

Rapid Identification of Methicillin-Resistant *Staphylococcus aureus* Transmission in Hospitals by Use of Phage-Derived Open Reading Frame Typing Enhanced by Multiplex PCR and Reverse Line Blot Assay[∇]

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The relatively high-level clonality of methicillin-resistant *Staphylococcus aureus* (MRSA) and its frequent high-level endemicity in nosocomial settings complicate the development of methods for rapid subtyping of MRSA strains that are capable of identifying person-to-person transmission in hospitals. Phage-derived open reading frame (PDORF) typing is an MRSA typing method targeting mobile genetic elements that was recently described and evaluated using a geographically restricted set of isolates. The objective of this study was to develop a multiplex PCR-reverse line blot (mPCR/RLB) assay for PDORF typing and to test its applicability on a broad range of isolates and in an environment where MRSA is highly endemic. The 16 targets were identified using a 23-primer-pair mPCR/RLB assay with two probes for each target. The method was evaluated using 42 MRSA reference strains, including those representing major international clones, and 35 isolates from episodes of suspected nosocomial transmission. *In vivo* stability was explored using 81 isolate pairs. Pulsed-field gel electrophoresis (PFGE) and *spa* typing were performed for comparison. Among the 42 reference strains, there were 33 PFGE subtypes, 30 PDORF types, and 22 *spa* types. Simpson's index of diversity was 0.987, 0.971, and 0.926 for PFGE subtyping, PDORF typing, and *spa* typing, respectively. Typing of clinical isolates by PDORF typing and PFGE demonstrated concordant results. mPCR/RLB-based PDORF typing has similar discriminatory power to that of PFGE, can assist in tracking MRSA transmission events in a setting of high-level endemicity, and has the advantage of being a high-throughput technique.

A range of methods for rapid identification and subtyping of methicillin-resistant *Staphylococcus aureus* (MRSA), which can improve the effectiveness of infection control procedures, have been reported (9, 13, 15, 16). However, their ability to detect person-to-person transmission in settings with high-level endemicity has not been evaluated fully. Phage-derived open reading frame (PDORF) typing is a typing method for MRSA that was recently described from Japan and was reported to have similar discriminatory power and stability to those of pulsed-field gel electrophoresis (PFGE) (16). PDORF typing interrogates the presence or absence of 16 variable elements within the *S. aureus* genome: 13 from integrated prophages, 1 from a genomic island, and 2 from the SCC*mec* element. In its original form, PDORF typing involves four 4-plex PCRs, each with product detection by agarose gel electrophoresis (16).

Reverse line blot (RLB) assay is a high-throughput and inexpensive method for product detection from multiplex PCR (mPCR) assays (6). Due to the high sensitivity of RLB probes, "megaplex" PCRs can be utilized, usually allowing amplification of all targets in a single reaction tube. Up to 43 isolates can be interrogated with up to 43 probes on a single reusable

membrane. Several membranes can be processed simultaneously, with a total turnaround time of 12 h. The objectives of this study were to develop a high-throughput mPCR-based RLB assay (mPCR/RLB) for PDORF typing and to determine whether PDORF typing would retain its discriminatory power in other geographic settings or in a setting with high-level MRSA endemicity.

MATERIALS AND METHODS

Selection of isolates. Isolates studied fell into the following four groups: (i) 5 *S. aureus* isolates for which whole genome sequences are publicly available (MRSA isolates COL, MW2, Mu3, and Mu50 [GenBank accession numbers CP000046, BA000033, AP009324, and BA000017] and methicillin-susceptible *S. aureus* strain NCTC8325 [GenBank accession number CP000253]), utilized for *in silico* analysis; (ii) 42 molecularly well-characterized MRSA reference strains which were temporally and/or geographically unrelated and represented major clones circulating in Australia and overseas (2); (iii) 35 MRSA clinical isolates from two Sydney tertiary hospitals, collected between September 2006 and July 2007; and (iv) pairs of isolates obtained from 81 patients at intervals ranging from 34 to 993 days (median, 146 days) for study of *in vivo* stability. The 35 clinical isolates consisted of 14 isolates from inpatients with nosocomial acquisition, that is, those who had had a documented negative MRSA screen (nose, axilla, and perineum) and subsequently acquired MRSA infection or colonization during the same admission, along with isolates with matching antibiograms from MRSA-colonized patients who shared the same ward at the time of MRSA acquisition and thus were likely sources of transmission. Both hospitals have a high prevalence of MRSA colonization and infection. At one hospital, a recent point prevalence survey indicated that 25% of patients within surgical wards carried MRSA, with a hospital-onset MRSA bacteremia rate of approximately 1.1 per 10,000 occupied bed days for the whole facility.

