2015

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Recommended Citation

Rodriguez, Marcela; Hogan, Patrick G.; Satola, Sarah W.; Crispell, Emily; Wylie, Todd; Gao, Hongyu; Sodergren, Erica; Weinstock, George M.; Burnham, Carey-Ann D.; and Fritz, Stephanie A., "Discriminatory indices of typing methods for epidemiologic analysis of contemporary Staphylococcus aureus strains." Medicine. 94, 37. (2015).
https://digitalcommons.wustl.edu/open_access_pubs/4911

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OBSERVATIONAL STUDY

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Discriminatory Indices of Typing Methods for Epidemiologic Analysis of Contemporary *Staphylococcus aureus* Strains

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Abstract: Historically, a number of typing methods have been evaluated for *Staphylococcus aureus* strain characterization. The emergence of contemporary strains of community-associated *S. aureus*, and the ensuing epidemic with a predominant strain type (USA300), necessitates re-evaluation of the discriminatory power of these typing methods for discerning molecular epidemiology and transmission dynamics, essential to investigations of hospital and community outbreaks. We compared the discriminatory index of 5 typing methods for contemporary *S. aureus* strain characterization.

Children presenting to St. Louis Children's Hospital and community pediatric practices in St. Louis, Missouri (MO), with community-associated *S. aureus* infections were enrolled. Repetitive sequence-based PCR (repPCR), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), staphylococcal protein A (*spa*), and staphylococcal cassette chromosome (SCC) *mec* typing were performed on 200 *S. aureus* isolates. The discriminatory index of each method was calculated using the standard formula for this metric, where a value of 1 is highly discriminatory and a value of 0 is not discriminatory.

Overall, we identified 26 distinct strain types by repPCR, 17 strain types by PFGE, 30 strain types by MLST, 68 strain types by *spa* typing, and 5 strain types by SCC*mec* typing. RepPCR had the highest discriminatory index (*D*) of all methods ($D = 0.88$), followed by *spa* typing ($D = 0.87$), MLST ($D = 0.84$), PFGE ($D = 0.76$), and SCC*mec* typing ($D = 0.60$). The method with the highest *D* among MRSA isolates was repPCR ($D = 0.64$) followed by *spa* typing ($D = 0.45$) and MLST ($D = 0.44$). The method with the highest *D* among MSSA isolates was *spa* typing ($D = 0.98$), followed by MLST ($D = 0.93$), repPCR ($D = 0.92$), and PFGE ($D = 0.89$). Among isolates designated USA300 by PFGE, repPCR was most discriminatory, with 10 distinct strain types identified ($D = 0.63$). We identified 45 MRSA isolates which were classified as identical by PFGE, MLST, *spa* typing, and SCC*mec* typing (USA300, ST8, t008, SCC*mec* IV, respectively); within this collection, there were 5 distinct strain types identified by repPCR.

The typing methods yielded comparable discriminatory power for *S. aureus* characterization overall; when discriminating among USA300 isolates, repPCR retained the highest discriminatory power. This property is advantageous for investigations conducted in the era of contemporary *S. aureus* infections.

(*Medicine* 94(37):e1534)

Abbreviations: CA = community-associated, HA = healthcare-associated, MLST = multilocus sequence typing, MRSA = methicillin-resistant *Staphylococcus aureus*, MSSA = methicillin-susceptible *Staphylococcus aureus*, PFGE = pulsed-field gel electrophoresis, repPCR = repetitive sequence-based PCR, SCC = staphylococcal cassette chromosome *mec*, *spa* = staphylococcal protein A.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have presented a significant burden in healthcare settings for >50 years, serving as a source of severe morbidity and mortality in compromised hosts and posing substantial financial strain on healthcare institutions. In the late 1990s, novel strains of MRSA emerged, affecting immunocompetent individuals without exposure to healthcare settings and thus were designated community-associated (CA) MRSA. These CA-MRSA strains are clinically and genetically distinct from traditional healthcare-associated (HA) MRSA strains and have caused a clonal epidemic of cutaneous abscesses as well as invasive, life-threatening infections among otherwise healthy individuals.[1–8] More recently, this lineage has entered healthcare settings and, in some regions, has become the predominant cause of nosocomial infections.[9–11] Worldwide outbreaks of MRSA infections in both community and healthcare settings necessitate optimized strain typing methods in order to elucidate pathogen transmission dynamics.[12–14]

A number of strain typing methodologies have been developed for investigations of *S. aureus* molecular epidemiology, each based on a slightly different principle. *S. aureus* strains most frequently become resistant to methicillin via

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Editor: Pablo Yagupsky.
Received: July 24, 2015; revised: August 13, 2015; accepted: August 14, 2015.

