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Research Article

Role and Risk Factors for Community-associated Methicillin Resistant Staphylococcus aureus USA300 Carriage in Children Presenting with and without Skin and Soft Tissue Infections in a Pediatric Emergency Department

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Abstract

The purpose of this study was to examine community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) carriage and infections and determine risk factors associated with specific *S. aureus* strains, specifically MRSA USA300. A case control study was conducted in a pediatric emergency department. Nasal and axillary swabs were collected, and participants were interviewed for risk factors for MRSA infections. The primary outcome was the proportion of *S. aureus* carriers among those presenting with and without a skin and soft tissue infection (SSTI). *S. aureus* carriers were further categorized into MRSA USA300 carriers or non MRSA USA300 carriers. MRSA USA300 carriage rate was higher in children less than 2 years of age, those with an SSTI, children with recent antibiotic use, and those with a family history of SSTI compared to non MRSA USA300 carriers. MRSA USA300 carriers were also more likely to have lower income compared to non MRSA USA300 carriers and no *S. aureus* carriers. Rates of presence of Panton-Valentine leukocidin (PVL) genes were higher in MRSA carriage isolates with an SSTI, with all 39 being USA300, compared to MRSA carriage isolates of patients without an SSTI. Our results indicate risk factors associated with MRSA USA300 carriage were age younger than two years, low income, recent antibiotic use, and previous or family history of SSTI. There is also an association between MRSA USA300 carriage and presence of PVL in those diagnosed with an abscess.

INTRODUCTION

The predominant pediatric community associated MRSA (CA-MRSA) clinical presentation, primarily in the ambulatory settings, is skin and soft tissue infections (SSTIs) [1-4]. The prevalence of MRSA SSTI is likely under reported in outpatient settings since many SSTIs are not submitted for culture testing. In Atlanta, Georgia, the MRSA carriage rate among adults seen in the ED was 7.3% [5,6] but the *S. aureus* carriage rate for children in Atlanta is unknown. In the US, the majority of CA-MRSA SSTIs have been attributed to pulsed-field type USA300 [7,1] but little is known about what are all the risk factors for CA-MRSA USA300 carriage [2,3] or what drives this carriage to then cause SSTIs in the pediatric population [4,8]. In order to explore from an epidemiological perspective how *S. aureus* is associated with development of SSTI in children, especially MRSA USA300,

we determined carriage rates and assessed for associated risk factors among a population of children with and without a *S. aureus* SSTI in a large emergency department (ED) in Atlanta. Based on what has been reported in the medical literature [9-11], we hypothesized that MRSAUSA300 carriage was more highly associated with those who presented with SSTIs compared to those who presented without a SSTI.

MATERIALS AND METHODS

Study Design

This was a case control study performed in the ED of a children's hospital in Atlanta, Georgia. During the study period the ED had 72,722 outpatient visits and 1,114 visits for SSTI.

Recruitment of Study Participants

Patients younger than 21 years of age, who accessed the ED for any condition and were determined to be clinically stable by the attending physician, were eligible to participate and approached by study personnel until 250 children with SSTI and 750 who lacked an SSTI were enrolled (Figure 1).

Study Procedures

After informed consent / assent were obtained, participants and legal guardians were administered a survey pertaining to their demographic, personal and household members' risk factors (Table 1). Two swabs were then collected, one each from the anterior nares and axillae. Moistened swabs were then transported immediately to the clinical microbiology laboratory for plating on selective and non selective media. This study was approved by Institutional Review Boards of participating institutions.

Assessment of Risk Factors for CA-MRSA Carriage and Infection

Medical records of study participants for demographic information and evidence of any previous hospital visits for *S. aureus* infections were reviewed. In the survey, information on age, race/ethnicity, gender, household income and household size was collected. Information on the participant and families' past medical history and living conditions was also collected. Participants were also asked about daycare or school attendance.

