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Validation of Virulence and Epidemiology DNA Microarray for Identification and Characterization of *Staphylococcus aureus* Isolates▽†

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The human pathogen *Staphylococcus aureus* is isolated and characterized using traditional culture and sensitivity methodologies that are slow and offer limited information on the organism. In contrast, DNA microarray technology can provide detailed, clinically relevant information on the isolate by detecting the presence or absence of a large number of virulence-associated genes simultaneously in a single assay. We have developed and validated a novel, cost-effective multiwell microarray for the identification and characterization of *Staphylococcus aureus*. The array comprises 84 gene targets, including species-specific, antibiotic resistance, toxin, and other virulence-associated genes, and is capable of examining 13 different isolates simultaneously, together with a reference control strain. Analysis of *S. aureus* isolates whose complete genome sequences have been determined (Mu50, N315, MW2, MRSA252, MSSA476) demonstrated that the array can reliably detect the combination of genes known to be present in these isolates. Characterization of a further 43 *S. aureus* isolates by the microarray and pulsed-field gel electrophoresis has demonstrated the ability of the array to differentiate between isolates representative of a spectrum of *S. aureus* types, including methicillin-susceptible, methicillin-resistant, community-acquired, and vancomycin-resistant *S. aureus*, and to simultaneously detect clinically relevant virulence determinants.

Staphylococcus aureus is a common human pathogen responsible for a plethora of infections, from superficial skin infections to life-threatening diseases such as endocarditis, sepsis, and pneumonia. Methicillin-resistant *S. aureus* (MRSA) is a major cause of morbidity and mortality in the hospital setting. The emerging threats of community-associated MRSA (CA-MRSA) and vancomycin-resistant *S. aureus* (VRSA) highlight the importance of rapid detection of such infections.

Most diagnostic microbiology laboratories continue to identify *S. aureus* using traditional culture and susceptibility methods that are slow (48 to 72 h) and provide only limited information. Molecular assays based on PCR have been reported for the detection of MRSA (4, 6, 7, 9, 10, 30), the identification of staphylococcal species (17, 20, 21), or the identification of specific virulence genes (5, 11, 14, 15, 18, 19, 22, 24, 26, 33). DNA microarrays can identify, subtype, and detect acquired antibiotic resistance determinants simultaneously (1, 23, 32, 35); however, their clinical value has been limited by a complicated methodology that is unsuitable for routine use in diagnostic microbiology laboratories.

We have developed an oligonucleotide-based microarray (designated VirEp, for virulence and epidemiology microarray) incorporating 84 clinically relevant gene targets for the characterization and molecular typing of clinical isolates of *S. aureus* in an economical, multiwell format enabling 13 *S. aureus* isolates to be analyzed simultaneously.

MATERIALS AND METHODS

Bacterial isolates and culture conditions. The isolates used in this study (Table 1) were grown at 37°C for 16 h on brain heart infusion agar, except for the VRSA isolates, for which 6 μg/ml of vancomycin was added to the brain heart infusion agar.

Oligonucleotide probe design and synthesis. Oligonucleotide probes were designed using OligoArray 2.0 software (28) and were synthesized at a 10-nmol scale with amino C6 modification (Operon Biotechnologies). Three 45- to 46-mer oligonucleotides with calculated melting temperatures of 68 to 72°C and minimal internal structure were selected for each gene target (see Appendix S1 in the supplemental material). Where this was not possible, shorter or longer oligonucleotides were used.

Slide printing. Oligonucleotides were printed onto amine silane-coated UltraGAPS slides (Corning B.V. Life Sciences) at a concentration of 20 μM in spotting buffer (3 × SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 1.5 M betaine) along with Universal ScoreCard controls (GE Healthcare UK Ltd.) and appropriate positive and negative controls (see the supplemental material). Fourteen replicates of the array were printed on each microarray slide using a QArray Lite robotic arrayer (Genetix Ltd.).

DNA extraction and labeling. Genomic DNA (gDNA) was extracted from *S. aureus* cultures using the DNeasy tissue kit (Qiagen) according to the manufacturer's instructions with the addition of 5 μl of lysostaphin (0.5 mg/ml) and 2 μl of RNase A (100 mg/ml) to the lysis buffer. The concentration of gDNA was determined using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies Inc.). gDNA and spike-in Universal controls (for details, see Appendix S3 in the supplemental material) were labeled with Cy3-dCTP using a protocol based on that described by Pearson et al. (27).