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Funding: This work was supported by the National Institutes of Health (NIH; UL1-RR024992, KL2-RR024994 and K23-AI091690 to SAF; RC2-HG005680 to GW); the Agency for Healthcare Research and Quality (AHRQ; R01-HS021736 to SAF); the Infectious Diseases Society of America/National Foundation for Infectious Diseases Pfizer Fellowship in Clinical Disease (to SAF); grant support from Pfizer, Inc; and the Children's Discovery Institute (CDI) of Washington University and St. Louis Children's Hospital. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or AHRQ. The funding sources had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

The authors have no conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000001534

acquisition of a staphylococcal cassette chromosome (SCC) *mec* element carrying *mecA*.[15] In the United States, HA- and CA-MRSA strains have classically been distinguished based on SCC*mec* types. SCC*mec* I, II, and III are most abundant in HA-MRSA strains, whereas SCC*mec* IV and V are present in most CA-MRSA strains.[16] Pulsed-field gel electrophoresis (PFGE), the now historical ''gold standard'' for bacterial typing, relies on restriction digestion and subsequent separation of genomic DNA fragments. Minor protocol differences or changes in electrophoresis parameters can result in poor interlaboratory reproducibility. This method is both time-consuming and technically demanding.[17–21] Whereas MRSA pulsotype USA100 represents the traditional strain type causing infections in healthcare settings, USA300 is the predominant MRSA strain type in the community and is also supplanting USA100 in many hospitals.[9–11] Multilocus sequence typing (MLST) is a sequence-based genotyping method, which compares single nucleotide variants within housekeeping genes to a reference database, providing a sequence type (ST).[16] Staphylococcal protein A (*spa*) typing analyzes the number and type of point mutations in the repeat region of the *spa* gene.[16] Repetitive element sequence-based PCR (repPCR) is based on genomic fingerprint patterns to infer relationships among microorganisms using primers that hybridize to intergenic repetitive sequences scattered throughout the genome. Combining repPCR with Diversilab analysis software uses semi-automated objective criteria for assigning strain similarity and provides the ability to simultaneously compare a large number of isolates. A disadvantage of repPCR is that there is no standardized nomenclature at present in use across laboratories for designation of distinct strain types.

Historically, these strain typing methods have been characterized and compared to one another in a variety of settings with classical strains of *S. aureus*.[9,22–26] Given the recent shift in circulating strain types in both community and healthcare settings, particularly the clonal epidemic of USA300 infections, it is essential to re-evaluate the discriminatory power (ie, the capability to identify distinct strains) of these methodologies in the contemporary era to inform approaches used in epidemiologic studies and outbreak investigations.[27] Indeed, in settings with a largely homogeneous population of bacterial strains, genomic sequencing has revealed overestimation of transmission events when methods with inadequate discriminatory power have been used.[24] Thus, the objective of this study was to evaluate the discriminatory index of a variety of strain typing methods in the context of contemporary *S. aureus*.

METHODS

Microbiology and Molecular Typing

RepPCR was performed at the Washington University School of Medicine (WUSM, St. Louis, MO) on 1527 infecting and colonizing *S. aureus* isolates obtained as part of a community-associated *S. aureus* colonization study.[28] The Washington University Human Research Protection Office approved study procedures and written informed consent was obtained from all participants. *S. aureus* isolates were identified and antibiotic susceptibility testing was performed in accordance with Clinical and Laboratory Standards Institute procedures.[29] DNA was extracted from *S. aureus* isolates using the BiOstic Bacteremia DNA Isolation Kit (MO BIO Laboratories, Inc, Carlsbad, CA) according to manufacturer's specifications. RepPCR was performed as previously described[30–32] using the primer RW3A.[33] PCR products were resolved using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc, Santa Clara, CA), and the resulting banding patterns were compared using the Diversilab System software (bioMérieux, Durham, NC). A similarity index of $>95\%$ was used to group isolates as identical strain types. Each distinct strain type (ie, ''reference strain'') was assigned a consecutive number (these repPCR strain types are local designations unique to this study).

