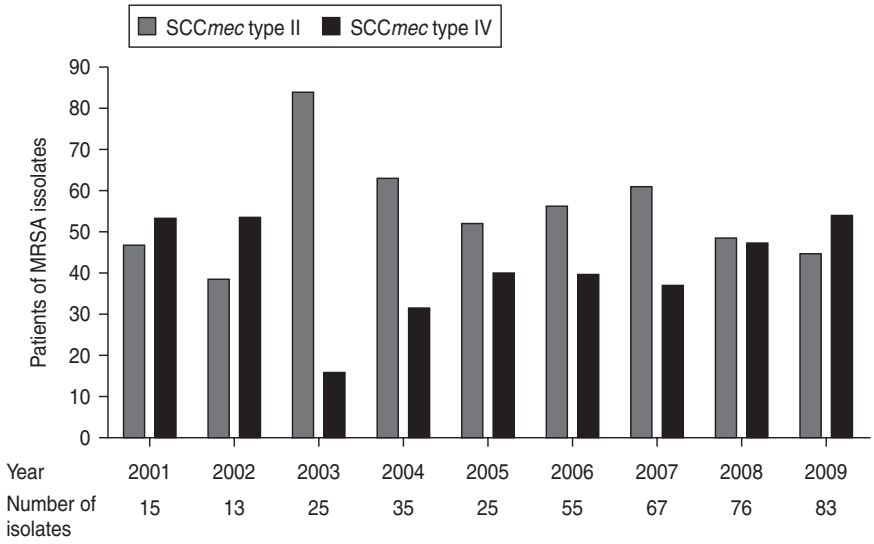

Fig. 2. Proportion of methicillin-resistant *Staphylococcus aureus* isolates from colonized children in intensive-care units between 2001 and 2009 carrying SCC*mec* types II and IV.

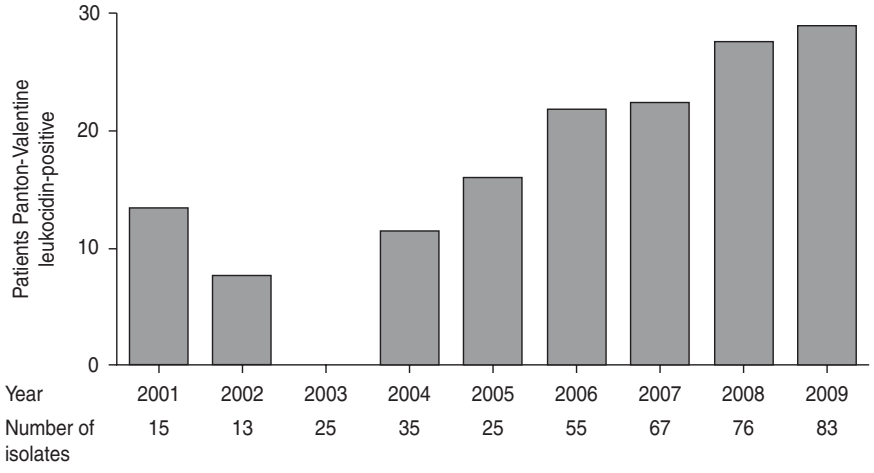

Fig. 3. Proportion of methicillin-resistant *Staphylococcus aureus* isolates from colonized children in intensive-care units between 2001 and 2009 carrying genes that encode Panton–Valentine leukocidin.

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*mec* types, we have also documented increasing prevalence of PVL and SCC*mec* type IV in colonizing MRSA isolates over time.

Recently, Milstone *et al*. reported a prevalence of MRSA colonization of 6% in children admitted to