Hybridization. Microarray slides were incubated in prehybridization solution (5× SSC, 0.1% sodium dodecyl sulfate [SDS], 0.1 mg/ml bovine serum albumin) for 60 min at 60°C, washed twice in 0.1× SSC for 5 min and once in purified water for 30 s at room temperature, and then dried by centrifugation. Prior to

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TABLE 1. *S. aureus* isolates used in this study

Isolate	Source[a]	Origin[b]	Site of isolation	Description[c]	Disease association
MRSA252[d,e]	NARSA	United Kingdom	Blood	Sequenced epidemic MRSA	Septicemia
MSSA476[d,e]	NARSA	United Kingdom	N/K	Sequenced CA-MSSA	Osteomyelitis
Mu50[d,e]	NARSA	Japan	Wound/skin	GISA	Wound infection
MW2[d,e]	NARSA	North Dakota	Blood	Sequenced CA-MRSA	Septic arthritis
N315[d,e]	NARSA	Japan	Pharyngeal smear	Sequenced MRSA	N/K
NRS4[d,e]	NARSA	New Jersey	Blood	GISA	Peritonitis
NRS77[d,e]	NARSA	United Kingdom	N/K	MSSA	N/K
NRS111[d,e]	NARSA	United States	N/K	TSST-1-positive MSSA	N/K
NRS157[d,e]	NARSA	France	N/K	CA-MSSA	Necrotizing pneumonia
NRS176[d,e]	NARSA	France	Abscess	TSST-1-positive MSSA	Nonmenstrual TSS
NRS179[d,e]	NARSA	France	Blood	TSST-1-positive MSSA	Scarlet fever
NRS182[d,e]	NARSA	France	Blood	MSSA	Endocarditis
NRS188[d,e]	NARSA	France	Pus	MSSA	Osteomyelitis
NRS192[d,e]	NARSA	Minnesota	Hip/blood	CA-MRSA	Septic arthritis, pneumonia
NRS194[d,e]	NARSA	North Dakota	Pleural fluid	CA-MRSA	Necrotizing pneumonia
NRS229[d,e]	NARSA	France	Blood	CA-MSSA	Necrotizing pneumonia
NRS231[d,e]	NARSA	France	Bone/joint	MSSA	Arthritis
NRS233[d,e]	NARSA	France	Wound/skin	MSSA	Bulbous impetigo
NRS248[d,e]	NARSA	Minnesota	Bronchoalveolar fluid	CA-MRSA	Necrotizing pneumonia
NRS249[d,e]	NARSA	France	Blood	MRSA	Endocarditis
NRS265[d,e]	NARSA	Switzerland	Wound/skin	MRSA	Bulbous impetigo
NRS272[d,e]	NARSA	Belgium	Sputum	GISA	Pulmonary exacerbation
NRS283[d,e]	NARSA	United Kingdom	Blood	GISA	Endocarditis
VRS1[d,e]	NARSA	Michigan	Catheter exit site	VRSA	Wound infection
VRS2[d,e]	NARSA	Pennsylvania	Wound/skin	VRSA	Wound infection
VRS3[d,e]	NARSA	New York	Urine	VRSA	Urinary tract infection
CC7[d,e]	QMC	Nottingham, United Kingdom	Nose	MRSA	Carriage
CC356[d,e]	QMC	Nottingham, United Kingdom	Nose	MRSA	Carriage
RSS035[d,e]	QMC	Nottingham, United Kingdom	Vagina	TSST-1-positive MSSA	TSS
RSS092[d,e]	QMC	Nottingham, United Kingdom	Blood	MSSA	Septic arthritis
RSS136[d,e]	QMC	Nottingham, United Kingdom	Blood	MRSA	Endocarditis
RSS161[d,e]	QMC	Nottingham, United Kingdom	Blood	MSSA	Septic arthritis
RSS199[d]	QMC	Nottingham, United Kingdom	Blood	MRSA	Endocarditis
RSS230[d,e]	QMC	Nottingham, United Kingdom	Blood	MSSA	Osteomyelitis
RSS242[d,e]	QMC	Nottingham, United Kingdom	Blood	MSSA	Endocarditis
RSS254[d,e]	QMC	Nottingham, United Kingdom	Wound	EMRSA-15 variant B1 (PFGE)	N/K
RSS255[d,e]	QMC	Nottingham, United Kingdom	Sputum	EMRSA-15 variant B5 (PFGE)	N/K
RSS256[d,e]	QMC	United Kingdom	N/K	EMRSA-15 UK	N/K
RSS257[d,e]	QMC	United Kingdom	N/K	EMRSA-16 UK	N/K
RSS258[d]	QMC	Nottingham, United Kingdom	Sputum	EMRSA-15 new variant (PFGE)	N/K
RSS289[d,e]	QMC	Nottingham, United Kingdom	Wound	CA-MSSA	N/K
RSS291[d,e]	QMC	Nottingham, United Kingdom	Wound	MRSA	Destruction of skin grafts
MSSA32130[d,e]	J. E. Corkill	Liverpool, United Kingdom	N/K	Pre-MRSA	N/K
MRSA32344[d,e]	J. E. Corkill	Liverpool, United Kingdom	N/K	MRSA corresponding to pre-MRSA	N/K
ND96[d]	N. Day, S. Peacock	United Kingdom	N/K	MRSA	Invasive disease
ND3026[d]	N. Day, S. Peacock	United Kingdom	N/K	Carriage MRSA	Carriage
RSSmec1	D. Morrison	N/K	N/K	MRSA containing SCC*mec* type I	N/K
RSSmec2	D. Morrison	N/K	N/K	MRSA containing SCC*mec* type II	N/K

