Molecular Epidemiology of Invasive *Staphylococcus aureus* Infections and Concordance with Colonization Isolates

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Objectives To characterize *Staphylococcus aureus* isolates recovered from hospitalized children and to determine the concordance between colonizing and invasive isolates.

Study design Children with culture-confirmed, community-onset, invasive *S aureus* infections were enrolled in this prospective case series from a large children's hospital over a 5-year period. Colonization isolates were obtained from the anterior nares, oropharynx, and inguinal folds and were compared with invasive isolates via repetitive-element, sequence-based polymerase chain reaction testing. Isolates with a ≥96% genetic match were characterized as concordant.

Results A total of 86 *S aureus* isolates (44 invasive, 42 colonization) were collected from 44 children with invasive infections. Clinical isolates were genetically diverse, 64% of invasive isolates were methicillin-susceptible *S aureus* (MSSA), and 59% of cases had a colonizing *S aureus* isolate at the time of hospitalization. Of those who were colonized, at least 1 of their colonization isolates was indistinguishable from the infecting isolate in 88% of cases. Patients with invasive MSSA were significantly more likely to have a concordant MSSA colonization isolate present compared with patients with invasive methicillin-resistant *S aureus* (MRSA) (61% vs 38%, $P < .05$).

Conclusions Invasive MSSA infection was more common than MRSA infection in this pediatric cohort, and patients with MSSA infection were significantly more likely than those with MRSA infection to have concordant colonizing isolates across multiple anatomic sites. These findings warrant larger scale validation and may have important infection control and epidemiologic implications, as unlike MRSA, transmissibility of MSSA largely is ignored in healthcare settings. *(J Pediatr 2019;210:173-7)*.

Staphylococcus aureus is a major cause of invasive hospital-associated and community-associated bacterial infections globally. From 1999 to 2005, the number of hospitalizations related to *S aureus* increased by 62%.[1] In the same period, the percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the lungs or blood leading to hospitalization more than tripled.[1] However, since 2015, the rate of invasive MRSA infections in the US has decreased, likely because of, in part, improved infection control practices.[2]

The burden of antimicrobial resistance in *S aureus* extends well beyond methicillin resistance. The rates of resistance to clindamycin, a commonly used antistaphylococcal agent, are increasing in both adults and children.[3] Moreover, not all strains of *S aureus* are equivalent in virulence. *S aureus* produces numerous, diverse virulence factors that facilitate establishment and persistence of infection in humans. Although only a small number of MRSA clones dominated the community-associated MRSA (CA-MRSA) epidemic of the 2000s, *S aureus* strains asymptomatically colonizing the nose of healthy individuals are much more genetically diverse.[4,5] It is unclear whether this degree of genetic variability exists between colonizing and invasive methicillin-susceptible *S aureus* (MSSA) isolates that have emerged in the last decade. A primary goal of the current study was to describe the current molecular epidemiology of invasive *S aureus* in a large pediatric medical center. This analysis included assessment for genes encoding Panton-Valentine leukocidin (PVL), a member of the bi-component leukocidin family of toxins produced by *S aureus* that has been closely associated with the CA-MRSA epidemic strain[6]; LukAB

ACME	Arginine catabolic mobile element
agr	Accessory gene regulator
CA-MRSA	Community-associated MRSA
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *S aureus*
PFGE	Pulsed-field gel electrophoresis
PVL	Panton-Valentine leukocidin
SCC*mec*	Staphylococcal cassette chromosome *mec*

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(also known as LukGH), the most recently described leukocidin that is critical for virulence in numerous animal models and is present in all clinical *S aureus* isolates studied to date[7-9]; and the accessory gene regulator (*agr*) locus, a global regulator of several *S aureus* virulence factors.[10]

The second major goal of this study was to examine paired invasive and colonizing *S aureus* isolates to determine the genotypic concordance between colonization and invasive isolates. Few data are available regarding genotypic concordance between invasive and colonizing MSSA (as opposed to studies of noninvasive MSSA clinical isolates or MRSA disease), or colonization characteristics across anatomic sites. Current infection control protocols at many institutions typically focus exclusively on isolating patients with MRSA infection, and suppressing or eradicating MRSA colonization, ignoring MSSA infection and colonization. Determining the prevalence of genotypically concordant MSSA colonization at various sites in patients with invasive infection may inform future infection control strategies.