As described below, 200 isolates were selected for further analysis. In addition to repPCR, each of these isolates underwent molecular typing by PFGE, *spa* typing, SCC*mec* typing, and MLST. SCC*mec* typing to detect SCC*mec* types I-V was performed at WUSM via multiplex PCR as described previously.[34]

MLST was performed at The McDonnell Genome Institute at WUSM. All *S. aureus* reagent files used in our *in silico* typing were downloaded on August 27, 2014 (http://saureus.mlst.net). These data included allele-specific FASTA nucleotide sequences across the 7 representative *S. aureus* genes (*arcC, aroE, glpF, gmk, pta, tpiA, yqiL*). For MLST review, all of the strain's FASTQ files were independently aligned to a single, complete *S. aureus* reference genome (USA300_TCH1516; GenBank accession no. CP000730.1)[35] using the Burrows-Wheeler Aligner algorithm.[36] Once sample reads were aligned to the reference genome, alignments were collapsed into consensus sequences using various tools (*mpileup, bcftools, vcfutils.pl vcf2fq*) in the samtools[37] package. Once all alleles were assigned a designation for the 7 MLST genes, an ST pattern was defined. Eighteen isolates required manual assignment due to no ST pattern match. For these samples, sequence quality, reference coverage, and read alignments were manually validated using the Tablet[38] genome assembly graphical viewer. For these 18 samples, there were more than adequate levels of high-quality coverage for calling consensus, but the resultant calls included sequence variants (or new ST patterns) not represented in our downloaded MLST identity information, and thus 8 new designations were characterized for the purposes of this study.

PFGE was performed at Emory University School of Medicine (Atlanta, GA) with the *SmaI* restriction enzyme as described previously,[39] now updated using *Salmonella enterica* serovar Braenderup H9182 as the normalization standard. Gel images were compared by using BioNumerics version 5.01 software (Applied Maths, Austin, TX) and assigned to pulsed-field types at 80% relatedness by use of Dice coefficients and the unweighted-pair group method using average linkages.[39]

Spa typing was performed at Emory University School of Medicine as recommended[40] on DNA prepared using the InstaGene Matrix (Bio-Rad, Hercules, CA) according to the manufacturer's instructions with an additional 30-min incubation at 65°C followed by 30 min at 37°C with 20 μg/ml of lysostaphin (Sigma-Aldrich, St. Louis, MO) between step 3 and step 4. PCR fragments were sequenced (Beckman Coulter Genomics, Beverly, MA), and sequences were queried and *spa* type assigned using BioNumerics version 5.01 software (Applied Maths). Although 15 isolates yielded no assigned *spa*-type designation, based on their complete repeat succession data, we were able to characterize these isolates into 9 novel groups for the purposes of this study.

Cohort Generation

It has been established that there is a predominant clone of MRSA circulating in the US at the present time. Therefore, we evaluated *S. aureus* isolates collected from patients with *S. aureus* cutaneous and invasive infections presenting to St. Louis Children's Hospital and community pediatric practices in metropolitan St. Louis, as well as household contacts of these patients, from 2008 to 2011. From an initial collection of 1527 *S. aureus* isolates, a subset of 100 isolates was chosen, selected to enrich for strain type diversity as determined by repPCR. These 100 isolates included up to 5 representatives of each of the less common repPCR strain types, with the remainder of isolates comprising the more common repPCR strain types. To avoid an inherent bias toward repPCR diversity, we selected another subset of 100 isolates, chosen from a distinct collection of 641 isolates amassed during a separate study,[41] that had all been analyzed by MLST. This cohort of 100 isolates was comprised of all the non-ST8 strains in the collection ($n = 69$), with the complement of isolates made up of ST8 strains. The combined cohort of 200 isolates (from 124 patients) was subjected to all typing methods described above and the identity of all isolates as *S. aureus* was confirmed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Discriminatory Index

The discriminatory index (D) of each method was calculated using the standard formula for this metric[27]:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} nj(nj-1)$$

where N is the total number of strains in the sample population, S is the total number of subtypes described, and n_j is the number of strains belonging to each of the subtypes. A value of 1 is highly discriminatory and a value of 0 is not discriminatory.[27] Confidence intervals (95%) for each D were calculated as previously described.[42]