Continued on following page

TABLE 1—*Continued*

Isolate	Source[a]	Origin[b]	Site of isolation	Description[c]	Disease association
RSSmec3	D. Morrison	N/K	N/K	MRSA containing SCC*mec* type III	N/K
RSSmec4	D. Morrison	N/K	N/K	MRSA containing SCC*mec* type IV	N/K
WIS	T. Ito	N/K	N/K	MRSA containing SCC*mec* V	N/K
H034820381[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H040380042[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H040380045[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H040680209[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H040680232[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H041000045[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H042340013[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H042340015[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H055000318[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H055000319[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H055000320[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H055180446[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H060620441[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H060620443[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H060140520[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H060140521[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H060140523[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K
H060480457[d]	A. Kearns	United Kingdom	N/K	*S. aureus* outbreak isolate	N/K

[a] NARSA, Network on Antimicrobial Resistance in *Staphylococcus aureus*, Focus Technologies Inc., Herndon, VA; QMC, Diagnostic Microbiology Laboratory, Queens Medical Centre, Nottingham, United Kingdom. The affiliations of individuals who supplied isolates are as follows: John E. Corkill, Department of Medical Microbiology, Royal Liverpool University Hospital, Liverpool, United Kingdom; Nick Day and Sharon Peacock, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Donald Morrison, Scottish MRSA Reference Laboratory, Department of Microbiology, Stobhill Hospital, Glasgow, United Kingdom; Teruyo Ito, Department of Bacteriology, Juntendo University, Tokyo, Japan; and Angela M. Kearns, *Staphylococcus* Reference Laboratory, Centre for Infections, Health Protection Agency, London, United Kingdom.
[b] N/K, not known.
[c] TSST-1, toxic shock syndrome toxin 1.
[d] Examined using the complete *S. aureus* microarray.
[e] Examined by microarray and PFGE.

hybridization, ProPlate superstructures (Stratech Scientific Ltd.) were attached to the slides to create a multiwell format. Forty picomoles of Cy3-labeled gDNA in 50 μl of hybridization solution (5× SSC, 0.1% SDS, and 0.1 mg/ml herring sperm DNA) was denatured at 95°C for 5 min. Hybridization mixtures were then added to individual wells on the slide before wells were sealed. Slides were hybridized at 60°C for 16 h in the dark with gentle agitation before being washed in 2× SSC–0.1% SDS at 42°C once to remove the superstructure and once for 5 min. Slides were then washed twice for 5 min in 0.1× SSC–0.1% SDS, five times for 1 min in 0.1× SSC, and once for 10 s in 0.01× SSC. Arrays were dried by centrifugation at 1,600 × *g* for 2 min.

Data analysis. Hybridized slides were scanned with an Axon 4000B slide scanner (Molecular Devices Corporation) using a resolution of 10 μm, and images were analyzed with GenePix Pro 6.0 software. Spots with a signal-to-noise ratio of ≥1 and a total median fluorescence at 532 nm of >1,000 after subtraction of the background fluorescence were classified as positive. For a gene target to be considered present, at least two-thirds of the spots corresponding to that gene target had to be positive. Microarray experiments were MIAME (minimum information about a microarray experiment) compliant, and experimental data were deposited in the ArrayExpress repository (2).

PFGE. *S. aureus* chromosomal SmaI digests were prepared with the GenePath group 1 reagent kit (Bio-Rad Laboratories), and pulsed-field gel electrophoresis (PFGE) patterns were obtained with a contour-clamped homogeneous electric field apparatus (Bio-Rad) as described previously (13). PFGE images were analyzed with BioNumerics 2.0 software (Applied Maths). Dendrograms were generated using the Dice coefficient and the unweighted-pair group method using average linkages (UPGMA) with 1% tolerance and 0.5% optimization. A similarity cutoff of 80% and a difference of ≤6 bands were used to define clusters (29, 31). The presence and absence of genes determined by microarray analysis were recorded in a binary format and processed using Bionumerics 2.0 software, and the results were presented as a dendrogram.