Methods

All patients under 19 years of age who were admitted within a 5-year period (January 2013 to December 2017) to the Upstate Golisano Children's Hospital in Syracuse, New York with culture-confirmed, community-onset invasive *S aureus* disease were eligible for the study. Invasive *S aureus* disease was defined as recovery of *S aureus* from a sterile site, including blood, bones, joint, lungs, and central nervous system. Cultures obtained within 48 hours of admission met criteria for community-onset disease. All protocols were reviewed and approved by the Upstate Golisano Children's Hospital Institutional Review Board. The sample size of the study represents all eligible subjects admitted during the study period.

Patients with invasive *S aureus* disease were identified through a daily laboratory report to capture all eligible patients. *S aureus* isolates were obtained directly from the clinical Microbiology Laboratory. In addition, swabs for culture were collected from the anterior nares, the oropharynx, and inguinal folds of each subject admitted with community-onset invasive *S aureus* infection within 7 days of the sterile-site culture collection. Specific clinical information including age, sex, discharge diagnosis, intensive care unit stay, and recent antibiotic use was recorded. Routine antibiotic susceptibility testing was performed according to the standards of the Clinical and Laboratory Standards Institute.

Isolates were stored in sterile skim milk at −70°C until subculture for molecular analysis was performed. For molecular analysis, genomic DNA was extracted as previously described,[4] using the QIAGEN MoBio DNA extraction kit. DNA was quantified using the NanoDrop 1000 (ThermoFisher, Wilmington, Deleware) to confirm a concentration of 25-50 micrograms/mL. Purified genomic DNA was used as the template for polymerase chain reaction detection of genes encoding PVL, LukAB, arginine catabolic mobile element (ACME), staphylococcal cassette chromosome *mec* (SCC*mec*), and the *agr* locus type. Previously identified *S aureus* strains containing or lacking the genes of interest, or of a particular SCC*mec* or *agr* locus type, were used as controls. Assignment of SCC*mec* type was determined using the multiplex strategy as previously reported.[11] Repetitive element, sequence-based polymerase chain reaction (Diversilab System; Biomerieux, Durham, North Carolina) was used to determine genetic relatedness and assign clonal type based on pulsed-field gel electrophoresis (PFGE) USA types for *S aureus*. The threshold for categorizing two isolates as concordant was a ≥96% genetic match.

Statistical analysis was performed using Prism6 by GraphPad (San Diego, California). Significance of associations was analyzed using the Fisher exact test, and differences with P value of $< .05$ were deemed significant.

Results

Characteristics of the Patient Population

In total, 143 cases of invasive *S aureus* disease were identified during the study period; 44 parents/caregivers consented for their children to participate in the study within the timeframe required for collection of swab samples to determine colonization. Twenty-six children (59%) had at least 1 colonization strain detected. The median patient age was 10 years; 35% of subjects were female and 65% were male. The most common infections identified were bacteremia, pneumonia, osteomyelitis, and septic arthritis. Hospital length of stay varied widely among patients, with 5 patients remaining in the hospital 3 days or less, 16 between 4 and 9 days, 8 between 10 and 13 days, and 14 greater than 14 days (**Table I**).

Molecular Characteristics of Clinical Isolates

Overall, 86 *S aureus* isolates were collected from 44 children (44 invasive isolates and 42 colonization isolates, **Table I**). Of invasive *S aureus* isolates 64% (28 of 44) were MSSA. The most frequent clonotype of the total 86 invasive and colonizing *S aureus* isolates was USA300, representing 34% (29) of all isolates (41% of invasive isolates and 26% of colonization isolates, **Figure**). Overall, 20% (17) of isolates were classified as USA200, 11% (9) USA800, and 7% (6) USA100; 21% (18) were nontypeable. Of the MRSA isolates, 56% of isolates were classified as USA300 (9 of 6 invasive disease and 6 of 11 colonization), and 15% were classified as USA100. MSSA isolates were more diverse, with USA200 most frequently encountered (13 of 59, 22% of isolates), followed by USA300 (12 of 59 [20%]).

Molecular characteristics of invasive and colonizing isolates are provided in **Table II**. Of 27 MRSA isolates, 18 (67%) contained the ACME locus and 20 (74%) were PVL-positive. Twenty-two (82%) were *mec* class B, and 82% possessed *agr* type I. Of 59 MSSA isolates, 8 (14%) were PVL positive, and 19 (32%) possessed *agr* type I. Two of the MSSA isolates were ACME-positive. All isolates (both MRSA and MSSA) contained the *lukAB* gene.