Definition of *S. aureus* Carriage

We assigned *S. aureus* carriage to enrolled participants, based on evidence of *S. aureus* detection from swabs taken from nasal or axillary areas, or specimens collected from cultured SSTIs. We then sub-categorized those identified as *S. aureus* carriers into 'MRSA USA300 carriers' (cases) and 'non MRSA USA300 carriers' (control group 1). MRSA USA300 carriers included any participant who had a MRSA isolate from nasal/axillary swabs which was typed USA300 and any participant without a positive MRSA nasal/axillary isolate who had an SSTI isolate predictably MRSA USA300. [8, 12-14] Non MRSA USA300 (control group 1) included all participants who had *S. aureus* isolate not MRSA USA300 isolate from nasal/axillary swabs and participants not found to have *S. aureus* nasal/axillary isolate but had an SSTI for MSSA. If there was no evidence of *S. aureus* either from nasal/axillary swabs or SSTI culture, then participant was categorized as not having *S. aureus* detected ('No *S. aureus*' carriage and assigned as control group 2).

Characterization of *S. aureus* SSTIs

We categorized SSTIs into the following conditions: abscess, cellulitis, infected wound, and other.

Statistical Analysis

Descriptive statistics were used to provide mean value and relative frequency of each variable for all study participants and then for subgroups based on definitions of *S. aureus* carriage and the presence or absence of *S. aureus* SSTI at the time of enrollment. The relationships between MRSA USA300 and non MRSA USA300 (MSSA USA300, MSSA not USA300, and MRSA not USA300) and presence or absence of SSTI, along with epidemiological risk factors were investigated by chi-square and t-test statistics as appropriate. Sensitivity analyses on risk factors were performed to compare MRSA USA300 cases to two different control groups as described above. Logistic regression was applied to assess the bivariate association between carriage status and the presence of risk factors. Bivariate logistic regression analysis was also applied for those factors a priori thought to be associated with risk of MRSA USA300 and then multivariate logistic regression analysis was performed to assess the association between MRSA USA300 and non MRSA USA300 carriage status adjusted for those risk factors. Similarly, multivariate logistic regression analysis was performed to assess MRSA USA300 and no *S. aureus* carriage adjusted for risk factors determined a priori. The log likelihood ratio test was used to assess the significance of variables on the odds of *S. aureus* carriage and specifically, MRSA USA300 carriage. Likewise, we calculated odds ratios as estimates of relative risks, indicating the magnitude of associations, along with corresponding 95% confidence intervals (CI). All tests for significance were two-tailed, and a p-value of < 0.05 was considered significant. Statistical analysis was performed using SAS 9.1 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Study Population Characteristics

From November, 2006 through April, 2008, 2,162 children were approached in the pediatric ED for enrollment. Sixty-six percent (250/380) of children with an SSTI and 42% (750/1,782) of children who lacked an SSTI agreed to participate (Figure 1). Reasons for declining enrollment were similar in both groups.

Risk factors for *S. aureus* Carriage

Participants identified as MRSA USA300 carriers compared to non MRSA USA300 (control group 1) were less than 2 years of age, presented with or had previous SSTI, had recent antibiotic use, and had a household member with past SSTI (Table 1). In comparison, non MRSA USA300 carriers, who were mostly MSSA carriers, were more likely to have a household member employed in healthcare field, have an atopic condition, and if they were less than or equal to 2 years of age, attended day care. When MRSA USA300 were compared to those who had no evidence of any *S. aureus* carriage (control group 2), receiving public health insurance and having lower income also were determined to be significant risk factors.

