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Staphylococcus aureus and methicillin resistance detection directly from pediatric samples using PCR assays with differential cycle threshold values for corroboration of methicillin resistance

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ABSTRACT

Staphylococcus aureus is a major human pathogen, causing a variety of nosocomial and community-acquired infections. While *S. aureus* usually grows well, there are situations where it cannot be isolated in culture, such as patients who have received prior antimicrobial therapy. There are commercially available tests for molecular identification of *S. aureus* and methicillin resistance; however, they often have limited utility due to restrictive specimen requirements, lack of data in pediatric populations and issues with specificity for methicillin resistance detections. Our objective was to evaluate the performance of laboratory-developed PCR assays that detect *S. aureus* and methicillin resistance directly from various specimen types. We developed two real-time PCR assays: 1) a singleplex assay targeting the *nucA* gene and 2) a multiplex PCR assay (mecA/SCC-orf PCRs) that detects the *mecA* gene and the conjunction region where SCC*mec* elements insert into the genome. A total of 538 pediatric specimens, including specimens from the lower respiratory tract (n = 149), abscess/wounds (n = 245), tissue and body fluids (n = 144), were tested and the results compared with culture and susceptibility testing. The nucA PCR is sensitive and specific for detection of *S. aureus* when compared with culture with an overall agreement of 93.1% and sensitivity and specificity of 93.5% and 93.0%, respectively. Among those culture-confirmed and nucA PCR positive specimens (n = 145), concordance between mecA/SCC-orf PCRs, using cycle threshold values for corroboration, and conventional methods was 98.6% and the sensitivity and specificity were 97.3% and 100%, respectively. The assays' performance suggests they are rapid, reliable tools to detect and differentiate between methicillin susceptible and methicillin resistant *S. aureus* in our pediatric patient population providing diagnostic impact when used in conjunction with culture.

1. Introduction

Staphylococcus aureus is a major human pathogen, causing a variety of nosocomial and community-acquired infections (Stryjewski and Corey, 2014). Rapid diagnosis for *S. aureus* and methicillin resistant *S. aureus* (MRSA) infection can result in more targeted antibiotic treatment and potentially reduce the development of antimicrobial resistance. The primary mechanism of resistance to methicillin in *S. aureus* is the production of altered penicillin binding proteins PBP2a or PBP2′ that have low affinity for β-lactam antibiotics (Hiramatsu et al., 2001). Molecular tests targeting both *S. aureus* and methicillin resistance genes may provide rapid results directly from patient specimens without relying on the growth of the organism in culture (Cercenado et al., 2012; Costa et al., 2005; Huletsky et al., 2004).

Traditional culture methods for detection of *S. aureus* and determination of methicillin resistance require up to 48–72 h. In contrast, molecular detection of *S. aureus* and methicillin resistance may take as little as 2 h from the time the sample is received in the laboratory. There are numerous commercially available molecular tests that allow detection of *S. aureus* and MRSA directly from clinical specimens (de San et al., 2007; Mehta et al., 2015; Rossney et al., 2008). However, they often have limited utility due to restricted applications such as screening for colonization, restrictive specimen types, or lack of specific clearance in the United States for use in pediatric populations.

In the study presented here, our aim was to evaluate the performance of two qualitative laboratory developed PCR assays that specifically detect *S. aureus* and methicillin resistance directly from various specimen types from pediatric patients. These PCR assays target a *S.*

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aureus-specific gene (nucA), the mecA gene and the region where SCC*mec* element inserts into the *Staphylococcus* genomes. Further, we used an analysis of cycle threshold (Ct) values of the 3 targets to provide specificity for the detection of MRSA. The performance of these PCR assays was compared with bacterial cultures along with a comparison of molecular detection of methicillin resistance versus in vitro susceptibility testing. In addition to the application of Ct value analysis, this study adds significantly to the limited published studies demonstrating the utility of PCR for of direct detection of *S. aureus* in specimens from a pediatric population.