Table I. Clinical characteristics and concordance of invasive and colonization *S aureus* isolates

Patient identification numbers	Age (years)	Duration of hospitalization (d)	Invasive isolate, PFGE type	Infection type	Number of colonization sites	Invasive concordance with colonization?
10	0.8	40	MRSA, USA300	Pneumonia	0	N/A
11	3.1	15	MRSA, USA300	SSTI	0	N/A
15	0.1	37	MRSA, USA200	Pneumonia	0	N/A
29	5.7	8	MRSA, USA300	MSKI	0	N/A
32	4.0	35	MRSA, NT	Pneumonia	0	N/A
34	10.2	9	MRSA, USA300	MSKI	0	N/A
41	0.6	33	MRSA, USA300	MSKI	0	N/A
43	10.1	Not available	MRSA, USA200	MSKI	0	N/A
16	9.4	6	MRSA, USA300	MSKI	1 (N)	No
25	9.3	10	MRSA, USA1000	MSKI	1 (T)	No
28	17.6	21	MRSA, USA100	CLABSI	1 (T)	Yes (T)
33	17.7	13	MRSA, USA100	MSKI	1 (T)	Yes (T)
37	12.8	7	MRSA, USA300	MSKI	1 (T)	Yes (T)
45	14.0	10	MRSA, USA300	Pneumonia	1 (T)	Yes (T)
38	2.2	102	MRSA, USA300	SSTI	3 (G, N, T)	Yes (G)
30	1.1	31	MRSA, NT	Pneumonia	2 (G, N)	Yes (G, N)
4	1.4	19	MSSA, USA400	CLABSI	0	N/A
9	16.8	10	MSSA, USA300	Peritonitis	0	N/A
12	3.3	23	MSSA, USA900	VPS	0	N/A
23	0.9	2	MSSA, USA300	CLABSI	0	N/A
24	17.5	6	MSSA, USA200	MSKI	0	N/A
27	17.0	13	MSSA, USA600	SSTI	0	N/A
31	13.9	5	MSSA, NT	MSKI	0	N/A
36	6.0	11	MSSA, USA700	VPS	0	N/A
40	13.8	30	MSSA, NT	MSKI	0	N/A
44	15.0	7	MSSA, USA300	Bacteremia	0	N/A
8	0.3	15	MSSA, USA300	UTI	1 (G)	Yes (G)
1	13.7	6	MSSA, USA300	MSKI	1 (N)	No
2	10.5	5	MSSA, NT	MSKI	1 (N)	Yes (N)
7	12.7	8	MSSA, NT	CLABSI	1 (N)	Yes (N)
14	5.2	10	MSSA, USA300	RPA	1 (N)	Yes (N)
18	8	3	MSSA, NT	CLABSI	1 (N)	Yes (N)
19	14.9	7	MSSA, USA800	MSKI	1 (N)	Yes (N)
20	16.6	5	MSSA, USA800	MSKI	2 (N, T)	Yes (N, T)
39	11.1	3	MSSA, USA200	MSKI	2 (N, T)	Yes (N, T)
13	12.9	4	MSSA, USA200	CLABSI	2 (G, N)	Yes (N)
6	16.2	17	MSSA, USA100	Endocarditis	2 (G, N)	Yes (G)
21	14.5	8	MSSA, USA800	Bacteremia	2 (G, N)	Yes (N)
22	11.4	4	MSSA, USA200	MSKI	2 (G, N)	Yes (G, N)
35	9.5	2	MSSA, USA200	MSKI	2 (G, N)	Yes (G, N)
42	17.4	6	MSSA, USA300	Bacteremia	2 (G, N)	Yes (G, N)
5	12.1	12	MSSA, USA300	MSKI	2 (N, T)	Yes (N, T)
17	0.01	33	MSSA, USA200	MSKI	3 (G, N, T)	Yes (G)
3	5.3	3	MSSA, NT	SSTI	3 (G, N, T)	Yes (G, N, T)

CLABSI, central line-associated bloodstream infection; *G*, colonization isolate obtained from the inguinal folds; *MSKI*, musculoskeletal infection; *N*, colonization isolate obtained from the nares; *NT*, nontypeable strain; *RPA*, retropharyngeal abscess; *SSTI*, skin and soft tissue infection; *T*, colonization isolate obtained from oropharynx; *UTI*, urinary tract infection; *VPS*, ventriculoperitoneal shunt.