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TABLE 1. Primers and probes used in this study

Target	Oligonucleotide ^a	Sequence (5'-3') ^b	GenBank accession no.
Tn554 <i>tnpB</i>	N046Fb	1746-TGATGGAGAGGAGTGGGATA-1765	X03216
	N046AP	1789-AGTTACGTCTATCCCAAACGTCTT-1766	X03216
	N046SP	2096-TGGAAGGACTATTTAGTACCCTTCTTA-2122	X03216
	N046Rb	2143-GAACATCATCCCATTCTAGCC-2123	X03216
Mu50 SaGI _m SAV0803	SAV0803Fb	881430-CAATATCCAAACCACGACCC-881411	BA000017
	SAV0803AP	881376-GATGAAAAGCAAATATACATATAACTCTACATG-881410	BA000017
	SAV0803SP	881245-TCTTTAATATTTCTTTATGATTGTATTATTATATGTATA TG-881203	BA000017
	SAV0803Rb	881183-AAAAATAGCGCCAACAGTCC-881202	BA000017
φMu50B SAV0881	SAV0881Fb	932269-TGCTTGTGTGCATATCGCC-932287	BA000017
	SAV0881AP	932316-TGTTTGGTAACTAGCCACTGTATAGATA-932288	BA000017
	SAV0881SP	932418-TCAAATTTCTTTTTGAATAGTAAGTCAGA-932446	BA000017
	SAV0881Rb	932468-CCTAGCTTGTATGTCTGCGCTA-932447	BA000017
φMu50B SAV0898	SAV0898Fb	24676-GAAGATGCAGTTGTAGATCGC-24696	AF424781
	SAV0898AP	24721-CAACTTCCCAAGCTTCATACAATA-24697	AF424781
	SAV0898SP	24781-GGTTTCCACAATAAATTTGAATTAATA-24807	AF424781
	SAV0898Rb	24829-CATCAATACCGTTAGCTTCTGC-24808	AF424781
φPV83 ORF 2	PV83ORF2Fb	1269-GGCGCTTCTTCTTACAGGAG-1288	AB044554
	PV83ORF2AP	1323-CATTGTTAGATATTTTATATGGTATGTAACCTAAAA-1289	AB044554
	PV83ORF2Rinner	1356-GATACTCTGTGTTTTTCTACTAACTAAACCTAT-1324	AB044554
	PV83ORF2Finner	1625-TGTTTAATAACAACGGTAAACCAGTATTT-1653	AB044554
	PV83ORF2SP	1654-ATAGTTATTAAAGACTTTGAAAACAGAATCATT-1686	AB044554
	PV83ORF2R2b	1715-GAATTATAGGTTTTAAGTTACCCCTCTTC-1687	AB044554
φMu50B SAV0858	SAV0858Fb	6334-ATCTAAATTGCTGTGCGAAGC-6314	AP001553
	SAV0858AP	6294-TATTTGCGGCTTTAGCGTAA-6313	AP001553
	SAV0858SP	6063-CATTTGAGAAAAGTCTTTTGTGCGATACT-6037	AP001553
	SAV0858Rb	6017-CCAAGAACAGGGACATCGAC-6036	AP001553
φMu50A SAV1998	SAV1998Fb	2122883-CAGTAACTCACGCCTCCAAG-2122903	BA000017
	SAV1998AP	2122928-TGCATAGTTAAGCACATTTTTTGT-2122904	BA000017
	SAV1998SP	2123071-CCGAAATGGTTAAAGCACCTAT-2123092	BA000017
	SAV1998Rb	2123111-TGCTAAATCATGTGGTGCG-2123093	BA000017
SCC _{mec} II <i>kpdC</i>	CN009F	80974-GGACAACAATGGACAGAACC-80993	BA000017
	CN009AP	81019-CACTGATACGTCCATGGAATATTTA-80994	BA000017
	CN009SP	81061-GGCGGACCTGCTTCAG-81076	BA000017
	CN009Rb	81097-AATTGCCGTAGTTTGAGCC-81079	BA000017
φ11 nt 4427-5251	phi11-4563Fb	4563-GATATGCAAGATCAGACAATGCC-4585	AF424781
	phi11-4610AP	4610-CCTCGCTATCAACATGATTTCTAAT-4586	AF424781
	phi11-4632Rinner	4632-CTAAATTGGTGCCTGATTTGT-4611	AF424781
	phi11-5026Finner	5026-CAAACACTACTACACGAAGCTAGACTACAAC-5054	AF424781
	phi11-5055SP	5055-GAAAAGTAAATAAACAGTGGGTGCTTTA-5082	AF424781
	phi11-5103Rb	5103-CTCTTGCCCATGTGTTCTGAG-5083	AF424781
φSLT ORF 257	SLTorf257Fb	26802-GTGTATCGCTATGAGTGGTGAC-26824	EF462198
	SLTorf257AP	26855-TTAAAAAACTATTTTTGTGCATAAAAAATAGT-26825	EF462198
	SLTorf257SP	27093-TCTCTAAAGAGCAATATAAGCGTTTC-27118	EF462198
	SLTorf257Rb	27142-CTTTAAATCTTCTGGGACGTTCTC-27119	EF462198
φMu50B SAV0850	SAV0850F2b	12192-ACCACAAGTTGACGTATGGC-12211	AF410775
	SAV0850AP	12236-TGAACTTCGATTGGTCTAAAATGTT-12212	AF410775
	SAV0850SP	12419-GGAACTCACTATGGCGAGTATTCTATTA-12446	AF410775
	SAV0850Rb	12467-AAACTCTCAACGGCTCAAATG-12447	AF410775
φSLT ORF 175	SLTorf175Fb	15837-AAATGCTAGAATGCCGAAC-15856	EF462198
	SLTorf175AP	15878-CCTGCATCCGTCTTATGATTTTC-15857	EF462198
	SLTorf175SP	15967-GGCTATGTGCGGGCTGTTAACTA-15988	EF462198
	SLTorf175Rb	16014-CGTTTTACTACTTACACCACTACGG-15990	EF462198
φN315 SA1801	SA1801Fb	5235-CAATCAGCGGTGCGAGAACT-5253	EF462197
	SA1801AP	5279-GAGTCTTAACCTCTAATGCTTGATGA-5254	EF462197