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*

		Spa type 1				*Spa* type 2				Other *spa* types			
Year	Total no. typed	No. *spa* type 1 (% of total)	PVL+ (% of *spa* type 1)	SCC*mec* II (% of *spa* type 1)	SCC*mec* IV (% of *spa* type 1)	No. *spa* type 2 (% of total)	PVL+ (% of *spa* type 2)	SCC*mec* II (% of *spa* type 2)	SCC*mec* IV (% of *spa* type 2)	No. other *spa* types (% of total)	PVL+ (% of other *spa* types)	SCC*mec* II (% of other *spa* types)	SCC*mec* IV (% of other *spa* types)
2001	15	1 (6·7)	1 (100)	0 (0)	1 (100)	6 (40·0)	0 (0)	6 (100)	0 (0)	8 (53·3)	1 (12·5)	1 (12·5)	7 (87·5)
2002	13	2 (15·4)	1 (50·0)	0 (0)	2 (100)	5 (38·5)	0 (0)	5 (100)	0 (0)	6* (46·2)	0 (0)	0 (0)	5 (83·3)
2003	25	2 (8·0)	0 (0)	1 (50·0)	1 (50·0)	18 (72·0)	0 (0)	16 (88·9)	2 (11·1)	5 (20·0)	0 (0)	4 (80·0)	1 (20·0)
2004	35	4† (11·4)	4 (100)	0 (0)	3 (75·0)	14 (40·0)	0 (0)	12 (85·7)	2 (14·3)	17‡ (48·6)	0 (0)	10 (58·8)	6 (35·3)
2005	25	3 (12·0)	3 (100)	0 (0)	3 (100)	12‡ (48·0)	0 (0)	10 (83·3)	1 (8·3)	10† (40·0)	1 (10·0)	3 (30·0)	6 (60·0)
2006	55	10 (18·2)	10 (100)	0 (0)	10 (100)	29 (52·7)	0 (0)	26 (89·7)	3 (10·3)	16§ (29·1)	2 (12·5)	5 (31·3)	9 (56·3)
2007	67	16 (23·9)	15 (93·8)	0 (0)	16 (100)	31* (46·3)	0 (0)	28 (90·3)	2 (6·5)	20 (29·9)	0 (0)	13 (65·0)	7 (35·0)
2008	76	17 (22·4)	14 (82·4)	0 (0)	17 (100)	37‖ (48·7)	2 (5·4)	29 (78·4)	6 (16·2)	22† (28·9)	5 (22·7)	8 (36·4)	13 (59·1)
2009	83	30 (36·1)	23 (76·7)	0 (0)	30 (100)	30 (36·1)	0 (0)	28 (93·3)	2 (6·7)	23‡ (27·7)	1 (4·3)	9 (39·1)	13 (56·5)
Total	394	85 (21·6)	71 (83·5)	1 (1·2)	83 (97·6)	182 (46·2)	2 (1·1)	160 (87·9)	18 (9·9)	127 (32·2)	10 (7·9)	53 (41·7)	67 (52·8)

* One isolate, SCC*mec* type indeterminate.
† One isolate, SCC*mec* type 6.
‡ One isolate, SCC*mec* type 1.
§ One isolate, SCC*mec* type 1 and one SCC*mec* type indeterminate.
‖ Two isolates, SCC*mec* 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, SCC*mec* 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

1. **Rosenthal VD, *et al.*** International Nosocomial Infection Control Consortium (INICC) report, data summary for 2003–2008, issued June 2009. *American Journal of Infection Control* 2010; **38**: 95–104 e102.
2. **National Nosocomial Infections Surveillance System.** National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *American Journal of Infection Control* 2004; **32**: 470–485.
3. **Davis KA, *et al.*** Methicillin-resistant *Staphylococcus aureus* (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. *Clinical Infectious Diseases* 2004; **39**: 776–782.
4. **Milstone AM, *et al.*** Methicillin-resistant *Staphylococcus aureus* colonization and risk of subsequent infection in critically ill children: importance of preventing nosocomial methicillin-resistant Staphylococcus aureus transmission. *Clinical Infectious Diseases* 2011; **53**: 853–859.
5. **Calfee DP, *et al.*** Strategies to prevent transmission of methicillin-resistant *Staphylococcus aureus* in acute care hospitals. *Infection Control and Hospital Epidemiology* 2008; **29** (Suppl. 1): S62–80.
6. **Siegel JD, *et al.*** Management of multidrug-resistant organisms in healthcare settings. Atlanta, GA: Centers for Disease Control and Prevention, Department of Health and Human Services, USA, 2006.
7. **King MD, *et al.*** Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. *Annals of Internal Medicine* 2006; **144**: 309–317.
8. **Francis JS, *et al.*** Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. *Clinical Infectious Diseases* 2005; **40**: 100–107.
9. **Nakamura MM, *et al.*** Prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in the community pediatric population. *Pediatric Infectious Disease Journal* 2002; **21**: 917–922.
10. **Hussain FM, Boyle-Vavra S, Daum RS.** Community-acquired methicillin-resistant *Staphylococcus aureus* colonization in healthy children attending an outpatient pediatric clinic. *Pediatric Infectious Disease Journal* 2001; **20**: 763–767.
11. **Cheng Immergluck L, *et al.*** Prevalence of Streptococcus pneumoniae and *Staphylococcus aureus* nasopharyngeal colonization in healthy children in the United States. *Epidemiology and Infection* 2004; **132**: 159–166.
12. **Fritz SA, *et al.*** Prevalence of and risk factors for community-acquired methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* colonization in children seen in a practice-based research network. *Pediatrics* 2008; **121**: 1090–1098.
13. **Creech 2nd CB, *et al.*** Increasing rates of nasal carriage of methicillin-resistant *Staphylococcus aureus* in healthy children. *Pediatric Infectious Disease Journal* 2005; **24**: 617–621.
14. **Tenover FC, *et al.*** Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. *Journal of Clinical Microbiology* 2006; **44**: 108–118.
15. **Otter JA, French GL.** Nosocomial transmission of community-associated methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infectious Diseases* 2006; **6**: 753–755.
16. **Patel M, *et al.*** USA300 genotype community-associated methicillin-resistant *Staphylococcus aureus* as a cause of surgical site infections. *Journal of Clinical Microbiology* 2007; **45**: 3431–3433.
17. **Seybold U, *et al.*** Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. *Clinical Infectious Diseases* 2006; **42**: 647–656.
18. **Maree CL, *et al.*** Community-associated methicillin-resistant *Staphylococcus aureus* isolates causing health-care-associated infections. *Emerging Infectious Diseases* 2007; **13**: 236–242.
19. **Liu C, *et al.*** A population-based study of the incidence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* disease in San Francisco, 2004–2005. *Clinical Infectious Diseases* 2008; **46**: 1637–1646.
20. **Klevens RM, *et al.*** Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *Journal of the American Medical Association* 2007; **298**: 1763–1771.
21. **McDougal LK, *et al.*** Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national