2. Materials and methods

2.1. Specimen collection

Clinical samples included in the study consisted of two groups: 1) samples submitted for standard of care bacterial culture (December 2014 to March 2015) were selected randomly based on availability, integrity and amount of remnant specimen; and 2) all samples submitted for *S. aureus* PCR after testing was in use for patient care between October 2015 to March 2017, and having concomitant bacterial culture ordered. Body source of the specimens included three specimen groups: non–sterile sites (wound/abscess; $n = 245$), lower respiratory tract specimens (LRT) [bronchoalveolar lavage (BAL), tracheal aspirate and pleural fluid; $n = 149$], and specimens from sterile sites [tissue, body fluid and cerebrospinal fluid (CSF); $n = 144$]. This study was approved by the Institutional Review Board at Nationwide Children's Hospital.

2.2. Bacterial culture

Culture was performed using standard microbiologic solid media (sheep blood, chocolate, MacConkey agars), and for sterile sites a thioglycolate broth was also included. Media were incubated at 37 °C in ambient air or 5% CO_2 as appropriate based on body site. Cultures were examined daily and standard methods were used for identification of any bacterial growth. For *S. aureus* these methods included catalase, latex agglutination and/or matrix assisted laser desorption ionization – time of flight mass spectroscopy (MALDI-TOF).

2.3. Detection of methicillin resistance using conventional methods

Methicillin (Nafcillin) susceptibility was based on cefoxitin screening(CLSI, 2018) or detection of the altered penicillin binding protein. Susceptibility testing for *S. aureus* was performed using the Vitek 2 system with the GP 68 card (BioMerieux, Durham NC) Altered penicillin binding protein testing was done using an immunochromatographic membrane assay (Alere PBP2a, Abbott, Waltham, MA).

2.4. Laboratory-developed S. aureus and methicillin resistance PCR

In-house real-time PCR assays for detection of *S. aureus* and methicillin resistance were developed: 1) a singleplex assay that targets a 90 bp fragment of the *S. aureus* specific *nucA* gene (thermonuclease, nucA PCR) and 2) a multiplex PCR assay (mecA PCR/SCC-orf PCR) that simultaneously detects the *mecA* gene and the conjunction region where the SCC*mec* element inserts into *Staphylococcus* genomes of SCC*mec* types. The SCC-orf PCR was based on the work of Kim with modification and can detect SCC*mec* types I, II, IV, VI and VIII which are the types most common in MRSA in the U.S. (Liu et al., 2016). Briefly, total nucleic acid was obtained by extracting 200-400 µL clinical samples using the NucliSENS easyMag platform (bioMerieux, Durham, NC). For the detection of *S. aureus,* 5 µL of the eluate was added to a 20 µL total volume reaction mixture [1XTaqMan Universal master mix (Life Technologies, Grand Island, NY), 0.25 µM of each primer: *nucA*-F, *nucA*-R and 0 .9µM of probe *nucA*-P. For the detection of methicillin resistance, 5 µL of the eluate was added to a 20 µL total volume reaction mixture [1XTaqMan Universal master mix, 0.25 µM of each primer: *mecA*-F, *mecA*-R, SCC-*orf*-F and SCC-*orf*-R; and 0 .9µM of probes: *mecA*-P and SCC-*orf*-P (Table 1).

Table 1
Primer and probe sequences for nucA, mecA and SCC-orf PCR assays.

Gene target	Oligonucleotide	Sequences (5′-3′)
nucA	*nucA*-F	5′- GGA AAA GGG ACT TGC TAG AGA GAA GT −3′
	nucA-R	5′- CTG TTG TTT AGC TTT ATT TTG TGC TTC TAT −3′
	nucA-P	5′- VIC-CCA AAT GGC AAA TAT –MGB -3′
mecA	*mecA*-F	5′- ACT GAT TAA CCC AGT ACA GAT CCT TTC -3′
	mecA -R	5′- TCC AAA CTT TGT TTT TCG TGT CTT T -3′
	mecA -P	5′- FAM- ATCTATAGCGCATTAGAAAA -MGB -3′
SCC-*orf*	SCC-*orf*-F	5′- TTA TGA AGC GGC TGA AAA AAC C -3′
	SCC-*orf*-R	5′- TCA GCA AAA TGA CAT TCC CAC A -3′
	SCC-*orf*-P	5′- VIC- TCA ATT AAC ACA ACC CGC ATC -MGB -3′

FAM, 6-Carboxyfluorescein; VIC, 2′-chloro-7'phenyl-1,4-dichloro-6-carboxyfluorescein; MGB, Minor Groove Binder.