Statistical analysis. The reproducibility of microarray data was examined by calculating the coefficient of variation, which is the standard deviation divided by the normalized mean for replicates hybridized to different microarrays. The discriminatory power of the VirEp (virulence and epidemiology) microarray as a typing method was determined by calculating Simpson's index of diversity (8).

RESULTS

Validation of the complete multiwell format *S. aureus* oligonucleotide microarray by analysis of sequenced *S. aureus* isolates. An initial list of 89 gene targets, including acquired antibiotic resistance determinants, toxins, adhesins, proteases, and other virulence genes, was selected on the basis of the significance of these genes in clinical disease and epidemiology. Four replicates of *S. aureus* isolates MW2, N315, MRSA252, MSSA476, and Mu50 (Table 1) were hybridized to UltraGAPS microarray slides printed with the VirEp microarray. Oligonucleotides for 84/89 genes examined generated the expected results compared to sequencing data. Five gene targets (*sea*, *seg*, *sep*, *edin-B*, and *sdrD*) gave discrepant results and were discarded. The specificity of oligonucleotides for staphylococcal cassette chromosome *mec* (SCC*mec*) types I to V were confirmed by hybridizing gDNA from isolates containing each SCC*mec* element to the VirEp microarray (Table 1). The experimental coefficient of variation for the VirEp microarray was found to be 0.14 (14%), indicating that the data generated by the VirEp microarray are reproducible.

Application of the VirEp microarray to the identification and characterization of clinical isolates of *S. aureus*. Labeled DNAs from a collection of 64 clinical isolates of *S. aureus* were

TABLE 2. Comparison of laboratory and VirEp microarray characterization for diagnosis of 64 clinical isolates of *S. aureus*

Isolate identification[a]	No. of isolates	No. (%) correctly assigned by ViREp microarray
MRSA (*mecA* positive)	38	30 (78.9)
MSSA (*mecA* negative)	10	10 (100)
tst$^+$	6	6 (100)
vanA$^+$	3	3 (100)
PVL$^+$	7	7 (100)

[a] Determined using standard phenotypic and genotypic techniques in the laboratories where the isolates were collected.

hybridized to the VirEp microarray with the remaining 84 gene targets (Table 1). All isolates were correctly identified as *S. aureus* based on the presence of *cap*, *coa*, *cpn60*, *femA*, *nuc*, and *tpi* genes. Ten of ten (100%) isolates were correctly identified as MSSA (Table 2), and 30 of 38 (78.9%) isolates were correctly identified as MRSA (Table 2). However, false-negative results for the *mecA* gene were obtained for eight MRSA isolates from the United Kingdom. Six of the eight isolates were confirmed to possess the *mecA* gene by PCR. We assume that isolates RSS257 and RSS258, which were *mecA* negative by PCR, had lost their *mec* element upon storage at −80°C, which has been reported to be common among MRSA isolates (34). All four CA-MRSA isolates and all three Panton-Valentine leukocidin (PVL)-positive MSSA isolates were correctly identified by the microarray, as were each of six *tst*-positive MSSA isolates and each of three VRSA isolates tested.

Analysis of microarray results revealed that 36/84 gene targets were present in all 64 isolates analyzed while 12 gene targets were absent in all isolates examined (see Appendix S2 in the supplemental material). The conserved genes included the identification genes (11%), genes encoding adhesins (25%), proteases (22%), and toxins (16.5%), acquired antibiotic resistance determinants (16.5%), and molecular typing genes (9%). Additional genes associated with adherence, antibiotic resistance, gene regulation, or production of extracellular virulence factors were found in >96% of isolates examined (*aapA*, *etc*, *hla*, *norA*, *sarA*, *spa*). Of the 12 genes determined to be absent in all isolates tested, 42% encoded antibiotic resistance genes, 33% encoded SCC*mec* type specific genes, 17% encoded toxin genes, and 8% encoded biofilm-related genes. Eight additional genes representing antibiotic resistance determinants (*ermB*, *msrB*, *smr*, *tet*, *tetM*, and *vanA* [identified only in the known VRSA isolates]), SCC*mec* type I, and enterotoxin B were identified in <10% of the isolates studied.