Continued on following page

TABLE 1—Continued

Target	Oligonucleotide ^a	Sequence (5'–3') ^b	GenBank accession no.
	SA1801Rinner	5305-CATTCTTTCAAACCATTTTTGTATG-5280	EF462197
	SA1801Finner	5687-CGAGATTGTTTGTAGTGGTTA-5707	EF462197
	SA1801SP	5708-CGTCAAACGATTCTTATTTAAA-5731	EF462197
	SA1801Rb	5751- <u>TT</u> AATAATCCACACCCTTGCG-5732	EF462197
φMu50B SAV0913	SAV0913Fb	958598- <u>TTATTC</u> ATAACGACGCAGGAAG-958619	BA000017
	SAV0913AP	958636-CTGCTGTTGCCCTTTG-958620	BA000017
	SAV0913SP	958868-GCGCTAGATTGTTGAAAAAATG-958889	BA000017
	SAV0913Rb	958910- <u>TTTCTG</u> TTTGCTGGTAATCCC-958890	BA000017
φMu50A SAV1974	SAV1974Fb	2110912-GCCACAAGAAAAGGCAGTG-2110894	BA000017
	SAV1974AP	2110869-TGCTTACAGCTACATCTGTTTGGAT-2110893	BA000017
	SAV1974SP	2110719-GATATGAGTAACTTTGGTCGGAGTC-2110695	BA000017
	SAV1974Rb	2110673- <u>ATAC</u> TTTCCATCTATCCCAGCAG-2110694	BA000017
φSLT ORF 182	SLTorf182Fb	1080359- <u>AAATG</u> ATAGGAAAGCGACACG-1080379	CP000736
	SLTorf182AP	1080413-TTGATATAACCTTTAATGTCTCTTACTAAATTGT-1080380	CP000736
	SLTorf182SP	1080493-CAACCTTGTTACCTACTAACCAAAAA-1080518	CP000736
	SLTorf182R2b	1080540- <u>GTTTG</u> CTACTATGTCGCAACCT-1080519	CP000736
<i>mecA</i>	<i>mecAP4b</i>	1190-TCCAGATTACAACCTTACCAGG-1211	Y00688
	<i>mecAAP</i>	1236-TGCTGTTAATATTTTTTGAGTTGAAC-1211	Y00688
	<i>mecASP</i>	1309-GATAAATCTTGGGGTGGTTACAAC-1332	Y00688
	<i>mecAP7b</i>	1357- <u>CATTTA</u> CCACTTCATATCTTGTAACG-1332	Y00688

^a Oligonucleotides whose names end in “b” are 5'-biotinylated primers. Oligonucleotides whose names end in “P” are 5'-amine-labeled probes. Inner primers were unlabeled. *mecA* primers were derived from the work of Oliveira et al. (11). Primers and probes for *nuc*, *ist*, and *pvl* were unchanged from those previously published (2).

^b Modifications of the primers originally published by Suzuki et al. (16) are indicated by underlines. Numbers flanking the sequences indicate the starting and ending nucleotide positions in the indicated GenBank sequence.

Primer and probe design. Primers were modified from the originally published set (16) to produce a melting temperature of approximately 60°C. When necessary, additional inner primers were designed to produce amplicon lengths of <400 bp. In addition to the PDORF targets, primers and probes for *nuc*, *mecA*, *ist*, and *pvl* were included in the mPCR/RLB assay. Two probes (one sense and one antisense) were designed for each target. Primer modification and probe design were based on bacteriophage and gene sequences referenced in the original paper (16) and published in NCBI GenBank (Table 1). Primer and probe specificities were verified using BLASTn searches of published sequences in NCBI GenBank.

In silico analysis. Results of the mPCR/RLB assay were predicted by performing *in silico* PCR and probe binding, using FastPCR software (PrimerDigital), against five published *S. aureus* genomes: Mu3, Mu50, MW2, COL, and NCTC8325. First, *in silico* mPCR was performed to predict PCR products for each genome. These products were then used to predict probe binding. The predicted results were compared to the *in vitro* findings as part of assay validation.

mPCR and reverse line blot assay. DNA was extracted by suspending 1 or 2 colonies in 400 μl molecular-grade H₂O and heating them to 100°C for 10 min. This solution was frozen at –20°C and then thawed and centrifuged at 16,100 × g for 5 min before the supernatant was used as a PCR template. A 23-plex PCR and product detection by RLB assay were performed as described previously (6). The positive control consisted of isolates which, in combination, produced positive results for each probe (Mu3, COL, SJOG30, and 14176–5170). The specificity of positive controls was confirmed by *in silico* analysis (Mu3 and COL) or by single PCR and sequencing (SJOG30 and 14176–5170). The negative control consisted of PCR master mix without a DNA template.