Thermocycling occurred using the ABI 7500 thermocycler (Life Technologies, Grand Island, NY) with the following running conditions: 50 °C for 2 min, denaturation at 95 °C for 10 min and 45 cycles of 95 °C for 15 s and 60 °C for 1 min.

2.5. Interpretation of PCR cycle threshold values

The combination of PCR results for the *nucA*, *mecA* and SCC-*orf* PCR targets were interpreted as shown in Table 2. In the situation where sterile specimens are tested and a single population of bacterium likely, detection of the *nucA* and *mecA* gene with the same relative quantity measured by Cycle threshold (Ct) values between targets being ≤ 2 was deemed sufficient for detection of MRSA. (Table 2A and Fig. 1)(Kim et al., 2013). When non-sterile clinical specimens were tested, where a mixed bacterial population may be present including both methicillin susceptible *S. aureus* (MSSA) and MR-coagulase-negative staphylococcus (CoNS), presence of all markers at the same relative quantity (Cts, ΔCt between any 2 or the three targets ≤ 2) was considered indicative of MRSA (Table 2B and Fig. 1). When ΔCt between any two targets is > 2, it is considered most likely that a mixed culture of MSSA and MR-CoNS is present in the specimen, less likely indicative of a culture of MRSA that harbors a SCC*mec* type other than those that SCC-orf PCR can detect (types I, II, IV,VI and VIII).

2.6. Clinical data for pediatric patients tested by PCR and culture

The electronic medical record (EMR) was reviewed by an Infectious Disease physician for a select group of patients. Review was limited to those with a positive result by PCR or culture in sterile and LRT samples submitted for *S. aureus* PCR after testing went live (October 2015 to March 2017). Data for demographics, treatment, and clinical course were collected to determine patient characteristics and to investigate the potential impact prior antimicrobial therapy had on testing results and any changes made to treatment as a result of the PCR results.

2.7. Statistical analysis

The median and interquartile range were used to summarize patient age. Comparison of the means of the nucA PCR Ct values in different semi-quantitative culture groups were carried out using one-way ANOVA analysis. *P*-values are presented at the nominal level.

Table 2
Interpretations of PCR results for detection of *S. aureus* and methicillin resistance.

A. Specimens from sterile or lower respiratory tract sites.

nucA	*mecA*	SCC-*orf*	Interpretation	Report
+	+	+	ΔCt between nucA and mecA ≤2, MRSA[a]	MRSA DNA detected
+	+	−	ΔCt between nucA and mecA ≤2, MRSA[b]	MRSA DNA detected
+	+	+/−	ΔCt between nucA and mecA > 2, MRSA or MSSA	*S. aureus* DNA detected
+	−	−	MSSA	MSSA DNA detected
+	−	+	MSSA with SCCmec remnants	MSSA DNA detected
−	+/−	+/−	No *S. aureus*	No *S. aureus* DNA detected

B. Specimens from non-sterile sites

nucA	*mecA*	SCC-*orf*	Interpretation:	Report:
+	+	+	ΔCt between each target ≤2, MRSA[a]	MRSA DNA detected
+	+	+	ΔCt between any target > 2, MSSA or MRSA[c]	*S. aureus* DNA detected
+	+	−	MSSA or MRSA[c]	*S. aureus* DNA detected
+	−	−	MSSA	MSSA DNA detected
+	−	+	MSSA with SCCmec remnants	MSSA DNA detected
−	+/−	+/−	No *S. aureus*	No *S. aureus* DNA detected

MSSA-methicillin susceptible *S. aureus*, MRSA- methicillin resistant *S. aureus*.
[a] MRSA that harbors a SCCmec type of I, II, IV, VI, or VIII.
[b] MRSA that harbors a SCCmec type of III, V, VII, IX, X or XI.
[c] Difference in Ct values (ΔCt) between any 2 of the targets being great than 2 cycles. Most likely mixed culture of MSSA and MR-CoNS, less likely MRSA that harbors a SCCmec type of III, V, VII, IX, X or XI.

3. Results

3.1. Patient population

A total of 538 samples were included in this analysis. All the samples were collected from patients < 21 years of age with a median age of 6.33 years (1.98–12.71 years, interquartile range) and 53.1% (n = 286) being male.