RESULTS

Number of Strain Types and Discriminatory Index *(D)* Per Method

The first cohort of 100 isolates, generated based on repPCR diversity, comprised 20 distinct strain types, as determined by repPCR. We identified 21 distinct strain types by MLST, 16 strain types by PFGE, 43 strain types by *spa* typing, and 5 strain types by SCC*mec*. The methods with the highest D were *spa* typing and repPCR ($D = 0.89$ and $D = 0.87$, respectively), followed by MLST ($D = 0.79$), PFGE ($D = 0.75$), and SCC*mec* ($D = 0.56$) (Table 1).

In the second cohort of 100 isolates, selected for MLST diversity, the number of strain types identified by repPCR was 16. Within this sample set, we identified 21 strain types by MLST, 14 strain types by PFGE, 38 strain types by *spa* typing, and 5 strain types by SCC*mec*. The method with the highest D was MLST ($D = 0.87$), followed by repPCR and *spa* typing (both $D = 0.86$), PFGE ($D = 0.77$), and SCC*mec* ($D = 0.63$) (Table 1).

When evaluating the entire collection of 200 *S. aureus* isolates, we identified 26 distinct strain types by repPCR, 17 strain types by PFGE, 30 strain types by MLST, 68 strain types by *spa* typing, and 5 strain types by SCC*mec*. The methods with the highest D were repPCR ($D = 0.88$) and *spa* typing ($D = 0.87$), followed by MLST ($D = 0.84$), PFGE ($D = 0.76$), and SCC*mec* ($D = 0.60$) (Table 1).

Stratified Analysis by Methicillin Resistance of the Isolates

Of the 200 *S. aureus* isolates, 78 (39%) were MRSA and 122 (61%) were methicillin-susceptible *S. aureus* (MSSA). Among the MRSA isolates, 9 distinct strain types were identified by repPCR, 9 by MLST, 5 by PFGE, 2 by SCC*mec*, and 13 by *spa* typing. The method with the highest D among MRSA isolates was repPCR ($D = 0.64$) followed by *spa* typing ($D = 0.45$) and MLST ($D = 0.44$). Among the MSSA isolates, 24 distinct strain types were identified by repPCR, 25 by MLST, 14 by PFGE, 4 by SCC*mec*, and 61 by *spa* typing. The method with the highest D among MSSA isolates was *spa* typing ($D = 0.98$), followed by MLST ($D = 0.93$), repPCR ($D = 0.92$), and PFGE ($D = 0.89$) (Table 1). We identified 45 MRSA isolates which were classified as identical by PFGE, MLST, *spa* typing, and SCC*mec* typing (USA300, ST8, t008, SCC*mec* IV, respectively); within this collection, there were 5 distinct strain types identified by repPCR.

Analysis of USA300 Subset

Of the 200 *S. aureus* isolates, 92 (46%) were designated USA300 by PFGE. Within these USA300 isolates, 10 distinct strain types were identified by repPCR, 13 by MLST, and 18 by *spa* typing. The method with the highest D among the USA300 isolates was repPCR ($D = 0.63$) followed by *spa* typing ($D = 0.44$) and MLST ($D = 0.42$) (Table 1).

The 108 non-USA300 *S. aureus* isolates represented 16 other PFGE strain types. Of the non-USA300 isolates, 24 distinct strain types were identified by repPCR, 26 by MLST, and 55 by *spa* typing. The method with the highest D among the non-USA300 isolates was *spa* typing ($D = 0.98$), followed by MLST ($D = 0.93$) and repPCR ($D = 0.91$) (Table 1).

Most Frequent Strain Types per Method

Within the collection of 200 *S. aureus* isolates, the most common strain type identified by PFGE was USA300 (46%), by MLST was ST8 (37%), by SCC*mec* was IV (44%), and by *spa* was t008 (35%). The 2 most common strain types identified by repPCR (repPCR type 16 and repPCR type 17) each made up 21% of the collection (Table 2).