Single PCR and sequencing. The specificity of probe signals was confirmed where necessary by using single-primer-pair PCR, with sequencing of the product in the forward and reverse directions on an Applied Biosystems 3730xl DNA analyzer.

Interpretation of results. Probe signals were considered strong if they were equal to or of greater intensity than the positive-control result for the corresponding probe. Probe signals were considered weak if they were present but of clearly lower intensity than that of the positive-control result. Probes for which no signal was detected were considered negative. Isolates belonging to the same PDORF type shared a unique pattern for the 16 genes. Since each gene was

represented by two probes, for the purposes of PDORF type assignment, a gene was considered present if at least one strong probe signal or two weak probe signals were found and absent if only one weak probe signal or no probe signal was found (i.e., a solitary weak signal for one probe with a negative signal for the second probe was considered nonspecific).

PFGE and *spa* typing. PFGE was performed according to the Harmony protocol (10), and fingerprint patterns were examined by Bionumerics v3.00 software (Applied Maths, Sint-Martens-Latem, Belgium). Optimization was set at 0.6%, with a band tolerance of 2% and a change toward the end of the fingerprint of 0.5%. Cluster analysis of PFGE patterns was performed using the Dice coefficient and the unweighted-pair group method using average linkages (UPGMA). PFGE subtypes were defined by patterns which were indistinguishable (100% similarity). *spa* sequencing and type assignment were performed as previously published (2).

Statistics. Simpson's index of diversity and 95% confidence intervals (95% CI) were calculated using standard methods (3, 4).

RESULTS

In silico analysis. Computational predictions confirmed *in vitro* experiments for isolates Mu3, Mu50, MW2, COL, and NCTC8325 (Fig. 1). It was noted that weak signal strength was associated with amplicons of 341 bp or more (Tn554 *tnpB*, 398 bp; φSLT ORF 257, 341 bp). No signal was found for the antisense probe for φMu50B SAV0858 in isolates Mu3 and Mu50, despite *in silico* analysis predicting only a single base-pair mismatch with the PCR product. However, this mismatch reduced the predicted melting temperature of the probe-PCR product binding from 52.1°C to 42.8°C, which was the likely explanation for the lack of signal on the RLB. This was confirmed by single PCR and sequencing of φMu50B SAV0858 in Mu3 and Mu50 in addition to isolate 14176–5170 (for which

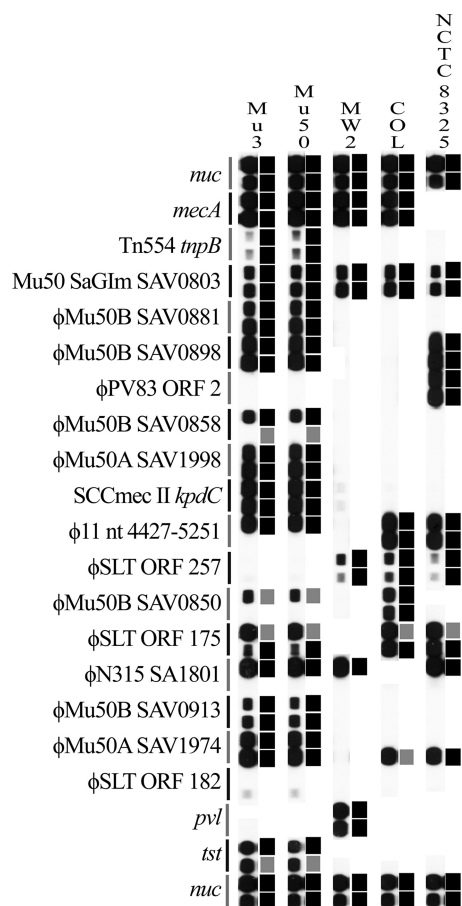


FIG. 1. Comparison of *in vitro* and *in silico* analyses of five isolates. For each isolate, the *in vitro* analysis is shown on the left and the *in silico* analysis is shown on the right. For the *in silico* analysis, black squares indicate a 100% match, while gray squares indicate a one- or two-base-pair mismatch between the predicted amplicon and the probe. Sense probes are shown above the antisense probes for each target gene.

both probes gave a strong signal and no mismatch was found using the antisense probe).

For isolates Mu3 and Mu50, weak, nonspecific probe signals were noted for the antisense probe for ϕ SLT ORF 182. Single PCR using primers against ϕ SLT ORF 182 did not yield any PCR product for these isolates.

Analysis of reference collection. Analysis of the 42 reference strains revealed 33 PFGE subtypes ($D = 0.9872$; 95% CI, 0.9765 to 0.9980) and 30 PDORF types ($D = 0.9710$; 95% CI, 0.9447 to 0.9973) (Fig. 2). There were two PFGE subtypes that were comprised of three isolates each and five subtypes that were comprised of two isolates each. The remaining 26 PFGE subtypes contained only one isolate each. The largest group of PDORF types contained six isolates. Four of the PFGE subtypes with multiple members corresponded exactly with groups of PDORF types. For two of the PFGE subtypes, the isolates differed by a single PDORF target, while for one PFGE subtype, one isolate contained three PDORF targets that were absent in the other two isolates (Fig. 2). *spa* sequence typing produced 22 *spa* types ($D = 0.9257$; 95% CI, 0.8791 to 0.9722),

and there were 15 types obtained by multilocus sequence typing (MLST) ($D = 0.8815$; 95% CI, 0.8225 to 0.9405).