3.2. Detection of S. aureus in various pediatric specimen types

The nucA PCR results were evaluated using standard culture methods as the performance comparator for all three specimen groups. Among the 538 clinical specimens, *S. aureus* was detected in 172 specimens by PCR. One hundred forty-five (83.8%) PCR positive specimens also had concomitant positive culture results. *S. aureus* was not detected in 366 specimens by PCR, among which 356 (97.3%) yielded negative culture results. The overall sensitivity, specificity, PPV and NPV of the nucA PCR were found to be 93.5%, 93.0%, 84.3% and 97.3%,

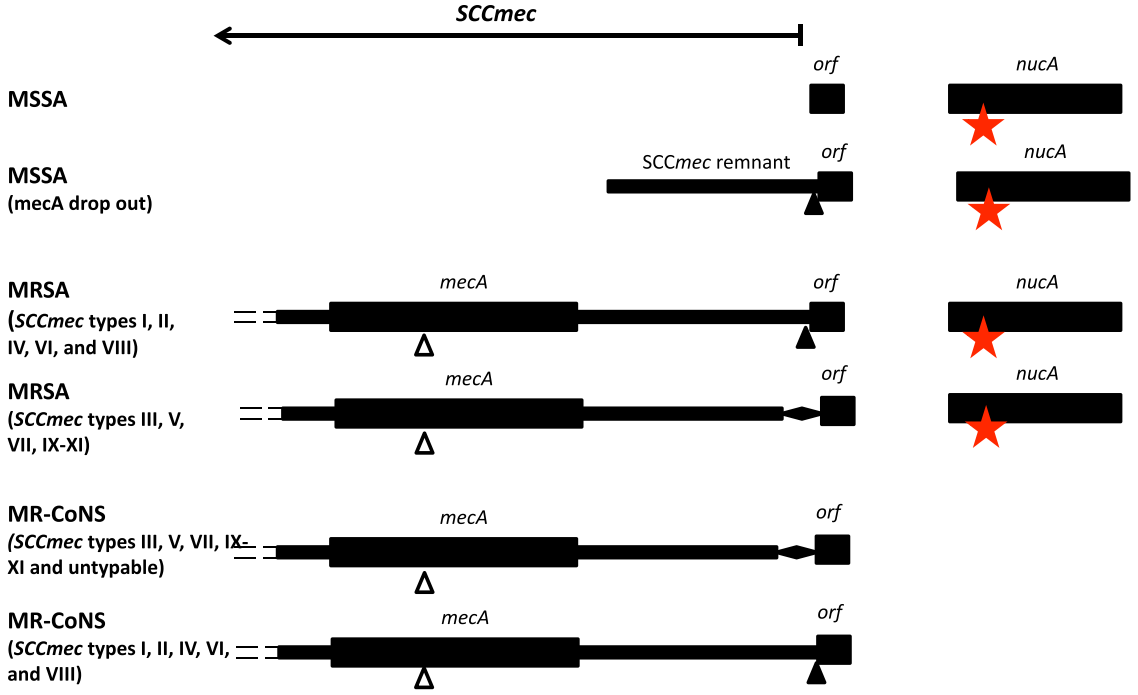

Fig. 1. Schematic diagram showing the genetic elements targeted by nucA PCR (★), mecA PCR (Δ) and SCC-orf PCR (▲). This figure is adapted from ref. (Dubouix-Bourandy et al., 2011).

Table 3
Performance characteristics for nucA PCR compared to culture for detection of *S. aureus* in pediatric specimens types.

		Culture					
		Sterile N = 144		LRT N = 149		Non-sterile N = 245	
		Positive	Negative	Positive	Negative	Positive	Negative
nucA PCR	Positive	11	4	12	6	122	17
	Negative	2	127	5	126	3	103
Sensitivity		84.6%		70.6%		97.6%	
Specificity		96.9%		95.5%		85.8%	
PPV		73.3%		66.7%		87.8%	
NPV		98.4%		96.2%		97.2%	
Concordance		95.8%		92.6%		91.8%	

LRT, lower respiratory tract; PPV, positive predictive values; NPV, negative predictive value.

respectively.