DISCUSSION

Staphylococcus aureus molecular typing is essential for epidemiologic studies and outbreak investigations. The optimal typing method may vary depending on the context, including the predominant strain types present in the population, relative clonality of the strains within a collection, and whether the desired resolution is on a local or more global level. Although accurate strain typing and robust discriminatory power is essential, there is at present no consensus in the field regarding a single best molecular typing method for comparing *S. aureus* strain types, and indeed an amalgamation of typing methods may be necessary for producing both high discriminatory power as well as inferring the relatedness of strains.[43] This lack of consensus makes the comparison of epidemiologic findings across studies difficult. Additionally, prior studies comparing molecular typing methods to determine *S. aureus* relatedness have produced widely varying results.[22,44]

Several studies have conducted epidemiologic investigations discriminating strain types based on a variety of phenotypic and genotypic factors, including methicillin resistance. Bocchini and colleagues investigated the similarity of *S. aureus* isolates recovered from recurrent CA *S. aureus* infections in 700 otherwise healthy patients presenting to Texas Children's Hospital. Classifying strains based only on methicillin resistance, this group reported that 90% of recurrences within the first 12 months after initial infection arose from the same strain, compared with 79% of recurrences observed > 12 months after

TABLE 1. Number of Strain Types and Discriminatory Index per Method, N = 200

	Total N = 200		Selecting for repPCR diversity N = 100		Selecting for MLST diversity N = 100		MRSA isolates only N = 78		MSSA isolates only N = 122		USA300 isolates only N = 92		Non-USA300 isolates only N = 108	
Method	**No. of unique strain types**	***D* (95% CI)**	**No. of unique strain types**	***D* (95% CI)**	**No. of unique strain types**	***D* (95% CI)**	**No. of unique strain types**	***D* (95% CI)**	**No. of unique strain types**	***D* (95% CI)**	**No. of unique strain types**	***D* (95% CI)**	**No. of unique strain types**	***D* (95% CI)**
Methicillin resistance	2	0.48 (0.45, 0.51)	2	0.47 (0.42, 0.52)	2	0.49 (0.45, 0.52)	–	–	–	–	2	.37 (0.28, 0.46)	2	.14 (0.05, 0.22)
SCC*mec*	5	0.60 (0.56, 0.63)	5	0.56 (0.52, 0.60)	5	0.63 (0.58, 0.69)	2	0.12 (0.02, 0.22)	4[‡]	0.41 (0.31, 0.51)	5[§]	.37 (0.26, 0.48)	5	.50 (0.40, 0.60)
repPCR	26	0.88 (0.86, 0.91)	20	0.87 (0.82, 0.92)	16	0.86 (0.81, 0.90)	9	0.64 (0.57, 0.70)	24	0.92 (0.90, 0.94)	10	.63 (0.57, 0.68)	24	.91 (0.88, 0.94)
MLST[*]	30	0.84 (0.79, 0.88)	21	0.79 (0.72, 0.86)	21	0.87 (0.82, 0.91)	9	0.44 (0.30, 0.58)	25	0.93 (0.91, 0.94)	13	.42 (0.29, 0.55)	26	.93 (0.90, 0.95)
PFGE	17	0.76 (0.70, 0.82)	16	0.75 (0.67, 0.83)	14	0.77 (0.69, 0.84)	5	0.19 (0.07, 0.31)	14	0.89 (0.87, 0.91)	–	–	16	.90 (0.87, 0.92)
spa[†]	68	0.87 (0.83, 0.92)	43	0.89 (0.83, 0.94)	38	0.86 (0.79, 0.92)	13	0.45 (0.30, 0.59)	61	0.98 (0.97, 0.99)	18	.44 (0.31, 0.57)	55	.98 (0.97, 0.99)

CI = confidence interval, *D* = discriminatory index, MLST = multilocus sequence typing, MRSA = methicillin-resistant *S. aureus*, MSSA = methicillin-susceptible *S. aureus*, PFGE = pulsed-field gel electrophoresis, repPCR = repetitive sequence-based PCR, SCC*mec* = staphylococcal cassette chromosome *mec*, *spa* = staphylococcal protein A.

[*] 18 isolates that do not match a known MLST (9% of the 200 strains overall) have been categorized into 8 novel types A-H based on sequences.

[†] 15 isolates that do not match a known *spa* type (8% of the 200 strains overall) have been categorized into 9 novel types 1–9 based on sequences.