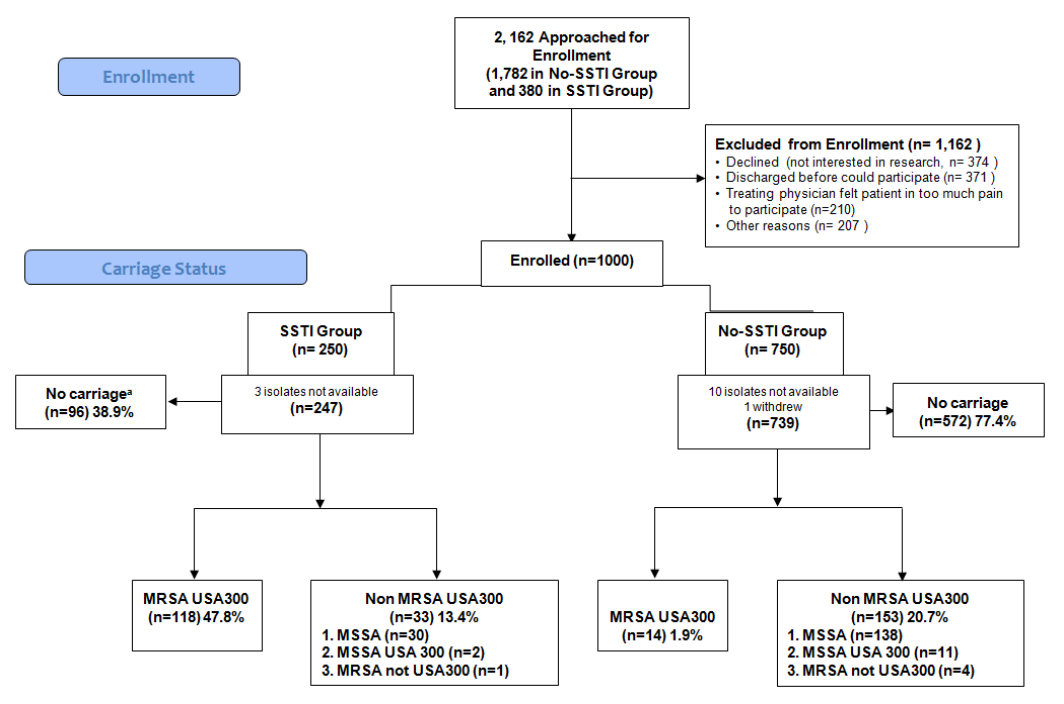

Figure 1 ***Staphylococcus aureus*** **Carriage Enrollment Flow Diagram.** Definition for 'No carriage': No detection of *S. aureus* from SSTI wound culture or no detection of *S. aureus* from cultures obtained from nasal or axillary swabs.

Table 1: Descriptive Characteristics of Population. Comparison of Risk Factors between MRSA USA300 Cases and non MRSA USA300 Controls (Control Group 1) and MRSA USA300 Cases and no evidence of *S. aureus* Controls (Control Group 2).

Characteristic Variable	Cases MRSA USA300 n=132(%)	Control 1 non MRSA USA300** n=186(%)	Control 2 No *S. aureus* n=572(%)
Demographics			
Gender		**P=0.0685**	**P=0.0747**
Male	63 (47.7)	108 (58.1)	322 (56.3)
Female	69 (52.3)	78 (41.9)	250 (43.7)
Age Distribution*		**P< 0.0001**	**P=0.0009**
Birth through 2 years	60 (45.5)	29 (15.6)	162 (28.3)
>2 through 5 years	28 (21.2)	21 (11.3)	139 (24.3)
>5 through 8 years	9 (6.8)	42 (22.6)	99 (17.3)
>8 through 12 years	15 (11.4)	51 (27.4)	81 (14.2)
>12 years	20 (15.2)	43 (23.1)	91 (15.9)
Family Size		**P=0.9164**	**P=0.584**
0-4	78 (59.1)	111 (59.7)	323 (56.5)
> 4	54 (40.9)	75 (40.3)	249 (43.5)
Race/Ethnicity		**P=0.4775**	**P=0.0777**
White	38 (28.8)	44 (23.7)	121 (21.2)
Black	91 (68.9)	135 (72.6)	421 (73.6)
Other	3 (2.3)	7 (3.8)	30 (5.2)
Insurance Type		**P=0.1111**	**P=0.0158**
Self pay	11 (8.3)	25 (13.8)	79 (13.9)
Private	32 (24.2)	54 (29.8)	183 (32.2)
Public	89 (67.4)	102 (56.4)	306 (53.9)
Annual Household Income		**P=0.0079**	**P=0.0027**
Not reported	31 (23.5)	32 (17.2)	126 (22.0)
<$20,000	80 (60.6)	98 (52.7)	264 (46.2)
$20,00-$75,000	13 (9.9)	21 (11.3)	105 (18.4)
>$75,000	8 (6.1)	35 (18.8)	77 (13.5)
Participant Risk Factors			