3.2.1. Specimens from sterile sites

Among 144 specimens collected from sterile sites, there were 15 samples with nucA PCR positive indicating *S. aureus* nucleic acid was present (Table 3). Of these 15, eleven were also positive by culture for *S. aureus*. Among the four presumed false positive samples, three were collected from two patients that had been treated with vancomycin prior to the collection. One was collected from a patient who had two courses of antibiotic treatment within 30 days prior to sample collection. Two culture positive specimens were negative by nucA PCR and had semi-quantitative assessments of "rare MSSA isolated". *S. aureus* prevalence based on *nucA* detection was 10.4%. Gram stain results done directly from the specimens were negative for GPC in all the discordant samples (data not shown).

3.2.2. Specimens from the lower respiratory tract

Among 149 specimens collected from the lower respiratory tract, 18 specimens were nucA PCR positive among which 12 had concomitantly positive culture results for *S. aureus* (Table 3). Among the 6 false positive specimens, four were collected from individual patients who received antibiotics prior to specimen collection. Among these four, two patients had *S. aureus* isolated from blood cultures prior to the respiratory specimen collection.

Five specimens were culture positive but negative by nucA PCR, suggesting a false negative PCR result. The corresponding cultures had semi-quantitative assessments of "rare MRSA" ($n = 2$) or" few MSSA" ($n = 3$) isolated. *S. aureus* prevalence was 12.1% based on nucA PCR.

3.2.3. Specimens from non-sterile sites (wound/abscess)

A total of 245 specimens collected from wounds or abscesses were tested. nucA PCR was positive for 139, of which 122 were also culture positive for *S. aureus*. There were 17 specimens that were positive with nucA PCR but culture negative, all but one had high Ct values > 37.0. *S. aureus* prevalence was 56.7% by nucA PCR. nucA PCR did not detect three *S. aureus* culture-positive specimens that had semi-quantitative assessment of "rare MRSA" ($n = 1$) and "few MSSA" (n = 2) (Table 3).

3.2.4. Correlation of Ct values for positive nucA PCR versus culture

The Cts determined by nucA PCR were compared to the semi-quantitative culture results as shown in Fig. 2. The mean Cts between each semi-quantitative culture group were significantly different ($p < .05$) except among groups of rare, few and culture negative.

3.3. Methicillin resistance detection using differential cycle threshold values

The presence of methicillin resistance by PCR was defined as 1) *mecA* detection for sterile and LRT samples or 2) detection of *mecA* and SCC-*orf* for non-sterile samples and the ΔCt among all three targets (*nucA*, *mecA* and *SCC*-orf) being < 2 (Table 2). Table 4 contains the sensitivity, specificity, PPV and NPV for all specimen types. In the sterile specimen group, results from PCR had a 100% agreement with conventional testing results. In LRT specimen group, one MRSA determined by susceptibility testing was identified as MSSA by PCR. This discordant specimen had a semi-quantitative culture assessment of "rare MRSA". In the non-sterile specimen group, one MRSA determined by susceptibility testing was identified as MSSA by PCR. This specimen had a positive nucA PCR (Ct 27.7), positive mecA PCR (27.6) and a negative SCC-orf PCR, which is considered negative for methicillin resistance as all three targets were not positive. We identified 10 samples from non-sterile sites that had all three positive targets but ΔCts > 2. The conventional susceptibility tests suggested they were all MSSA. They would have been misidentified as MRSA without using the Ct analysis and the overall specificity in this specimen type would have dropped from 100% to 83.9%.

3.4. Clinical characteristics of patients with sterile and LRT specimens with S. aureus detected

There were 22 samples from 21 patients in total with a positive result for either PCR or culture, all ordered and reported for patient care (Table S1). All of the samples were collected from in-patients and 10 (47.6%) were previously healthy. There were seven specimens that were PCR positive and culture negative [sterile sites ($n = 4$) and LRT ($n = 3$)] (Table S1). Of these seven specimens, six (85.7%) were from patients who had received antibiotics in the seven days prior to collection.

Twelve specimens were PCR positive and culture positive [sterile site ($n = 8$), LRT (n = 4)]. Nine (75%) specimens were from patients who received antibiotics in the seven days prior to collection. Seven (58.3%) specimens were from patients who were previously healthy. In 1 out of 12 cases, the patient did not receive antibiotics directed to the *S. aureus* that was detected [BAL sample (patient 17), Table S1].There were three samples where the nucA PCR was negative but the culture grew *S. aureus*, all at rare quantitation. All three patients were treated for *S. aureus* infection.