Among the toxin genes, those encoding two exfoliative toxins (*eta* and *etc*), alpha-hemolysin (*hla*), beta-toxin (*hlb*), delta-toxin (*hld*), and gamma-hemolysin (*hlgA*, *hlgB*, *hlgC*) were found to be present in almost all of the isolates examined. The leukocidin encoded by *lukD* and *lukE* was present in 45.3% of isolates, and PVL, encoded by the *lukS* and *lukF* genes, was identified in 10.9% of isolates. Each enterotoxin gene included on the microarray was identified in 6.3% to 67.2% of the isolates examined.

The frequency of antibiotic resistance genes in the isolates studied differed greatly. Genes involved in trimethoprim resistance (*dfrA* and *dfrB*), penicillin resistance (*fmt*), sulfonamide resistance (*folP*), streptogramin A resistance (*lsa*, *vga*), and macrolide resistance (*msrA*) were identified in all isolates examined. Some antibiotic resistance genes (*ereA*, *ereB*, *ermC*, *vat*, and *vgb*) were absent from our study isolates, whereas others (e.g., *blaZ*) were found in the majority of isolates examined (87.5%). Of the three genes encoding components of multidrug efflux pumps screened, *norA* was identified in 98.4% of the isolates examined. In contrast, *qacA* and *qacB* were found in only 11%, and *smr* in only <2%, of the isolates. Although we have included a number of acquired antibiotic resistance genes among the gene targets used, we have not attempted at this stage to use them as predictors of antibiotic susceptibility, because considerable further work is required to confirm that the presence of a resistance gene is correlated with phenotypic resistance in *S. aureus* isolates.

Nine of eleven adhesins included on the microarray were identified in the study isolates. In addition, *spa*, encoding protein A, was found in 98.4% of isolates, and *cna*, encoding a collagen adhesin protein, was found in 75% of isolates. All six proteases included on the microarray were present in all study isolates. Of the three gene targets involved in biofilm formation, one (*icaA*) was present in all isolates, one (*aapA*) was present in 98.4% of isolates, and one (*bap*) was absent from the study isolates. The three genes linked to capsular polysaccharide synthesis (*cap1A*, *cap5A*, and *cap8A*) were identified in all the study isolates. The virulence gene regulator *sarA* was identified in 63 of 64 (98.4%) isolates.

Among the MRSA isolates examined, there were a number of discrepancies with the SCC*mec* types identified by the VirEp microarray. First, 19 of 38 MRSA isolates failed to hybridize with any of the SCC*mec* oligonucleotides included on the microarray. Second, for three isolates, oligonucleotides specific for both SCC*mec* types II and IVc were identified as positive (see Appendix S2 in the supplemental material). Third, five isolates determined to be *mecA* negative generated positive results for SCC*mec* type IVc. Finally, isolate MRSA32344, which was identified as possessing SCC*mec* type I (3), produced a positive result with oligonucleotides specific for SCC*mec* type IVa. The corresponding pre-MRSA isolate MSSA32130 was correctly identified as *mecA* negative, but the SCC*mec* element was not detected.

Analysis of the population structure of clinical *S. aureus* isolates using PFGE and the VirEp microarray. All replicates of the internal-control *S. aureus* isolate (NCTC8325) included in the PFGE analysis produced identical restriction fragment profiles clustering at 100% similarity, demonstrating the reproducibility of the method. The 43 test isolates generated 34 PFGE profiles according to the criteria of Tenover et al. (31) and were separated into seven clusters containing 41 out of 43 isolates (Fig. 1). Control isolate NCTC8325 and test isolate NRS265 were the only isolates found to be outliers. Cluster 1 contained 14 isolates (33%) from the United Kingdom, the United States, and France associated with a variety of diseases and included VRSA, MRSA, MSSA, CA-MRSA, and PVL-positive MSSA. Cluster 2 contained two isolates (5%): one from Nottingham, United Kingdom, associated with septic arthritis and one from France linked to endocarditis. Cluster 3 contained seven isolates (16%) from France, Japan, and the United States that were associated with different disease outcomes and included VRSA, glycopeptide-intermediate *S. aureus* (GISA), and MSSA. Cluster 4 consisted of two isolates (5%), one from France (MRSA) and one from Belgium