Presence of SSTI		**P<0.0001**	**P<0.0001**
No	14 (10.6)	153 (82.3)	572 (100)
Yes	118 (89.4)	33 (17.7)	0 (0.0)
Prior Atopic Condition ***		P=0.0062	P=0.5466
No	114 (86.4)	137 (73.7)	482 (84.3)
Yes	19 (13.6)	49 (26.3)	90 (15.7)
Recent Hospitalization or Surgery		**P=0.9416**	**P=0.1127**
No	109 (82.6)	153 (82.3)	502 (87.8)
Yes	23 (17.4)	33 (17.7)	70 (12.2)
Recent Antibiotic Use		**P<0.0001**	**P<0.0001**
No	65 (49.2)	134 (72.0)	418 (73.1)
Yes	67 (50.8)	52 (28.0)	154 (26.9)
History of SSTI		**P<0.0001**	**P<0.0001**
No	102 (77.3)	174 (93.6)	538 (94.1)
Yes	30 (22.7)	12 (6.5)	34 (5.9)
Daycare attendance, ≤2 years of age		**P<0.0001**	**P=0.0098**
No	51 (38.6)	29 (15.6)	156 (27.3)
Yes	81 (61.4)	157 (84.4)	416 (72.7)
Daycare attendance, >2 through 5 years of age		**P=0.0789**	**P=0.4948**
No	34 (56.7)	22 (75.9)	100 (61.7)
Yes	26 (43.3)	7 (24.1)	62 (38.3)
Immunizations up to date		**P=0.1256**	**P=0.0554**
No	14 (10.6)	11 (5.9)	34 (5.9)
Yes	118 (89.4)	175 (94.1)	538 (94.1)
Household Member Risk Factors			
Recent Antibiotic Use		**P=0.4048**	**P=0.0349**
No	69 (52.3)	106 (57.0)	356 (62.2)
Yes	63 (47.7)	80 (43.0)	216 (37.8)
History of SSTI		**P<0.0001**	**P<0.0001**
No	93 (70.5)	173 (93.0)	512 (89.5)
Yes	39 (29.6)	13 (7.0)	60 (10.5)
History of Hospitalizations		**P=0.3942**	**P=0.2929**
No	102 (77.3)	151 (81.2)	465 (81.3)
Yes	30 (22.7)	35 (18.8)	107 (18.7)
History of Surgeries		**P=0.8999**	**P=0.6766**
No	110 (83.3)	154 (82.8)	485 (84.8)
Yes	22 (16.7)	32 (17.2)	87 (15.2)
History of Dialysis		**P=0.5001**	**P=0.6487**
No	131 (99.2)	183 (98.4)	565 (98.8)
Yes	1 (0.8)	3 (1.6)	7 (1.2)
Daycare Attendance		**P=0.5089**	**P=0.7910**
No	84 (63.6)	125 (67.2)	371 (64.9)
Yes	48 (36.4)	61 (32.8)	201 (35.1)
History of Indwelling Catheter		**P=0.5417**	**P=0.5762**
No	125 (94.7)	173 (93.0)	548 (95.8)
Yes	7 (5.3)	13 (7.0)	24 (4.2)
Employed in Healthcare		**P=0.0131**	**P=0.3174**
No	109 (82.6)	131 (70.4)	450 (78.7)
Yes	23 (17.4)	55 (29.6)	122 (21.3)
History of Residing in Congregate Setting		**P=0.111**	**P=0.0728**
No	130 (98.5)	177 (95.2)	543 (94.9)
Yes	2 (1.5)	9 (4.8)	29 (5.1)

* For the multivariate analyses, the age groups were re categorized into 3 groups (birth through 2 years, >2 through 5 years, and >5 years) and Control Group 1, p=0.9129, and for Control Group 2, p=0.0359.