4. Discussion

There is scant data on the detection of *S. aureus* and methicillin resistance using molecular methods in infections among a pediatric population. To our knowledge, this study provides the largest analysis of pediatric specimens providing performance comparisons of PCR and conventional testing for detection of *S. aureus* in addition to the use of differential Ct values for accurate determination of methicillin resistance. While *S. aureus* is not fastidious and is isolated in culture relatively easily, there are situations where it cannot be isolated, such as in patients receiving antimicrobial therapy prior to specimen collection.

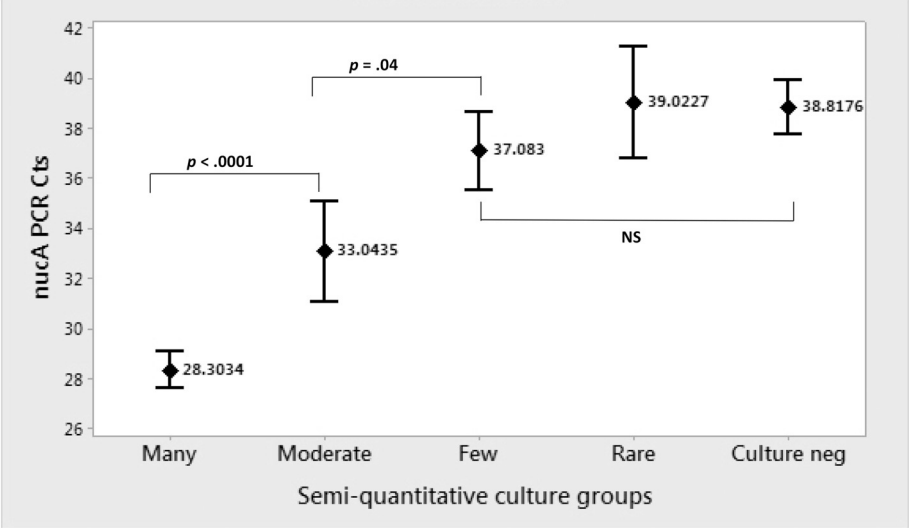

Fig. 2. Comparison of nucA Ct values among different semi-quantitative culture groups. The mean Ct, 95% confidence intervals, and *p*-values between groups are shown. NS: non-significant.

Table 4
Performance characteristics for mecA/SCC-orf PCRs compared to conventional susceptibility testing for detection of methicillin resistance.

		Conventional susceptibility testing					
		Sterile group $N = 9$		LRT group $N = 12$		Non-sterile group $N = 124$	
		MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
PCR	MRSA	2	0	9	0	61	0
	MSSA	0	7	1[a]	2	1[b]	62
Sensitivity		100%		90%		98.4%	
Specificity		100%		100%		100%	
PPV		100%		100%		100%	
NPV		100%		66.7%		98.4%	
Concordance		100%		91.7%		99.2%	

[a] This specimen has a PCR profile as nucA 40.4, mecA Not-detected and SCC-orf 41.7.
[b] This specimen has a PCR profile as nucA 27.7, mecA 27.6 and SCC-orf Not-detected.

As an example of the utility of PCR, during the study period, we identified a 10 year old patient who had a secondary pneumonia after Influenza A virus infection (patient 1, Table S1). The patient had been empirically treated with Vancomycin and Ceftriaxone before she was diagnosed with influenza A at which time the antibacterial coverage was discontinued. The patient returned with ongoing symptoms. A pleural fluid culture was collected and was negative for bacterial growth however, the PCR was positive for MRSA. Based on the PCR result, the patient was restarted on treatment specifically for MRSA and responded. A brief description of clinical findings for all patients with positive results for *S. aureus* by PCR and culture are provided in Table S1.