Genetic Relatedness Between Colonization and Invasive *S aureus* Isolates

In 18 of 44 patients (41%), *S aureus* was not isolated from 1 or more colonization sites. For the 26 (59%) patients colonized with *S aureus* at the time of hospitalization, at least 1 colonization isolate was concordant with the corresponding invasive isolate in 23 of 26 cases (88%, Table I). Concordance between disease isolates and nasal colonization isolates (56%) was higher than for the inguinal folds (36%) or oropharynx (28%, $P < .05$). Colonization with a concordant strain was significantly more common for children with MSSA infections than those with MRSA infection (17 of 28 [61%] vs 6 of 16 [38%]; $P < .05$).

Discussion

In our study, analyzing genetic relatedness between invasive and colonizing strains of *S aureus*, we found that invasive MSSA was significantly more likely to be associated with concordant colonization compared with invasive MRSA, and patients with invasive *S aureus* infections were significantly more likely to have a genetically matched nasal colonization strain than concordant throat or inguinal skin colonization. Furthermore, we observed substantial genetic diversity among both MRSA and MSSA invasive isolates, reflecting increased genetic diversity across invasive *S aureus* infections than was seen during the height of the CA-MRSA epidemic in the US. The prevalence of the PVL in

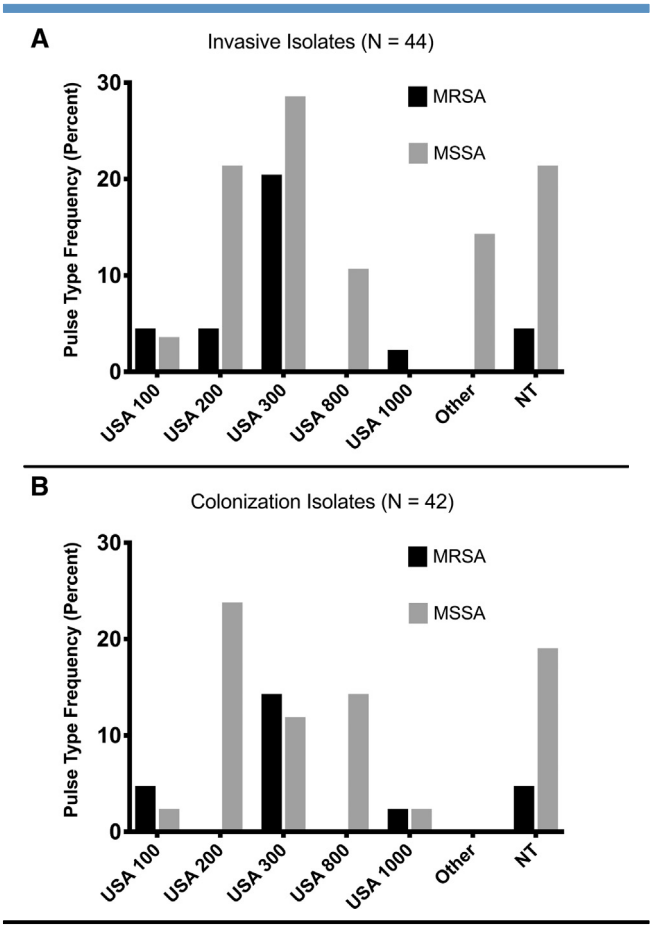

Figure. Genetic diversity of *S aureus* colonization and invasive disease isolates obtained in this study, by PFGE type*, stratified by invasive and colonization isolates. **A,** Each bar represents the relative percentage of a given PFGE type among all invasive disease isolates (n = 45). Although USA300 was the most common pulsotype among both MRSA and MSSA populations (~20% of each), isolates were diverse, with no clone representing the majority of isolates. **B,** Each bar represents the relative percentage of a given PFGE type among all colonization isolates (n = 42). Colonization isolates were quite diverse, with no pulsotype representing more than a one-quarter of the group. *Not assignable to any PFGE type.

invasive MSSA strains has remained low, whereas it remained high in invasive MRSA strains, underscoring an uncertain role of PVL in *S aureus* pathogenesis.

In this series of hospitalized children with culture-proven, invasive *S aureus* infection, MRSA accounted for 30% of isolates, consistent with recent national data.[12] Of the MRSA isolates, USA300 remained the most common clonotype, accounting for 62% of MRSA isolates; however, this is a departure from our 2009 findings in which >80% of invasive isolates were classified as USA300,[4] suggesting that invasive MRSA disease is becoming more genetically diverse. In addition, USA300 was infrequently identified as a colonization isolate in our study, and MSSA isolates represented ~70% of the isolates across both colonization and invasive sites.

