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Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Costa Rican Children

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Abstract

We collected all *Staphylococcus aureus* isolates from the National Children's Hospital in Costa Rica to evaluate the prevalence and molecular epidemiology of MRSA. Of 299 *S. aureus* isolates, 61% were MRSA. Most MRSA isolates (94.5%) carried SCCmec IV, and 45.6% carried Panton-Valentine leukocidin-encoding genes. The high prevalence of MRSA in this population highlights the need for improvement of antibiotic prescription and infection control measures.

Keywords

Staphylococcus aureus; methicillin-resistant *Staphylococcus aureus*; child; colonization; epidemiology

Introduction

Staphylococcus aureus is the leading cause of skin and soft tissue infections, bacteremia, and osteoarticular infections. Methicillin-resistant *S. aureus* (MRSA) has become the most frequently encountered clone of *S. aureus* in many areas, and community-associated MRSA (CA-MRSA) has become the leading cause of skin and soft tissue infections.¹ CA-MRSA strains are typically characterized by the presence of staphylococcal cassette chromosome *mec* (SCCmec) type IV or V and a range of exoproteins that are distinct from those produced by typical hospital-associated MRSA (HA-MRSA) strains, such as Panton-Valentine leukocidin (PVL), exfoliative toxins, and enterotoxins.^{1,2} In the US, CA-MRSA also appears to preferentially infect children and young adults without risk factors for antibiotic-resistant organisms.

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While the epidemic of CA-MRSA has been well documented in the US, the burden and molecular epidemiology of MRSA in Latin America remains largely unknown. Older reports have suggested that MRSA may represent as many as 30–50% of *S. aureus* isolates in Latin America, and Loureiro et al reported that nearly 50% of newborns in a Brazilian hospital were colonized with MRSA.³ The first report of CA-MRSA disease in South America (Brazil) was published in 2005. These isolates were obtained from two patients with skin and soft tissue infections and one isolate from a patient with septic arthritis.⁴

There have been no published studies regarding the molecular epidemiology of MRSA in Costa Rica, and little is known about the epidemiology of this microbe in Costa Rica and the surrounding areas of Central America. Hence, the aim of this study was to analyze *S. aureus* isolates from the National Children's Hospital in Costa Rica to determine the frequency of MRSA and to define the molecular epidemiology of these isolates.

Materials and Methods

All *S. aureus* isolates from the Bacteriology Laboratory at the National Children's Hospital in Costa Rica were prospectively collected during a 10-month period. Identification of *S. aureus* was performed using an automated VITEK system (bioMérieux, Inc., Durham, NC). We obtained information only on the source of the isolates; patients from whom the isolates were obtained were not identified.

Samples were shipped to Vanderbilt University Medical Center for confirmation and molecular analysis. Upon arrival, isolates were placed in tryptic soy broth with 6.5% NaCl and incubated overnight at 37°C to improve bacteriologic yield. After broth enrichment, a 10 µL inoculum was plated onto mannitol salt agar plates with and without 6 µg/mL of oxacillin and incubated for 48 hours at 37°C. If yellow growth was observed, colonies were plated onto blood agar plates and incubated overnight at 37°C. Coagulase latex agglutination testing was performed (Staphaurex, Remel, Lenexa, KS), and the presence of the *nuc* gene (specific to *S. aureus*) was confirmed by polymerase chain reaction (PCR).

Isolates confirmed to be MRSA by PCR detection of the *mecA* gene were further characterized by SCC_{mec} typing, using a multiplex approach, or *ccr* and *mec* class typing when necessary, as previously described.^{5,6} Detection of the PVL-encoding genes was performed, as described elsewhere,⁷ as well as determination of *agr* locus type⁸ and the presence of enterotoxins A, B and C,⁹ and toxic shock syndrome toxin 1.¹⁰ Genotyping of MRSA isolates was performed by repetitive element, sequence-based PCR (rep-PCR), using the commercially available DiversiLab system (bioMérieux, Inc., Durham, NC).¹¹ Isolates with >95% similarity were defined as indistinguishable and PFGE-types were assigned based upon best-fit analysis.

Fisher's exact test and Pearson's chi-squared test were used to determine differences between groups. A p-value <0.05 was considered statistically significant. Stata 11.2 for Mac was used for statistical analysis. For rep-PCR typing and assessment of overall genetic relatedness, DiversiLab software was used. The study was approved by the ethics committee

at the National Children's Hospital in Costa Rica and exempt by the Vanderbilt Institutional Review Board as non-human subjects research.

Results

A total of 301 samples were available for analysis; however, 2 samples did not yield *S. aureus* upon confirmatory testing. Of these 299 samples, the source of infection was known in 296: 128 (43.2%) were obtained from skin and soft tissue infections (SSTIs), 79 (26.7%) from invasive infections, and 89 (30.1%) from miscellaneous infections. Invasive-infection sources included blood (42), synovial fluid (10), cerebrospinal fluid (9), bronchoalveolar lavage (7), catheter tip (3), bone (1), peritoneal (5), and pleural fluid (1). SSTIs included burns (13), skin (16), abscess (34), wounds (26), pustules (4), cellulitis (7), impetigo (3), ulcer (10), lesion (5), and others (13). Miscellaneous infections included inner ear (13), eye (12), nasal (17), sputum (5), oral (2), urine (2), tracheal (19), gastric (2), secretions (5), and eczema (7),

Over half of all samples (60.9%) were MRSA. Of these, 94.5% carried *SCCmec* type IV (Table 1). Two isolates that were not typeable by multiplex PCR carried *ccr* type 2 and *mec* class C, a combination of elements in the staphylococcal cassette chromosome not previously described. Approximately 45% of the MRSA isolates carried PVL-encoding genes, and 50% carried staphylococcal enterotoxin C (SEC; Table 1). No statistically significant differences were observed when comparing MRSA isolates from different infection sites, except for PVL (Table 1).