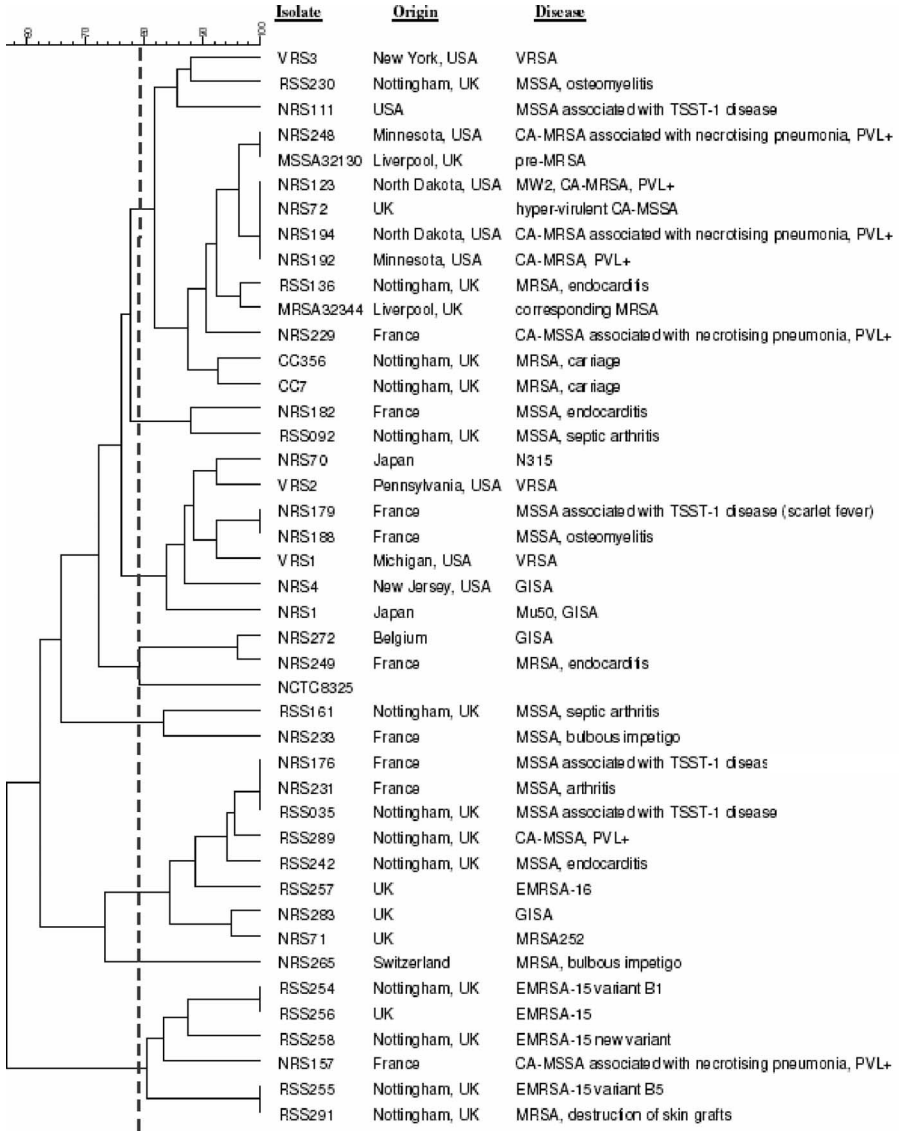

FIG. 1. Dendrogram of 43 *S. aureus* isolates examined by PFGE, produced with Bionumerics (version 2.0) software using the Dice coefficient and UPGMA. Isolates were clustered using the criteria of Tenover et al., where 80% similarity is the cutoff for differentiating closely related isolates (31). Clusters 1 through 7 are shown.

(GISA). Cluster 5 was also made up of two isolates (5%), one from France and one from Nottingham, both MSSA. Cluster 6 contained eight isolates (19%) from France and the United Kingdom and included two epidemic MRSA-16 (EMRSA-16) isolates, as well as PVL-positive MSSA and MSSA isolates. Cluster 7 contained five EMRSA-15 isolates from the United Kingdom and one PVL-positive MSSA isolate from France.

Replicates of sequenced isolates (Mu50, N315, MW2, MRSA252, and MSSA476) clustered together at 100% similarity when analyzed by the VirEp microarray, demonstrating the reproducibility of the assay. As expected, the closely related isolates Mu50 and N315, as well as isolates MW2 and MSSA476, were found to group together (16). The population structure of the 43 *S. aureus* clinical isolates as determined by the VirEp microarray is shown in Fig. 2. The similarity cutoff for distinguishing genotypes using the VirEp microarray (93.5%) was established by determining the percentage of similarity that grouped the sequenced *S. aureus* isolates correctly, as elucidated by PFGE (Fig. 2). Genotype A contained 10 isolates (23%), mainly CA-MRSA and MSSA from the United Kingdom, the United States, and France. Genotype B comprised eight MRSA and MSSA isolates (19%) from Nottingham, France, and Switzerland. Genotype C was composed of two isolates (5%), a GISA isolate from Belgium and a MRSA isolate from France. Genotype D contained eight isolates (19%), including all three VRSA, GISA, and MSSA isolates from the United States, France, and Japan. Finally, genotype E contained 14 isolates (33%) from the United Kingdom and France, including both EMRSA-15 and -16 isolates, PVL-positive MSSA, and MSSA.