Analysis of clinical isolates. Analysis of the epidemiologically restricted set of 35 clinical isolates demonstrated 19 PFGE subtypes, 8 PDORF types, and 4 *spa* types (Fig. 3). All of these isolates were multiresistant, with 32 carrying *spa* type t037, which is typical of the MLST239-SCCmec III MRSA clone that is currently endemic in many Australian hospitals. Figure 4 shows data for the same isolates, with 14 isolates representing cases of nosocomial acquisition grouped with isolates from MRSA carriers who had contact with the patients around the time of acquisition. In 8 of these acquisition events, an MRSA carrier with an identical PFGE subtype was identified. Of these, 6 also had identical PDORF types; for the remaining 2, the PDORF type differed only by the absence of one marker. There was only one instance when isolates from a newly colonized patient and his contact had identical PDORF types but different PFGE types (minimum similarity, 82.1%). One novel *spa* type was identified, with the repeat sequence 15-12-16-02-25-17-12-16-02-25-17-24.

***In vivo* stability and reproducibility.** For 42 of the 81 stability isolate pairs, the typing results were identical by PFGE and PDORF typing, while for 9 pairs the typing results were different by both methods (Table 2). For 22 pairs, PDORF typing results were indistinguishable but PFGE results differed. For 6 of these 22 pairs, the PFGE similarity was >95%, equivalent to a single band difference; for another 14, the PFGE similarity was 80 to 95%. Eight isolate pairs had indistinguishable PFGE patterns but different PDORF patterns. Three of these differed by two PDORF targets, and the remaining five differed by one PDORF target. The median time between collections for pairs that had identical patterns for both PFGE and PDORF typing was 140.5 days; for those with different PDORF patterns, it was 243 days, while it was 197 days for those that had different PFGE patterns (Mann-Whitney test; $P = 0.99$).

Forty-two isolates had DNA extracted on two separate occasions and were tested by PDORF typing, using two separate PCRs with product detection on two identical RLB membranes prepared on different days. There was 100% concordance between the results produced on the two occasions.

DISCUSSION

Despite a long turnaround time, low throughput level, and labor-intensive analysis procedure, PFGE remains the gold standard for typing of *S. aureus* outbreaks due to its high discriminatory power and its proven reflection of epidemiologic relationships. With the aim of overcoming the limitations of PFGE, newer, PCR-based typing methods have been developed, such as *spa* sequence typing (9), multilocus variable-number-tandem-repeat analysis (MLVA) (14), and repetitive sequence-based PCR (rep-PCR) (13). However, no method has yet been accepted as superior to PFGE, since the newer methods either are less discriminatory, require analysis of a gel-based fingerprint, lack reproducibility, or are too expensive.

Binary typing involves interrogating the bacterial genome for the presence or absence of a set of markers. It has theoretical advantages over other typing methods because of the ability to express results as positive or negative for each