[‡] 4 MSSA isolates were SCC*mec* type I, 12 III, and 14 IV; the remaining 92 isolates were negative for SCC*mec*.

[§] 1 isolate each of SCC*mec* type I (MSSA), II (MRSA), and III (MSSA); 18 isolates were negative for SCC*mec* (all MSSA); the remaining isolates were SCC*mec* type IV (69 MRSA, 2 MSSA).

TABLE 2. Most Frequent Strain Types per Method, N = 200

Methicillin resistance (%)	SCC*mec* (%)	repPCR[*] (%)	MLST (%)	PFGE (%)	*spa* (%)
MSSA (61)	IV (44)	RT 16 (21)	ST 8 (37)	USA 300 (46)	t008 (35)
MRSA (39)	III (6)	RT 17 (21)	ST 5 (9)	USA 200 (11)	t002 (5)
	NEG[†] (46)	RT 3 (13)	ST 30 (9)	USA 800 (9)	
		RT 20 (10)	ST 72 (6)	USA 600 (8)	
		RT 4 (5)	ST 45 (5)	USA 900 (5)	
		RT 24 (5)	ST 15 (5)		
			ST 59 (5)		

NOTE: strain types accounting for < 5% of sample not shown. MLST = multilocus sequence typing, MRSA = methicillin-resistant *Staphylococcus aureus*, MSSA = methicillin-susceptible *Staphylococcus aureus*, NEG = negative, PFGE = pulsed-field gel electrophoresis, repPCR = repetitive sequence-based PCR, RT = repPCR type, SCC*mec* = staphylococcal cassette chromosome *mec*, *spa* = staphylococcal protein A.

[*] repPCR strain types are local designations unique to this study.

[†] All were MSSA isolates.

the baseline infection. From the patients whose recurrent infecting isolate was discordant (by methicillin resistance) from the initial infecting isolate, a random sample (n = 44) of isolates underwent PFGE analysis, which revealed that 98% of the MRSA isolates and 59% of the MSSA isolates were USA300.[22] The findings from this study demonstrate that simple phenotypic distinctions do not necessarily translate to strain type discordance by typing methods that account for a greater proportion of genetic material. In other words, 2 isolates with an identical genetic ''backbone'' as determined by molecular typing methods, which query the entire chromosome within a bacterium (eg, repPCR or PFGE strain type) could be discordant for the *mecA* gene (encoding methicillin resistance), such that MRSA and MSSA isolates may in fact represent concordant strain types. Indeed, several investigations have described MSSA strains with the USA300 genetic lineage,[45,46] as well as those possessing remnants of the SCC*mec* element, as detected in the present study.[47–50]

PFGE has traditionally been considered the gold standard typing method; a standardized typing classification scheme has been established and the nomenclature of this method is widely understood by investigators and clinicians.[39] As the technical aspects of this method are cumbersome and expensive,[51] several groups have attempted to use a combination of molecular markers as surrogate designations for the most frequently encountered PFGE pulsotypes. For example, based on molecular and phenotypic characterization (SCC*mec* type, presence of the Panton-Valentine leukocidin [PVL] and toxic shock syndrome toxin [TSST] genes, and susceptibility to trimethoprim-sulfamethoxazole) of >3500 MRSA isolates submitted from the Active Bacterial Core Invasive MRSA Surveillance Program, the Centers for Disease Control and Prevention (CDC) developed an algorithm to infer PFGE types. MRSA isolates possessing SCC*mec* IV, negative for TSST, and PVL positive (or PVL negative but trimethoprim-sulfamethoxazole susceptible) were inferred to be USA300. Using this algorithm, 87% of isolates were correctly inferred as USA300. Additionally, the criteria of SCC*mec* II and absence of the genes conferring PVL and TSST correctly inferred 98% of USA100 isolates.[52] David and colleagues aimed to compare PFGE with several other genotyping methods (*spa* typing, MLST, SCC*mec* typing, and presence of the PVL, *arcA*, and *opp3* genes) in a sample of 149 MRSA isolates. Within 102 isolates classified by PFGE as USA300, 94% were ST8, 92% were *spa* type t008, and 98% possessed SCC*mec* IV. Among the 24 USA100 isolates, 79% were ST5, 88% were *spa* type t002, and 96% carried SCC*mec* II. The investigators then evaluated the specificity, sensitivity, and positive and negative predictive values of these other typing methods (*spa* type t008, presence of the PVL, *arcA*, and *opp3* genes, MLST ST8, and SCC*mec* type IV) to predict the USA300 pulsotype. The optimal combination of methods by receiver operator characteristic analysis was the presence of the *arcA* and PVL genes (area under the curve 0.98, 95% confidence interval 0.95 – 1.0).[53]