** This analyses was re-run excluding those which were determined to be MSSA USA300 (n=12) from the *S. aureus* non MRSA USA300 cohort, and the significance levels (p<0.05) for the risk factors remained unchanged

***Prior atopic condition: eczema, allergies, and asthma

Abbreviations: MRSA: Methicillin-Resistant *Staphylococcus aureus*; *S. aureus*: Staphylococcus aureus; MSSA: Methicillin-Sensitive *Staphylococcus aureus*

Table 2 shows the adjusted odds ratio for the epidemiological risk factors associated with MRSA USA300 carriers compared to non MRSA USA300 carriers and to those with no evidence of *S. aureus* carriage. In these multivariate analyses, we observed that those younger than 2 years of age who attended daycare were almost 4 times more likely to be MRSA USA300 carriers (aOR 3.67, 95% CI 1.07-12.57) compared to non MRSA USA300 carriers. Similarly, MRSA USA300 carriers had an adjusted odds ratio of 2.51(95% CI 1.47-29) compared to non MRSA USA300 carriers for recent antibiotic use, 4.88 (95% CI 2.08-11.43) for past history of SSTI and 3.91 (95% CI 1.76-8.69) for family history of SSTI. Among those with an SSTI, 48% (118/247) were MRSA USA300 carriers compared to 13% (33/247) non MRSA USA300. In contrast, among those without SSTI at enrollment, only 2% (14/739) were MRSA USA300 carriers compared to 21% (153/739) non MRSA USA300 (Table 3).

No MRSA carriers were found among those who had SSTI cultures which yielded no growth or *Streptococcus pyogenes*. MRSA USA300 carriers (71.2%, 84/118) were also more likely than non MRSA USA300 carriers (39.4%, 13/33) to have an SSTI located below the waist than above the waist (p=0.0008).

S. aureus Carriage Rates Based on Nasal and Axilla Cultures

The positivity rate was 25% (246/986) for *S. aureus* based only from nasal or axilla cultures. These carriage rates for MRSA USA300 and MSSA USA300 were 5.5% (54/986) and 1.3% (13/986), respectively; the remaining non USA300 were mostly all MSSA (17.5%, 173/986) and very few MRSA (0.6%, 6/986). Significant risk factors for nasal/axillary MRSA USA300 carriage were the same as stated previously (data not shown).

Table 2: Multivariate Logistic Regression Analysis of Risk factors Associated with MRSA USA300, Non MRSA USA300 Carriage, and No *S. aureus* Carriage.

Risk Factor	Odds Ratio (non MRSA USA300)	95% CI (non MRSA USA300)	Odds Ratio (No *S. aureus*)	95% CI (No *S. aureus*)
Interaction between Age and Daycare				NS
>2 through 5 years	1.00			
Birth through 2 years	3.67	1.07-12.57		
>5 years	1.00		1.00	
Birth through 2 years	11.47	4.33-30.42	2.14	1.32-3.48
>2years through 5 years	3.13	1.29-7.56	1.02	0.58-1.79
Interaction between Age and No daycare				NS
>2 through 5 years	1.00			
Birth through 2 years	0.78	0.23-2.67		
>5 years	1.00			NS
Birth through 2 years	1.13	0.23-5.52		
>2years through 5 years	1.45	0.19-11.03		
Income				
>$75,000	1.00		1.00	
Not reported	3.21	1.09-9.49	2.13	0.87-5.21
<$20,000	4.18	1.57-11.12	3.13	1.37-7.16
$20,000-$75,000	3.54	1.06-11.82	1.37	0.51-3.68
Prior Atopic Condition *				NS
Yes	1.00			
No	2.47	1.19-5.12		
Recent Antibiotic Use				
No	1.00		1.00	
Yes	2.51	1.47-2.90	2.42	1.58-3.71
Past History of SSTI				
No	1.00		1.00	
Yes	4.88	2.08--11.43	4.45	2.46-8.05
Family History of SSTI				
No	1.00		1.00	
Yes	3.91	1.76-8.69	3.42	2.06-5.67

Abbreviations: *S. aureus*: *Staphylococcus aureus*; SSTI: Skin and Soft Tissue Infection; MRSA: Methicillin-Resistant Staphylococcus aureus; CI: confidence interval

Table 3: Relationship of *S. aureus* Carriage and Presence of SSTI.