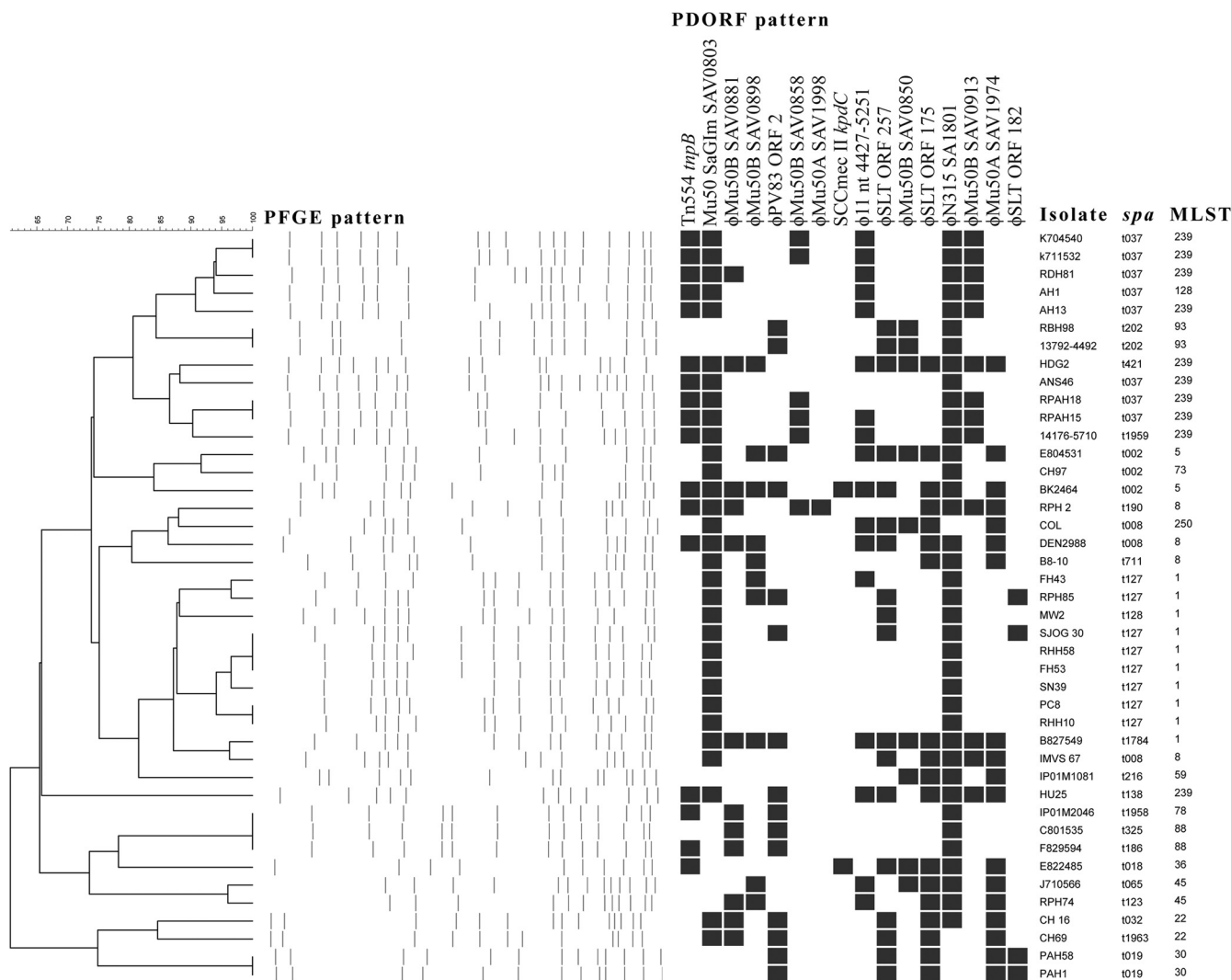


FIG. 2. PFGE, PDORF typing, *spa* typing, and MLST results for 42 reference strains. Clustering is based on the PFGE pattern. A black rectangle indicates detection of the relevant PDORF target by mPCR/RLB assay.

marker, which facilitates harmonization of test protocols and creation of typing databases for interrun and interlaboratory comparisons of results. Furthermore, no specialized equipment is needed, with the minimum requirements being a PCR cyler and detection of amplicons by agarose gel electrophoresis.

We use the mPCR/RLB format for binary typing because it offers a number of advantages over other methods. The probe hybridization with chemiluminescence detection which is utilized in the mPCR/RLB assay is highly sensitive, permitting somewhat inefficient PCR amplification conditions; this allows amplification of a large number of targets in a single megaplex PCR. At the same time, probe-based detection, particularly with two probes per product, is highly specific. The membrane and its attached probes can be re-used more than 20 times (after a simple washing step), up to 43 isolates may be tested on a single membrane, and multiple membranes can be processed simultaneously by one technician. This makes mPCR/RLB assay a cost-effective

(approximately \$2 [U.S. currency] per sample) and high-throughput option. Furthermore, the flexibility of the method allows for inclusion of additional targets of interest, such as *pvl* or *tst* (6).

Comparative genomic techniques have demonstrated a substantial array of genetic loci that are variably present among even otherwise closely related strains of *S. aureus*, raising the prospect of a highly discriminatory binary typing system based on such targets (7). We previously developed mPCR/RLB binary typing methods for detection of antibiotic resistance genes (12), exotoxin genes (2), and SCCmec elements (1) in MRSA. Another approach has been to identify discriminatory sequences by randomly amplified DNA analysis and then to clone sequences, label them, and interrogate their presence in genomic DNAs of test isolates by Southern blot hybridization (18, 19). This technique has also been adapted for reverse hybridization (17). Huygens et al. combined five binary targets (*pvl*, *cna*, *sdrE*, pUB110, and pT181) and single nucleotide polymorphism (SNP)-based MLST for an MRSA typing sys-