Using rep-PCR, all 182 MRSA isolates were grouped into 14 clusters and 15 unique strains, using both the R74 DL MRSA and the R11 DL MRSA-Harmony libraries as references. Eight of these clusters contained a single USA type. Using the former library, nearly 25% of the MRSA isolates were classified as USA700 (Table 2, Supplemental Digital Content 1), with USA400 being the second most common type (19.78%), followed by USA300 (18.68%) and USA800 (14.84%). Using the Harmony library, most isolates were classified into sequence type (ST) 80, clonal complex (CC) 80 (25.82%; Table 3, Supplemental Digital Content 2); these included USA400, USA700 and USA800 strains. The second most common classification was ST247, CC8 (12.09%), and included USA300, USA500, Iberian and Brazilian strains (Table 3, Supplemental Digital Content 2).

Discussion

To our knowledge, this is the first study to evaluate the molecular epidemiology of infections due to MRSA in Costa Rica, specifically in a pediatric population. For Latin America, published studies on the molecular epidemiology of MRSA are only available for eight countries.¹² It is noteworthy that well over half (61%) of all cultured staphylococcal infections in this pediatric population were due to MRSA. Previous studies in other Latin American countries have reported the prevalence of MRSA among staphylococcal infections to be between 30% and 50%.¹³ However, few reports have included pediatric populations. This high prevalence of MRSA in Costa Rica is concerning and highlights the need for better antibiotic prescription measures and infection control strategies in this setting.

It is also important that nearly all MRSA isolates (95%) carried *SCCmec* type IV, a characteristic of CA-MRSA. These strains were not limited to the USA300 PFGE type, as is typically the case in the US; rather, multiple genetic lineages, including those typically considered to be of hospital-origin, were seen in this collection of strains. These results are similar to those described in South America, as nosocomial infections due to *SCCmec* IV isolates are well described, implying the transmission of CA-MRSA in the healthcare setting.¹⁴

In previous Latin American studies, a specific clone is often found to prevail in each setting. For instance, in Brazil, from 1990 to 2004, the Brazilian epidemic clonal complex predominated, though it was not the only one circulating.¹² Additionally, a recent study in Argentina that recovered 312 CA-MRSA isolates from several hospitals revealed that CA-MRSA infections in children increased from 24% to 49% from 2005 to 2008 and a single genotype predominated (ST5, *SCCmec* IV, PVL positive).¹⁵ Although USA700 was the most commonly found strain in our study, it did not represent the majority of the MRSA isolates (24.7%). A larger study, potentially including an adult population, may elucidate whether there is a strain that prevails in Costa Rica.

A limitation of this study is that we did not review the medical records of the patients from whom the isolates were obtained, to evaluate the clinical features of the infections and compare the outcomes, such as length of stay, for the different strains of MRSA identified. Thus, we could not gain insight on the severity of disease as a function of the molecular epidemiology of the infecting isolate. Future studies will focus on linking clinical outcomes and bacterial characteristics.

Costa Rican staphylococcal isolates are distinctly different than those that circulate to the North (in the US) and to the South (in Brazil and other South American countries). While there is significant molecular heterogeneity, many features are shared between strains, including a predominance of *SCCmec* IV, PVL-negative strains. Given the overall prevalence of MRSA (>50%), providers in this region should appreciate that beta-lactam therapy will be insufficient in the majority of patients. Whether the precise molecular characteristics govern disease presentation or severity will be the focus of ongoing work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Molecular characteristics of 182 pediatric MRSA isolates by type of infection

	MRSA Isolates N (%)	SSTIs N (%)	Invasive N (%)	Miscellaneous N (%)	P
<i>SCC_{mec}</i>	182 (60.9)	76 (41.8) ^a	50 (27.5) ^a	53 (29.1) ^a	0.396 ^b
IV	172 (94.5)	73 (96.1)	48 (96.0)	48 (90.6)	
V	2 (1.1)	1 (1.3)	1 (2.0)	0 (0.0)	
<i>ccr 2/mec C2</i>	2 (1.1)	0 (0.0)	0 (0.0)	2 (3.8)	
NT	6 (3.3)	2 (2.6)	1 (2.0)	3 (5.7)	
<i>agr</i> type					
I	122 (67.0)	51 (68.9)	35 (71.4)	36 (67.9)	0.925 ^c
II	28 (15.4)	8 (10.8)	7 (14.3)	13 (24.5)	0.107 ^c
III	26 (14.3)	15 (20.3)	7 (14.3)	4 (7.5)	0.136 ^c
NT	1 (0.5)				
PVL positive	83 (45.6)	38 (50.0)	26 (52.0)	16 (30.2)	0.040 ^c
TSST-1 positive	15 (8.2)	7 (9.2)	3 (6.0)	5 (9.4)	0.835 ^b
SEA positive	24 (13.2)	13 (17.1)	3 (6.0)	8 (15.1)	0.181 ^b
SEB positive	41 (22.5)	22 (28.9)	11 (22.0)	8 (15.1)	0.180 ^c
SEC positive	92 (50.5)	40 (52.6)	29 (58.0)	20 (37.7)	0.097 ^c

^a: Percentages do not add to 100% because 3 isolates did not have information on the site of infection. These percentages refer to MRSA isolates only.^b: Fisher's exact test^c: Pearson's chi-squared