Similar to several published studies, in the present study of community-associated *S. aureus*, considering the entire cohort of 200 isolates, all molecular typing methods (repPCR, *spa* typing, PFGE, and MLST) yielded comparable discriminatory power.[51,54] RepPCR performed superiorly when evaluating a homogenous population of isolates, such as might be studied in an outbreak setting or in discerning transmission dynamics. Specifically, within a cluster of 45 MRSA isolates classified as 1 identical strain type by a combination of typing methods (USA300, ST8, t008, and SCC*mec* IV), repPCR offered further discrimination among these isolates, discerning 5 distinct strain types. Strikingly, within our population of MSSA isolates, *spa* typing yielded the highest discriminatory power ($D = 0.98$). However, the findings in our study of contemporary *S. aureus* strains recovered from otherwise healthy children are in contrast to several other studies, which have found superior discriminatory power of PFGE compared to repPCR.[21,44,55–57] The discrepancies between these studies could be attributable to methodological variation (eg, primers used and automated kits), study populations (hospitalized patients vs individuals in the community), geographic and temporal differences, and predominant circulating strain types.

Whole genome sequencing (WGS) is emerging as the ultimate strain typing tool.[58] Using WGS, Köser and colleagues conducted a case-control study of MRSA isolates recovered from patients in the neonatal intensive care unit in the United Kingdom associated with an outbreak situation. Of 14 isolates sequenced (7 from cases associated with the outbreak and 7 isolates unaffiliated with the outbreak), 10 isolates had identical sequence types by MLST (of note, these were consistent with the most common MRSA clone recovered from hospitals in the UK). Within this group of 10 isolates deemed identical by MLST, phylogenetic analysis generated by WGS grouped the 7 outbreak-associated isolates as identical and differentiated these isolates from the 3 strains not associated with the outbreak. Additionally, WGS illuminated a separate transmission event among the nonoutbreak isolates that had not been previously detected.[25] Similarly, Price and colleagues conducted an evaluation of *S. aureus* transmission and acquisition among ICU patients in the UK. Isolates recovered from surveillance cultures from these patients were subjected to *spa* typing, epidemiologic evaluation (ie, determination of overlapping patient time in the ICU), and WGS. On the basis of WGS, 3 transmission events detected by the combination of *spa* typing and epidemiologic evaluation were discounted; WGS also detected additional acquisition and transmission events that were missed by conventional criteria.[24] Although WGS provides ultimate discriminatory power among genotyping methods, a major challenge with WGS is determining the definition of a ''strain type,'' taking into consideration to which ''gold standard'' strain all isolates should be compared, what degree of genetic variation defines a distinct strain type, and what number of single nucleotide variants are expected due to evolution during microbial DNA replication. At present, given the specialized equipment, expense, time, and technical expertise required to conduct sequencing and analysis, WGS is not yet practical for most routine clinical settings.[53,59,60]

The strengths of the present study are the large number of isolates evaluated and the comprehensive comparison of multiple typing methods with formal calculation of discriminatory index. This study also has several limitations. First, the MRSA isolates were all recovered in 1 geographic region (metropolitan St. Louis, MO). Additionally, the selection of isolates for the first cohort on the basis of diversity as determined by repPCR may have biased our results toward repPCR having a superior discriminatory index; however, within the second cohort of isolates, chosen based on MLST diversity, repPCR produced an almost identical discriminatory index to that of the first cohort of isolates, minimizing the likelihood of this bias.

In conclusion, in our study comparing molecular typing methods for *S. aureus* characterization in the contemporary era, whereas all methods yielded comparable results overall, repPCR demonstrated the highest discriminatory power within the USA300 subset. When planning and implementing epidemiologic studies and outbreak investigations, the discriminatory index of typing methods is an important consideration, particularly in the context of a predominant circulating clone.

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