database. *Journal of Clinical Microbiology* 2003; **41**: 5113–5120.

22. **Koreen L, *et al.*** spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *Journal of Clinical Microbiology* 2004; **42**: 792–799.
23. **Harmsen D, *et al.*** Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *Journal of Clinical Microbiology* 2003; **41**: 5442–5448.
24. **Shopsin B, *et al.*** Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *Journal of Clinical Microbiology* 1999; **37**: 3556–3563.
25. **Carey AJ, *et al.*** Changes in the molecular epidemiological characteristics of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Infection Control and Hospital Epidemiology* 2010; **31**: 613–619.
26. **Popovich K, *et al.*** Phenotypic prediction rule for community-associated methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* 2007; **45**: 2293–2295.
27. **David MD KA, *et al.*** Community-associated methicillin-resistant *Staphylococcusl aureus*: nosocomial transmission in a neonatal unit. *Journal of Hospital Infection* 2006; **64**: 244–250.
28. **Healy CM, *et al.*** Emergence of new strains of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Clinical Infectious Diseases* 2004; **39**: 1460–1466.
29. **McAdams RM, *et al.*** Spread of methicillin-resistant *Staphylococcus aureus* USA300 in a neonatal intensive care unit. *Pediatrics International* 2008; **50**: 810–815.
30. **Seybold U, *et al.*** Emergence of and risk factors for methicillin-resistant *Staphylococcus aureus* of community origin in intensive care nurseries. *Pediatrics* 2008; **122**: 1039–1046.
31. **Flayhart D, *et al.*** Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. *Journal of Clinical Microbiology* 2005; **43**: 5536–5540.
32. **Clinical and Laboratory Standards Institute.** Performance standards for antimicrobials susceptibility testing; seventeenth informational supplement M100-S17 2007; **27**: 1–182.
33. **Oliveira DC, de Lencastre H.** Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 2002; **46**: 2155–2161.
34. **McClure JA, *et al.*** Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. *Journal of Clinical Microbiology* 2006; **44**: 1141–1144.
35. **Nair N, *et al.*** Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among patients admitted to adult intensive care units: The STAR*ICU Trial. *Infection Control and Hospital Epidemiology* 2011; **32**: 1057–1063.
36. **Milstone AM, *et al.*** Community-associated methicillin-resistant *Staphylococcus aureus* strains in pediatric intensive care unit. *Emerging Infectious Diseases* 2010; **16**: 647–655.
37. **Geva A, *et al.*** Spread of methicillin-resistant *Staphylococcus aureus* in a large tertiary NICU: network analysis. *Pediatrics* 2011; **128**: e1173–1180.
38. **Gerber JS, *et al.*** Trends in the incidence of methicillin-resistant *Staphylococcus aureus* infection in children's hospitals in the United States. *Clinical Infectious Diseases* 2009; **49**: 65–71.
39. **Larsen AR, *et al.*** Two distinct clones of methicillin-resistant *Staphylococcus aureus* (MRSA) with the same USA300 pulsed-field gel electrophoresis profile: a potential pitfall for identification of USA300 community-associated MRSA. *Journal of Clinical Microbiology* 2009; **47**: 3765–3768.
40. **Huskins WC, *et al.*** Intervention to reduce transmission of resistant bacteria in intensive care. *New England Journal of Medicine* 2011; **364**: 1407–1418.
41. **Jain R, *et al.*** Veterans Affairs initiative to prevent methicillin-resistant *Staphylococcus aureus* infections. *New England Journal of Medicine* 2011; **364**: 1419–1430.
42. **Gorwitz RJ.** Understanding the success of methicillin-resistant *Staphylococcus aureus* strains causing epidemic disease in the community. *Journal of Infectious Diseases* 2008;**197**: 179–182.