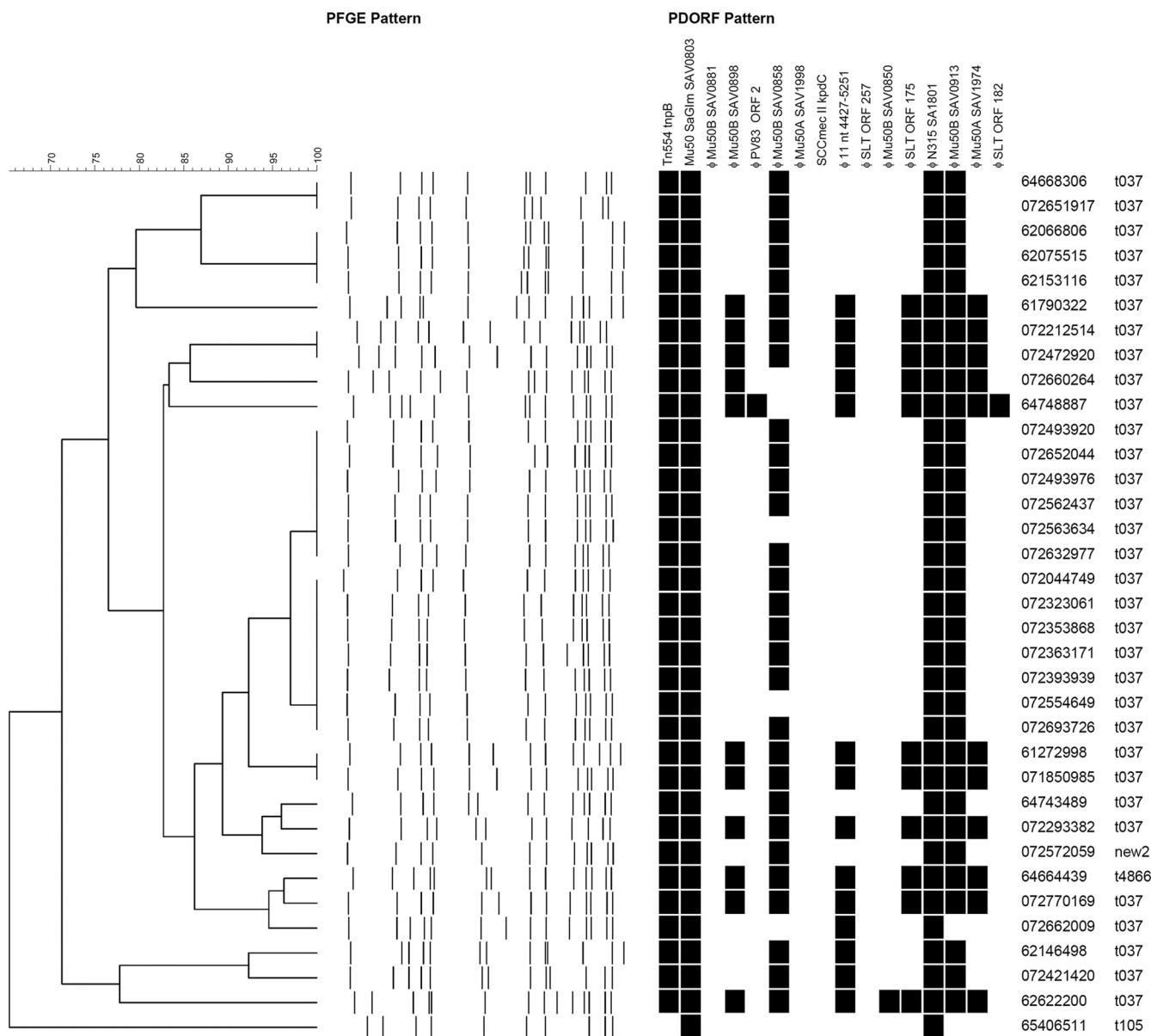


FIG. 3. PFGE, PDORF typing, and *spa* typing results for 35 clinical isolates. Clustering is based on the PFGE pattern. A black rectangle indicates detection of the relevant PDORF target by mPCR/RLB assay.

tem with improved discriminatory power compared with that of the SNP-based method alone (5).

Mobile genetic elements such as integrated prophages, transposons, and genomic islands have also attracted attention as potentially highly discriminatory typing targets (8). Such markers were used in the typing system developed by Suzuki et al. (16) that has been adapted in this study. In the original study, 60 ORFs were identified by comparative analysis of staphylococcal phage genomes, and locally circulating strains were surveyed for the presence or absence of these markers. A subset of the most discriminatory set of 16 markers was chosen for use in the typing system, which consisted of four 4-plex PCRs and product detection by electrophoresis on four agarose gels. This phage-derived ORF typing system had a similar discriminatory power to that of PFGE (16).

Our findings confirmed that PDORF typing had a similar discriminatory power to that of PFGE for a large collection of epidemiologically unrelated isolates ($D = 0.9710$ versus 0.9872). In testing the clinical isolates, two of the targets were absent in all clinical isolates tested (ϕ Mu50 SAV1998 and *SCCmec II kpdC*), and three others contributed little to discriminatory power (Mu50 SaGIm SAV0803, N315 SA1801, and ϕ Mu50B SAV0850). We did not survey locally circulating Australian strains for the relative frequencies of all 60 ORFs explored in the original study, but if this were done, there would be the potential to identify other binary targets that may further improve the discriminatory power of PDORF typing in our setting.

Mobile genetic elements can be relatively unstable due to the high molecular clock speeds of those components of the