4.1. Detection of S. aureus by nucA PCR

These data demonstrate that the nucA PCR is sensitive and specific for the identification of *S. aureus* directly from various pediatric clinical specimens. Overall, nucA PCR achieved a high agreement of 93.1% with culture and high negative predictive values among different specimen types. The performance of nucA PCR did vary among the specimen types. It was most specific for specimens collected from sterile sites with lowest specificity from non-sterile sites. Among the 10 specimens that were nucA PCR positive and culture negative from sterile and LRT specimens (Table 3), seven specimens were from patients pretreated with antibiotics, and four of these patients had *S. aureus* isolated from the same or other sites prior to the antibiotic treatment (data not shown). In these cases, the positive nucA PCR suggests that PCR detection is associated with true *S. aureus* infections. On the other hand, in the non-sterile specimens, nucA PCR detected 17 PCR positives that were culture negative while only three culture positive specimens were negative by nucA PCR. This difference between culture and PCR could be more problematic for interpretation in non-sterile specimens and may lead to over treatment due to detection of nucleic acid that does not represent true infection (Wolk et al., 2009). For the sterile and LRT sites, the sensitivity of PCR was not as high as non-sterile sites (84.6, 70.6 and 97.6%, respectively; Table 3). Other studies have evaluated the performance of commercial tests for detection of SA directly from clinical specimens and compared it with culture results, some with sensitivities higher than those reported here (Dubouix-Bourandy et al., 2011; Mehta et al., 2015; Sambri et al., 2017). As with our assays, PCR assays targeting various organisms yield varying sensitivities and specificities in diverse specimen types, possibly due to PCR-inhibiting material in each specimen type (Kern et al., 2009; Levidiotou et al., 2003). Taken together, this suggests that the PCR should only be used in conjunction with routine bacterial culture to reliably detect *S. aureus* in these sample types.

4.2. Detection of methicillin resistance by mecA/SCC-orf PCRs

To date, eleven SCC*mec* types and various subtypes have been reported. The prevalence of each of these types differs geographically. Type II and type IV SCC*mec* are detected as the predominant type in *S. aureus* in the U.S. and other countries (Liu et al., 2016). The SCC*mec* element is also prevalent in non—*S. aureus* staphylococci, even more commonly than in MRSA (Shore and Coleman, 2013), and is less well defined in CoNS as they are often non-typeable due to sequence divergence. Among those typeable SCC*mec* in MR-CoNS, type II and III predominate in Europe and type V predominates in the U.S. Our mecA/SCC-orf multiplex PCRs were carefully designed to simultaneously detect the *mecA* gene and the conjunction region of the SCC*mec* element of types I, II, IV, VI and VIII. The SCC-orf PCR can therefore detect the two SCC*mec* types that predominate in U.S. *S. aureus* isolates but does not detect the type V SCC-orf region in both *S. aureus* and non—*S. aureus* staphylococci.

Table 5
Differences in Ct values between the PCR targets for select clinical samples from Non-sterile sites (MRSA and MSSA) and an ATCC strain of MRSA.

Specimens	Ct values			ΔCt[a]
	nucA	*mecA*	SCC-*orf*	
MSSA from all sites with all targets being positive				
S1	22.1	35.5	40.8	18.7
S2	22.4	32.9	34.8	12.4
S3	23.5	36.4	42.7	19.2
S4	24.9	30.4	42.4	17.5
S5	26.7	40.1	42	15.3
S6	30.3	35.3	42.7	12.7
S7	31.1	33.3	42.9	11.8
S8	33.8	37.5	40.5	6.7
S9	34.1	38.4	39.7	5.6
S10	35.7	38.3	39.4	3.7
S11	43.0	38.4	39.8	4.1
MRSA from Non-sterile sites				
S12	19.6	20.0	20.9	1.3
S13	27.3	27.3	28.8	1.5
S14	35.2	34.5	35.4	0.9
MRSA ATCC 43300				
5000 cfu	27.3	27.4	28.1	0.8
500 cfu	30.4	30.6	31.1	0.7
50 cfu	34.5	34.3	34.9	0.4

[a] ΔCt = the difference in Ct values between the nucA target and the other highest Ct values for the other two targets; S = sample, cfu = colony forming units.

