Carriage of the Toxic Shock Syndrome Toxin Gene by Contemporary Community-Associated *Staphylococcus aureus* Isolates

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We report here the prevalence of the *tst-1* gene among 252 methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates and 458 methicillin-resistant *S aureus* (MRSA) isolates collected from 531 subjects between 2008 and 2017, one of which was recovered from a child with MRSA toxic shock syndrome. *tst-1* was encoded by 43 (6%) *S aureus* isolates overall: 42 (16.7%) MSSA isolates and 1 (0.2%) MRSA isolate ($P < .001$).

Keywords. methicillin-resistant *Staphylococcus aureus*; methicillin-susceptible *Staphylococcus aureus*; staphylococcal cassette chromosome *mec*; toxic shock syndrome; *tst-1*.

Toxic shock syndrome (TSS) is a life-threatening condition most commonly caused by the superantigen toxic shock syndrome toxin 1 (TSST-1), encoded by the *tst-1* gene [1]. This entity was first described in children in 1978 and is generally observed after a surgical procedure, in association with an open wound, or during menstruation in combination with superabsorbent-tampon use [2]. Traditional dogma asserts that *tst-1* carriage was more common among strains of methicillin-susceptible *Staphylococcus aureus* (MSSA) than those of methicillin-resistant *S aureus* (MRSA). Several studies and case reports recently indicated that virulence factors, including TSST-1, have been increasingly associated with clinical isolates of MRSA [1, 3–5].

To date, few studies have investigated the prevalence of *tst-1* and the molecular epidemiology of community-associated MSSA and MRSA clinical isolates in a predominantly pediatric population [6–8]. The objective of this study was to determine the prevalence of *tst-1* in MSSA isolates versus that in MRSA isolates and the molecular epidemiology of these isolates in the context of *tst-1*. We employed a biorepository of samples collected from colonized individuals and pediatric patients with skin and soft-tissue infection (SSTI) or invasive infection. These data ultimately can inform empiric antibiotic therapy for patients with TSS.

METHODS

Specimens for this study were sourced from children with *S aureus* infection, including those with a cutaneous abscess or invasive infection (eg, bacteremia, osteomyelitis, or septic arthritis), who presented to Saint Louis Children's Hospital (SLCH) and/or enrolled in an *S aureus* research study between 2008 and 2017 and from the household contacts of these children [9–11]. At the time of enrollment, colonization swabs from the anterior nares, axillae, and inguinal folds were collected from each participant, and their household contacts when applicable, as previously described [9–11]. The infecting isolate was obtained from the SLCH clinical microbiology laboratory. In total, 531 subjects and their colonizing ($n = 355$) and infecting ($n = 355$) isolates were compiled for this study. Each condition (ie, colonization, SSTI, invasive infection) from which *S aureus* was recovered was considered a unique event for the purposes of this analysis. Subjects from whom more than 1 isolate was recovered contributed 1 isolate per type of condition (eg, 1 colonization and 1 infection isolate) and/or contributed more than 1 isolate of *S aureus* per condition when differing strains were present (eg, 1 MSSA and 1 MRSA isolate or *S aureus* isolates carrying different Staphylococcal cassette chromosome *mec* [SCC*mec*] elements). Demographic and clinical data including age, sex, race, ethnicity, infection type, and isolate source obtained from participant interviews and medical record reviews were documented. The Washington University Human Research Protection Office approved all study procedures.

The identification of *S aureus* isolates and antibiotic-resistance profiling were performed using established procedures [12]. *S aureus* DNA was extracted using the BiOstic bacteremia DNA-isolation kit (Mo Bio Laboratories, Inc., Carlsbad, California). SCC*mec* typing via multiplex polymerase chain reaction (PCR) to detect SCC*mec* types I through V was completed as described previously [13]. SCC*mec* typing was completed for all the MSSA isolates ($n = 252$) and 46% of the MRSA isolates ($n = 209$). Whole-genome sequencing was performed on 436 *S aureus* isolates on the Illumina HiSeq 2000 platform. To determine the

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presence of *tst-1* in each isolate, sequences were aligned to a *tst-1* reference sequence (GenBank accession number J02615.1) using the Burrows–Wheeler Aligner MEM algorithm [14]. Sequence coverage of *tst-1* was reviewed using SAMtools [15], BEDTools [16], and Tablet [17]. *tst-1* detection was performed via PCR using an adaptation of a previously published assay amplifying a 350-bp fragment of *tst-1* that was visualized with a 2% agarose gel on 274 isolates [18]. In addition, PCR was used to validate a subset of genome-sequenced isolates, including all isolates in which the *tst-1* sequence was present.

Descriptive statistics, the Fisher exact test, the Pearson χ^2 test, and logistic regression analysis were performed to determine the prevalence of the *tst-1* gene across subject- and isolate-level factors using SPSS 25 for Windows (IBM SPSS, Chicago, Illinois). *P* values of <.05 were considered significant.