Previous studies have reported a concordance between colonization strains of *S aureus* and strains causing noninvasive disease, such as skin and soft tissue infections. Evidence is conflicting, however, regarding the most likely site of concordance between noninvasive disease isolates and colonization isolates. In 1 study of deep skin abscesses, inguinal isolates were most likely to be concordant,[13] and a similar study found that the throat was the most frequently concordant site of colonization.[14] Even less is known about concordance between colonization isolates and invasive disease, particularly regarding invasive MSSA infection. This is an important distinction as the relative frequency of MSSA infections has increased (compared with MRSA) over the last decade.[4] We observed a high degree of concordance between MSSA invasive isolates and colonization isolates, particularly those isolates from the anterior nares, similar to what has been previously demonstrated for MRSA.[15] This has important infection control implications, as nasal decolonization procedures are currently used in many hospitals for patients colonized with MRSA but not with MSSA.[16]

Our data indicate a low prevalence of the leukotoxin PVL in invasive MSSA isolates (only 16% of isolates harbored the *pvl* locus, compared with 80% of invasive MRSA isolates), consistent with previous reports.[17] Although PVL has been well-described as an epidemiologic marker for community-onset MRSA infection, its role in pathogenesis is unclear, as invasive MSSA isolates typically lack this toxin.[18] Of note,

Table II. Prevalence of purported virulence genes of all isolates collected from 44 children hospitalized with invasive *S aureus* infection, 2013 through 2017*

Molecular characteristics	All N = 86	MRSA N = 27	MSSA N = 59	All invasive N = 44	All colonization N = 42	Invasive MRSA N = 16	Invasive MSSA N = 28	Colonization MRSA N = 11	Colonization MSSA N = 31
agr Type I	47.7	81.5	32.2	56.8	38.0	87.5	42.9	81.8	25.8
agr Type II	24.4	11.1	30.5	20.4	28.6	6.3	25.0	18.2	29.1
agr Type III	27.9	3.7	39.0	22.7	33.0	6.3	32.1	0	45.1
pvl Presence	38.3	74.1	13.6	38.6	26.2	81.2	17.9	72.7	9.6
lukAB Presence	100	100	100	100	100	100	100	100	100
acme Presence	23.3	66.7	3.4	27.3	19.0	68.8	7.1	72.7	0
SCC*mec* IVB	22.1	81.5	N/A	29.5	21.4	87.5	N/A	81.8	N/A
SCC*mec* IIA	3.5	11.1	N/A	2.3	4.8	6.3	N/A	18.2	N/A
SCC*mec* V/VIIC	1.2	3.7	N/A	2.3	0	6.3	N/A	0	N/A

*All values in %.

the *lukAB* gene, which encodes another bi-component leukocidin with potential significance in invasive human disease, was present in all clinical isolates in this study. LukAB is known to be produced in the setting of invasive human disease[9] and may play an important role in virulence in the setting of human infection.

Our study has limitations. The study included children admitted with culture-proven, invasive *S aureus* infection in whom we were able to obtain colonization swabs. As a result, the sample size is limited and precluded a comprehensive analysis for multiple covariates. A larger, multicenter study would be needed to assess subtle epidemiologic differences. Sites chosen for sampling were nares, oropharynx, and inguinal folds; other relevant sites, such as axilla and rectal areas, were not tested. Colonizing sites or density of colonization may differ for MSSA and MRSA. An additional limitation is delay in sampling specified mucocutaneous sites until after beginning parenteral antibiotic therapy in most cases. In addition, all patients were enrolled from a single US medical center and the results may not be generalizable to a wider population.

The epidemiology of invasive, pediatric *S aureus* infections in the US continues to evolve, with a rising role of MSSA. We observed that children with invasive MSSA infections were more likely to have a corresponding colonization isolate, a finding that warrants validation on a larger, multicenter scale so that molecular epidemiologic findings can optimally inform infection control practices. Large-scale validation would also determine whether the detection of specific *S aureus* isolates from colonizing sites can reliably predict the presence of *S aureus* from a sterile site, specifically in patients for whom antibiotic therapy prior to sampling of a sterile site may reduce culture positivity.[19] ■

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