Carriage Status	SSTI, n= 247 (%)	No SSTI, n=739 (%)	Odds Ratio (95%, CI)	P-value
No *S. aureus* (n=668)	96 (38.9)	572 (77.4)	1.0	
MRSA USA300 (n=132)	118 (47.7)	14 (1.9)	50.21 (27.71-91.01)	<0.0001
Non MRSA USA300 (n=186)	33 (13.4)	153 (20.7)	1.29 (0.83-1.98)	0.26

Abbreviations: *S. aureus*: *Staphylococcus aureus*; SSTI: Skin and Soft Tissue Infection; MRSA: Methicillin-Resistant *Staphylococcus aureus*

Types of *S. aureus* SSTIs

Abscesses accounted for the majority of SSTIs (75.3%, 186/247). Among patients with an SSTI, only 66.4% (164/247) had a culture submitted from the site of infection. The SSTI culture results were MSSA (18.3%, 30/164), MRSA (65.2%, 107/164), *Streptococcus pyogenes* (2.4%, 4/164), and other (14.0%, 23/164).

Concordance between Nasal and Axillary *S. aureus* Carriage Isolates. Among 237 with positive *S. aureus* nasal isolates, 183 (77.25%) had positive *S. aureus* axillary isolates. Conversely, 183 of the 192 (95.3%) axillary carriers were also nasal carriers. Among the 57 *S. aureus* nasal and axillary pairs designated for typing, there was concordance of PFGE types in 53 pairs (93.3%). Discordant pulsed field types were found for 3 MSSA carriers who lacked an SSTI (USA300, nasal and MSSA novel type, axilla; MSSA Group A, nasal and USA300, axilla; USA700, nasal and USA600, axilla) and one MRSA carrier with an SSTI (USA300, nasal and USA800, axilla).

DISCUSSION

Our study found that children younger than two years were 3.67 times more likely to be MRSA USA300 carriers than all other *S. aureus* PFGE types; this observation persisted even after adjusting for factors such as daycare. Most likely, this is related to the naturally higher bacterial load and moist environment of the diapered area [15]. MRSA USA300 carriers were also more likely than all other *S. aureus* carriers to have SSTIs below the waist, even though the overall distribution of SSTI types was similar between MSSA and MRSA carriers.

Not surprising, we also found that MRSA USA300 carriage was at least 10 fold higher in children with SSTI compared to those who lacked an SSTI. In contrast, the non MRSA USA300 carriage rates (which were mostly MSSA non USA300 strains) were similar between SSTI and no SSTI, suggesting that MSSA carriage is not predictive of development of an SSTI. Our MRSA carriage rate was lower than the 61% observed among those with SSTIs reported by Fritz, S, et al [16]. However, in our study, we also addressed the impact of specific strain types, namely the impact of MRSA USA300 carriage. There was also more heterogeneity in pulsed field types among MRSA carriers who lacked an SSTI compared to those with an SSTI. This also suggests that MRSA USA300 carriage is predictive of development of a MRSA SSTI, particularly of abscesses large enough to warrant the clinician's decision to culture.

LIMITATIONS

This study was limited since it was a convenience sample, and thus, a point-prevalence determination of MRSA nasal and axillary carriage on the day patients were enrolled.

CONCLUSION

Our finding of higher MRSA USA300 carriage in children younger than two years with SSTIs needs to be further explored. Additional studies are also needed to define what host and what specific pathogenic factors might distinguish those who become infected to continue to become persistent MRSA USA300 carriers from those who are merely transient MRSA carriers. Given the strain diversity for both MRSA and MSSA and the variability in which strains spread among household members, more studies are needed to help understand the virulence and host factors which allow certain strains to move from carriage to primary and recurrent infections if we are to wage a successful battle to decrease SSTI in the pediatric population.

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