RESULTS

The participants were predominantly white (58.6%) or black (35.4%); 17 (3.2%) participants were of Hispanic or Latino ethnicity (Table 1). The median age among the 531 subjects was 7 years (range, 0.17–76 years), and 50.3% were male. Among the subjects from whom an MSSA or MRSA isolate was recovered, the median ages were 11 and 5 years, respectively ($P < .001$); among subjects with *tst-1* positive ($n = 42$) or *tst-1* negative ($n = 489$) strains, the median ages were 12 and 7 years, respectively ($P = .03$). In our population, 184 (34.7%) subjects provided only colonization isolates, 203 (38.2%) provided only infection isolates, and 144 (27.1%) provided isolates from sites of colonization and infection (Table 1).

Of 710 isolates, 50% were collected from a site of colonization, whereas 36.5% and 13.5% of the isolates were recovered from a cutaneous abscess or site of invasive infection, respectively

(Table 2). Overall, 252 (35.5%) isolates were MSSA and 458 (64.5%) were MRSA (Supplementary Figure). Colonizing and invasive isolates were more often *tst-1* positive (8.6% and 6.3% respectively) than were SSTI isolates (1%) ($P < .001$ and $P = .01$, respectively); *tst-1*–positivity rates between the colonizing and invasive isolates did not differ statistically ($P = .42$). Forty-two (16.7%) MSSA isolates and 1 (0.2%) MRSA isolate were *tst-1* positive ($P < .001$); stated another way, of 43 *tst-1*–positive isolates, 42 (98%) were MSSA. The 1 *tst-1*–positive MRSA isolate was recovered from a previously healthy 3-year-old girl with probable TSS (per the Centers for Disease Control and Prevention case definition [19]), who presented with fever, rash, hypotension, and involvement of 4 organ systems (gastrointestinal, mucous membrane, hepatic, and hematologic). In a logistic regression analysis, recovery from a site of colonization and methicillin susceptibility remained independently associated with carriage of the *tst-1* gene ($P = .03$ and $P < .001$, respectively).

Among the 252 MSSA isolates, those in which SCCmec type III was detected by PCR were most likely to be *tst-1* positive. Specifically, 27 (62.8%) of 43 MSSA isolates carrying SCCmec III were *tst-1* positive, whereas none of the MSSA isolates with an SCCmec I, IV, or V element and only 15 (8.2%) of 184 MSSA isolates not encoding any SCCmec element were *tst-1* positive (both $P < .001$; Table 2). Among the MRSA isolates that underwent typing, SCCmec elements were exclusively types IV (96%) and II (4%); the 1 *tst-1*–positive MRSA isolate was SCCmec type IV.

DISCUSSION

In this study, we evaluated the carriage of *tst-1* in MSSA and MRSA isolates among a predominantly pediatric population

Table 1. Subject-Level Data According to *tst-1* Prevalence

Subject-Level Data	All Subjects (N = 531) (%)	<i>tst-1</i> ⁺ (N = 42) (%)	<i>tst-1</i> [−] (N = 489) (%)	<i>P</i>
Age (median [range]) (years)	7.0 (0.17–76)	12.0 (1–76)	7.0 (0.17–61)	.03
Sex (n)				.08
Female	264 (49.7)	15 (35.7)	249 (50.9)	
Male	267 (50.3)	27 (64.3)	240 (49.1)	
Race (n)				.78
White	311 (58.6)	27 (64.3)	284 (58.1)	
Black	188 (35.4)	12 (28.6)	176 (36)	
Biracial	31 (5.8)	3 (7.1)	28 (5.7)	
Asian	1 (0.2)	0 (0)	1 (0.2)	
Hispanic/Latino (n)				.14
Yes	17 (3.2)	3 (7.1)	14 (2.9)	
No	514 (96.8)	39 (92.9)	475 (97.1)	
Subject type (n) ^a				<.001
Colonization	184 (34.7)	27 (64.3)	157 (32.1)	
Infection	203 (38.2)	8 (19)	195 (39.9)	
Both	144 (27.1)	7 (16.7)	137 (28)	

^aCategorization refers to isolates contributed according to subject (isolate from a site of colonization only, a site of infection only, or both a site of colonization and a site of infection).