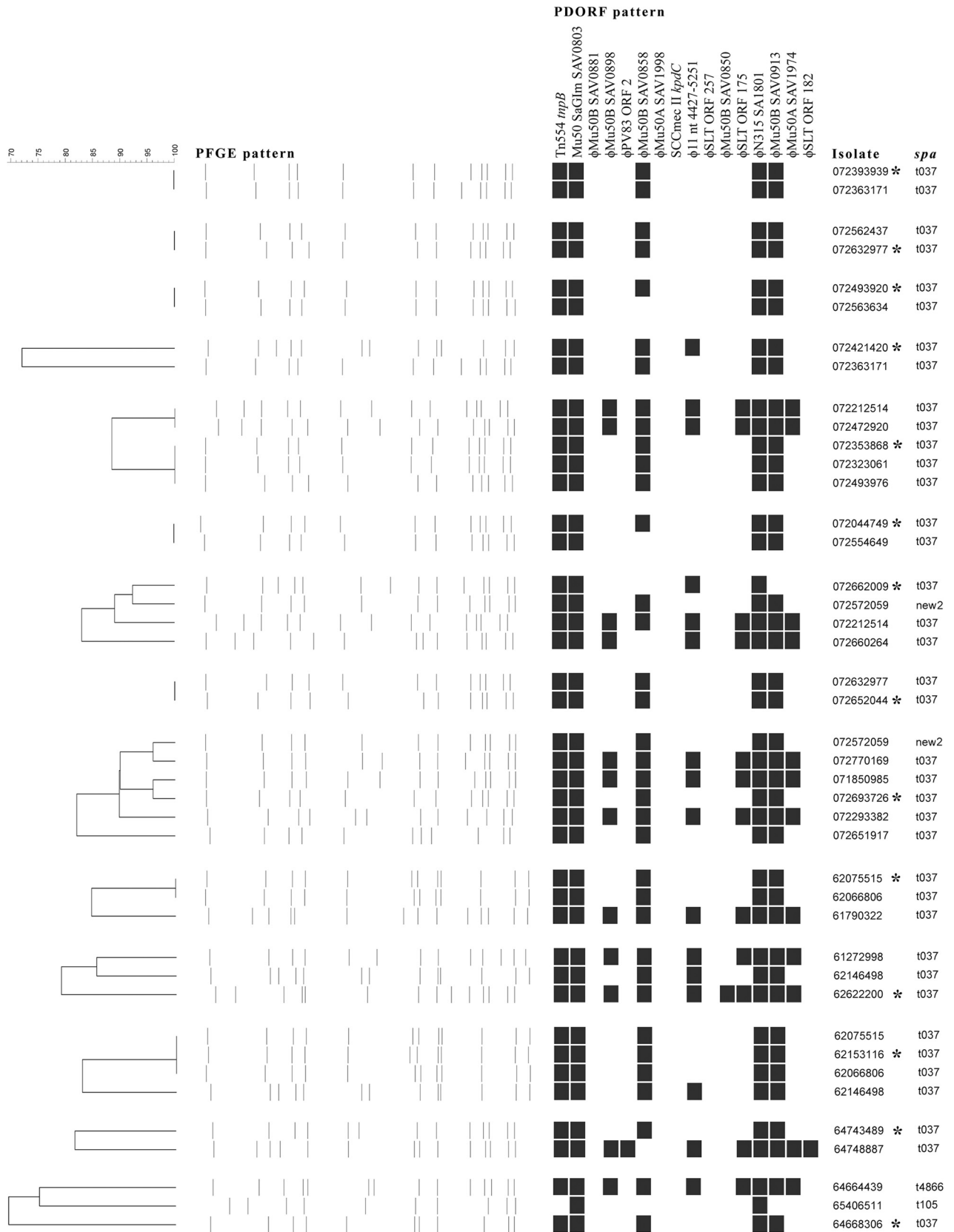


FIG. 4. Results of typing of clinical isolates involved in suspected nosocomial transmission events. Isolates marked with asterisks are from patients who had negative MRSA screens after admission. Each of these is grouped with isolates with the same antibiogram from patients in the same ward at the time of acquisition. Some isolates appear more than once in different groups.

TABLE 2. Results of *in vivo* stability study of pairs of isolates from 81 patients

PDORF result	No. of isolate pairs with PFGE result (median time [days] between collections)		Total no. of isolate pairs (median time [days] between collections)
	Identical	Different	
Identical	42 (140.5)	22 (107.5)	64 (135)
Different	8 (100)	9 (355)	17 (243)
Total	50 (138)	31 (197)	81 (146)

genome. While this is exactly what confers the high level of discriminatory power, it could also potentially result in misclassification of the epidemiologic relationships between isolates. It is important that the stability of new typing systems based on mobile elements be assessed comprehensively to ensure that the loci remain unchanged, at least over time frames which are relevant to the proposed application of the method. Studies of *in vivo* stability seem more relevant for this purpose than *in vitro* studies, since the acquisition of exogenous DNA and evolutionary changes due to ecological pressures are unlikely to occur simply with serial passage in the laboratory. For the purposes of monitoring hospital epidemiology of MRSA over a short-term period (several months; this time frame is relevant for investigation of nosocomial outbreaks), we have shown that PDORF typing is at least as stable as PFGE, a similar finding to that of Suzuki et al. (16). The binary format of this system lends itself to establishing databases of PDORF typing results, which can be useful for easy comparison of isolates from a suspected outbreak. However, the utility of PDORF typing for studying long-term epidemiology and evolution of MRSA and its relationship with other methods traditionally used for this purpose (such as MLST) need further study.

While PDORF typing separated 35 clinical isolates into fewer groups than PFGE subtyping did (8 versus 19), examination of subgroups of these isolates in an attempt to identify nosocomial transmission events suggested that the ability of PDORF typing to identify related isolates is similar to that of PFGE. Based on these data and the mobile nature of the loci utilized, we suggest that identical PDORF patterns can be used to define epidemiologically related isolates among isolates from an outbreak of MRSA. A difference at a single locus would suggest that the isolates are possibly related in this setting.

Conclusions. PDORF typing of MRSA has a similar discriminatory power to that of PFGE, but with better stability, can identify nosocomial transmission events in settings of high-level endemicity, and is highly reproducible. mPCR/RLB-based PDORF typing improves throughput compared with that of the original gel-based method. Its main advantages over PFGE include a short turnaround time, high throughput level, low cost, and binary result format. While the method as described utilizes stable targets and is useful for identifying nosocomial transmission events, surveying locally circulating strains for a wider range of phage-derived targets may identify additional markers which could be included in the system for further improvements in discriminatory power.

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