Analysis of 18 *S. aureus* outbreak isolates (Table 1) alone, using an arbitrary cutoff of 96% similarity, indicated that the isolates fall into two genotypes with one outlier. Comparison of

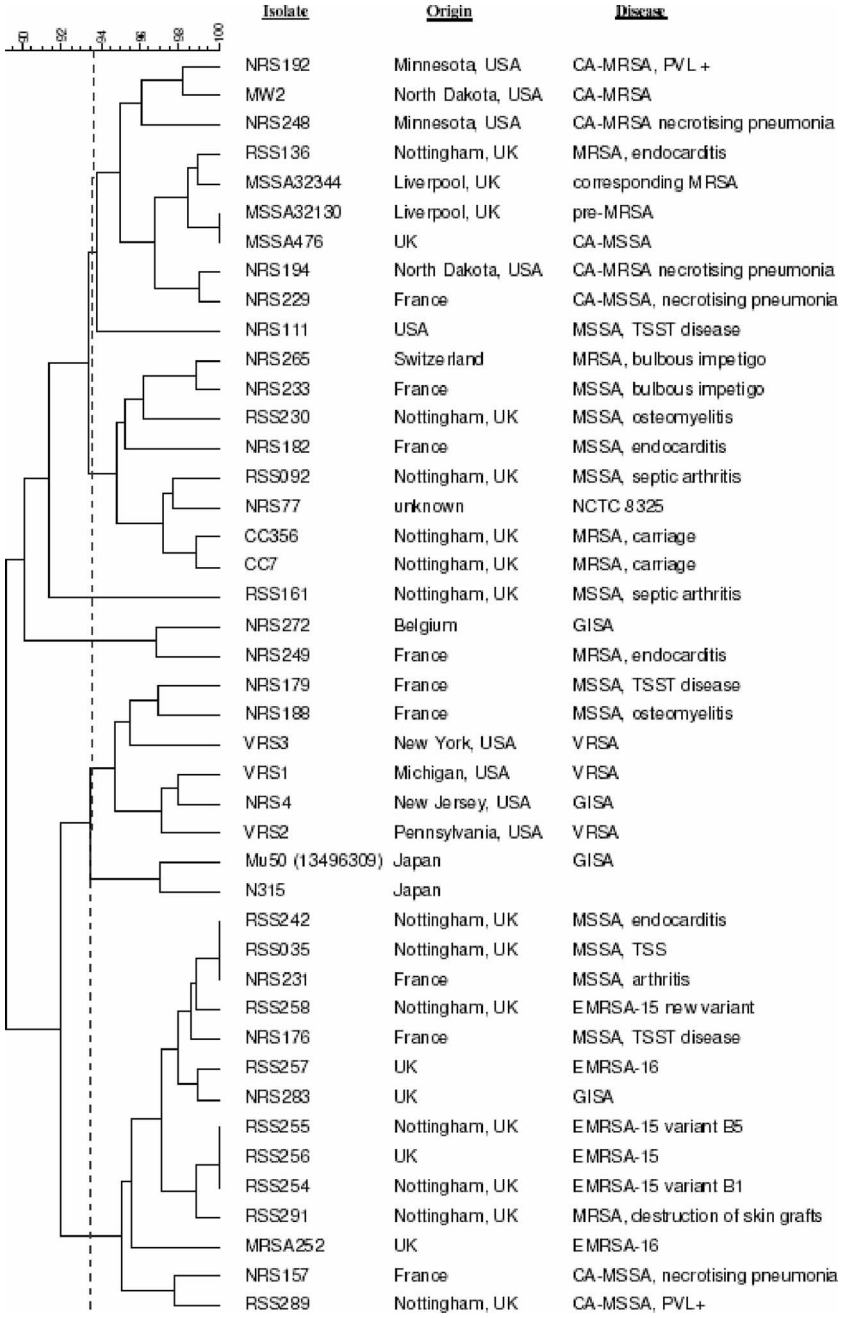

FIG. 2. Dendrogram of 43 clinical *S. aureus* isolates examined by the VirEp microarray, produced with Bionumerics (version 2.0) software using the Dice coefficient and UPGMA. A cutoff value of 93.5% was used to distinguish genotypes. Genotypes A through E were distinguished.

microarray and PFGE results for these isolates revealed that 17 of the 18 isolates examined grouped in agreement (Table 3).