4.2.1. False negative methicillin resistance

False-negative results for methicillin resistance may occur if a MRSA strain harbors a SCC*mec* element with a type that this test cannot detect (e.g. SCC*mec* types III, V, VII, IX, X or XI). Therefore, the result would be a positive mecA PCR and negative SCC-orf PCR. In this study, one such specimen was identified that had PCR results with nucA Ct 27.7, mecA Ct 27.6 and SCC-orf not-detected (Table 4), while the susceptibility test suggested MRSA. We believe this MRSA strain may harbor a SCC*mec* type that cannot be detected by the current PCRs but did not have an isolate available for confirmation. This discordance has also been shown with commercial tests that detect MRSA (Mehta et al., 2015; Mehta et al., 2017; Patel et al., 2014; Peterson et al., 2010). The Xpert MRSA/SA nasal assay (Cepheid, Sunnyvale, CA) targets the *mecA* and SCC*mec* genes for methicillin resistance detection. The rate of false negative MRSA using this test varies geographically with a sensitivity of 79% for MRSA in a French study to 92% in a U.S. population(Belmekki et al., 2013; Patel et al., 2014).

More recently, a highly divergent *mecA* gene termed *mecC* was identified in patients from many countries in Europe (Cuny et al., 2011; Ford, 2018; Garcia-Alvarez et al., 2011; Ito et al., 2012; Paterson et al., 2013; Paterson et al., 2014). *mecC* may not be identified by our mecA PCR or many of the currently available commercial tests (Becker et al., 2014; Belmekki et al., 2013). In our study, only one specimen was identified as MRSA by susceptibility testing and had a negative mecA PCR result (Table 4). The semi-quantitative culture assessment suggested a very low bacterial burden that may be below the LOD of the mecA PCR. While this isolate is unlikely to contain *mecC* gene, it is important to be aware of the emergence of new strains and of the need to continue phenotypic testing using conventional susceptibility methods.

4.2.2. False positive methicillin resistance

Based on the hypothesis that a pure MRSA strain has equivalent copies of *mecA*, SCC-*orf* and *nucA* [18], any specimen with ΔCt > 2 cannot be interpreted as MRSA. In our data among non-sterile samples, there were a total of 10 *S. aureus* isolates determined to be MSSA by susceptibility testing that had positive results in all three targets; all had ΔCts > 2 (Table 5). Among the sterile and LRT samples, there was only one specimen out of the 33 with ΔCt > 2, that was found to be MSSA by conventional testing. In all 11 instances, our ΔCt interpretation prevented miscalling methicillin resistance. There is one FDA-cleared test (MRSA/SA ELITe MGB Assay) using this concept of ΔCt between *S. aureus* and *mecA* targets to determine the presence of MRSA. Published data using this assay have demonstrated better concordance with phenotypic methods than another molecular test that did not account for these factors(Belmekki et al., 2013).

Another common concern relating to false positive results is the increasing incidence of *mecA* dropouts (*S. aureus* lacking a functional *mecA* gene but containing a SCC*mec* junction) (Blanc et al., 2011; Donnio et al., 2002; Donnio et al., 2005) which are estimated to occur in a rate of 7.1% in the MSSA strains in U.S (Mendes et al., 2016). These strains can be identified as MRSA by an assay that does not detect the *mecA* gene directly. Our mecA/SCC-orf PCRs were designed to detect these variants. Of note, among 145 MSSA and MRSA culture isolates, we identified only one isolate (0.7%) that had the empty cassette profile with positive nucA and SCC-orf results and a negative mecA result, which we correctly identified as MSSA.

4.3. Limitations

This is single center and retrospective in nature. The samples were chosen based on a convenience sampling but did cover multiple years. We did not determine the SCC*mec* types in this study so a true prevalence for each type is not known. Due to the complexity of assay design, the testing was set up as two separate assays targeting the most common SCC*mec* types seen in our geographic location. The mecA/SCC-orf PCRs only detect SCC*mec* types I, II, IV, VI, and VII which can prevent some false positive detection when both MSSA and MR-CoNS with other types are present; false negative results will arise when MRSA with a type of SCC*mec* III, V, VIII, IX, X and XI present. In addition, the mecA PCR is not designed to detect the *mecC* gene. Therefore, a negative result of methicillin resistance should be interpreted with caution.

5. Conclusions

In this study, nucA PCR was both sensitive and specific in comparison to culture for the identification of *S. aureus* from pediatric samples, particularly from sterile and non-sterile sites. These PCRs can be used as an adjunct to bacterial culture and routine antimicrobial susceptibility testing particularly in situations where pretreatment may impact culture. The PCR results should be interpreted with careful consideration of the local epidemiology and the patient's complete medical history.

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