Table 2. Isolate-Level Data According to *tst-1* Prevalence^a

Isolate-Level Data	All Isolates (N = 710) (n)	<i>tst-1</i> ⁺ (N = 43) (n)	<i>tst-1</i> ⁻ (N = 667) (n)	P
<i>S aureus</i> isolate (%)				<.001
MSSA	252	42 (16.7)	210 (83.3)	
MRSA	458	1 (0.2)	457 (99.8)	
Isolate type ^b (%)				<.001
Colonizing	355	34 (9.6)	321 (90.4)	
SSTI	259	3 (1.2)	256 (98.8)	
Invasive infection	96	6 (6.3)	90 (93.8)	
Isolate source (%)				<.001
Axilla ^c	87	7 (8.0)	80 (92.0)	
Nares ^c	178	23 (12.9)	155 (87.1)	
Inguinal fold ^c	90	4 (4.4)	86 (95.6)	
Cutaneous abscess	259	3 (1.2)	256 (98.8)	
Blood	50	2 (4)	48 (96)	
Pleural fluid	6	0 (0)	6 (100)	
Joint fluid	13	0 (0)	13 (100)	
Bone aspirate	21	4 (19.0)	17 (81.0)	
Muscle aspirate	3	0 (0)	3 (100)	
Tracheal aspirate	1	0 (0)	1 (100)	
Peritoneal fluid	1	0 (0)	1 (100)	
Pericardial fluid	1	0 (0)	1 (100)	
SCCmec (%)				
MSSA				<.001
None	184	15 (8.2)	169 (91.8)	
I	11	0 (0)	11 (100)	
II	0	0 (0)	0 (0)	
III	43	27 (62.8)	16 (37.2)	
IV	13	0 (0)	13 (100)	
V	1	0 (0)	1 (100)	
MRSA ^d				1.00
II	8	0 (0)	8 (100)	
IV	201	1 (0.5)	200 (99.5)	

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S aureus*; SCCmec, staphylococcal cassette chromosome *mec*; SSTI, skin and soft-tissue infection.

^aDifferences between *tst-1*-positive and *tst-1*-negative isolates were determined via the Pearson χ^2 and Fisher exact tests when appropriate.

^bOne hundred fifty subjects contributed more than 1 isolate: 123 subjects contributed 2 isolates, 25 subjects contributed 3 isolates, and 2 subjects contributed 4 isolates. Subjects with more than 1 isolate contributed 1 isolate per type of condition, except when they had more than 1 strain of *S aureus* per condition. Results of individual comparisons (via the Fisher exact test) between isolate types were as follows: colonizing versus SSTI isolates, $P < .001$; invasive versus SSTI isolates, $P = .01$; and colonizing versus invasive isolates, $P = .42$.

^cAll axilla, nares, and inguinal fold isolates represented colonizing isolates.

^dNone of the MRSA isolates possessed the type I, III, or V SCCmec element. SCCmec typing was performed on 209 (46%) MRSA isolates.

and found that the vast majority (98%) of *tst-1*-positive isolates were MSSA. The only *tst-1*-positive MRSA isolate was recovered from a patient with probable TSS. *tst-1*-positive isolates were more likely to have been recovered from a site of colonization than from a site of SSTI or invasive infection, even when we controlled for methicillin susceptibility.

In contrast to our US study, in which *tst-1* was found in 16% of MSSA isolates and 0.2% of MRSA isolates, the prevalence of *tst-1* in MRSA isolates in other countries has reportedly been higher. Between 2008 and 2012, studies in Iran, China, and France reported 63.5%, 18%, and 60.6% of MRSA isolates possessing *tst-1*, respectively [1, 20, 21]. In addition, in our study, 62.7% of *S aureus* isolates that possessed the SCCmec type III element were *tst-1* positive, which is similar to results from an Iranian study in which 68.4% of SCCmec type III *S aureus* isolates possessed *tst-1* [1]. In a study from Palestine, of the MRSA isolates studied (almost all of which were SCCmec type

IV or V), 40% carried *tst-1* [5]. In conjunction with our study findings, the results of these studies reveal the genetic and geographic heterogeneity of *tst-1*-positive strains.

The major strength of this study is the large number of isolates collected over an extended time period. However, limitations of this study include the convenience sampling of participants who presented with an *S aureus* infection and/or enrolled in an *S aureus* study in the metropolitan St. Louis area, which potentially limits extrapolation of the results to other geographic regions. In addition, the household contacts of children with an *S aureus* infection might have been colonized with a strain concordant with the index patients' disease-causing strain; thus, our study might not have captured the molecular epidemiology of non-disease-causing isolates.

Although studies in other countries have found an increase in the prevalence of *tst-1* carriage in MRSA isolates, the overwhelming majority of *tst-1*-positive isolates in our study were

methicillin susceptible. Our findings suggest that even when MRSA coverage (eg, with vancomycin) is desired, empiric antimicrobial therapy for patients with a clinical presentation consistent with TSS should include a β -lactam antibiotic, given the enhanced bactericidal activity of β -lactams against susceptible strains. Additional research regarding non-disease-causing strains of *S aureus* could further elucidate the contemporary prevalence of *tst-1* carriage in MSSA and MRSA isolates.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Notes

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