DISCUSSION

The VirEp microarray described in this paper represents a new tool for the identification and characterization of *S. aureus* isolates. The benefit of the VirEp microarray for *S. aureus* lies in its ability to simultaneously identify numerous virulence genes while avoiding the complexities of high-density microarray analysis. The VirEp microarray successfully identified all PVL-positive isolates, all *tst*-positive isolates, and all VRSA isolates (Table 2), demonstrating that these clinically relevant *S. aureus* virulence genes can be detected by this assay. This contrasts with the limited number of genes that can be detected by PCR-based assays. While the presence of such genes does not necessarily equate with expression of the protein product, it does give a good indication to the clinician of the pathogenic potential of the isolate, which can be used to guide appropriate antimicrobial chemotherapy and infection control measures.

Furthermore, the fact that six of the eight MRSA isolates

TABLE 3. Comparison of PFGE and VirEp microarray results for 18 *S. aureus* outbreak isolates

Isolate	Result by:	
	HPA PFGE (cluster)[a]	ViREp microarray (genotype)[b]
H034820381	1	E2
H040380042	1	E2
H040380045	1	E2
H040680209	1	E2
H040680232	1	E2
H041000045	1	E2
H042340013	1	E2
H042340015	1	E2
H055000318	2	E1
H055000319	2	E1
H055000320	2	E1
H055180446	2	E1
H060620441	2	E1
H060620443	2	E1
H060140520	2	E1
H060140521	2	Outlier
H060140523	2	E1
H060480457	2	E1

[a] Standard PFGE methodology was used at the *Staphylococcus* Reference Laboratory, Health Protection Agency (HPA), London, United Kingdom. Clusters were defined using the criteria of Tenover et al. (31).
[b] Genotypes were differentiated using a 96% similarity cutoff.

from the United Kingdom failed to hybridize to the *mecA* probes on the VirEp microarray yet contained the *mecA* gene by PCR indicated that the DNA sequences of *mecA* in the regions corresponding to the three probes are not conserved among all MRSA isolates. This suggests that for some genes, including *mecA*, sequence variation may be much greater in the wider population than among the few *S. aureus* isolates that have been sequenced to date. As a consequence, it is clear that further oligonucleotides are required to increase the robustness of *mecA* detection, a problem that is being encountered with all rapid MRSA detection systems.

The dendrogram generated from the VirEp microarray results illustrates that in general, the grouping of isolates was highly congruent with that observed with PFGE (Fig. 1 and 2). However, all three VRSA isolates were assigned to the same genotype as the EMRSA-15 and -16 isolates (Fig. 1 and 2). It is noteworthy that the percentage of similarity required to differentiate the sequenced *S. aureus* isolates by using the microarray results is 93.5%, whereas for PFGE it is 80% (31). This indicates that the microarray analysis provides slightly less discrimination than PFGE, probably due to the limited number of gene targets included in the microarray. This was confirmed when values for Simpson's index of diversity were calculated for the VirEp microarray and PFGE (0.771 versus 0.811). Unlike PFGE, however, the microarray provides biologically meaningful data in addition to the typing data. When isolates from two epidemiologically distinct outbreaks were examined by the VirEp microarray and PFGE, only a single incongruent isolate was found. The differences observed with this isolate were due to a difference in gene content (*lukD* positive, *sem* negative) (see Appendix S2 in the supplemental material) that could not be detected by PFGE. There were three differences in gene content between genotypes E1 and E2. Genotype E1 lacked the *ermA*, *mupA*, and *tst* genes, whereas genotype E2 possessed these genes (see Appendix S2 in the supplemental material). These data provide evidence that the VirEp microarray may be capable of distinguishing *S. aureus* isolates from different outbreaks. The VirEp microarray could be refined by the inclusion of additional selected targets that could improve the discriminatory power of the microarray. A recent study has demonstrated the potentially superior resolving power of microarrays compared to PFGE and multilocus sequence typing for typing of CA-MRSA isolates (12).

We anticipate that the VirEp assay could be used after presumptive staphylococci (gram-positive cocci in clusters) have been observed in a positive blood culture and species identification has been confirmed by an alternative methodology, such as a rapid PCR-based assay or fluorescence in situ hybridization with peptide nucleic acid probes (25). The inclusion in the array of oligonucleotides to detect coagulase-negative staphylococci would indicate if the blood culture was a mixture of *S. aureus* and coagulase-negative staphylococci. Our current development work has been successful in reducing the time taken to perform the VirEp assay to <24 h.

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