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Enhanced Discrimination of Highly Clonal ST22-Methicillin-Resistant *Staphylococcus aureus* IV Isolates Achieved by Combining *spa*, *dru*, and Pulsed-Field Gel Electrophoresis Typing Data▽†

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ST22-methicillin-resistant *Staphylococcus aureus* type IV (ST22-MRSA-IV) is endemic in Irish hospitals and is designated antibiogram-resistogram type-pulsed-field group (AR-PFG) 06-01. Isolates of this highly clonal strain exhibit limited numbers of pulsed-field gel electrophoresis (PFGE) patterns and *spa* types. This study investigated whether combining PFGE and *spa* typing with DNA sequencing of the staphylococcal cassette chromosome *mec* element (SCC*mec*)-associated direct repeat unit (*dru* typing) would improve isolate discrimination. A total of 173 MRSA isolates recovered in one Irish hospital during periods in 2007 and 2008 were investigated using antibiogram-resistogram (AR), PFGE, *spa*, *dru*, and SCC*mec* typing. Isolates representative of each of the 17 pulsed-field group 01 (PFG-01) *spa* types identified underwent multilocus sequence typing, and all isolates were ST22. Ninety-seven percent of isolates (168 of 173) exhibited AR-PFG 06-01 or closely related AR patterns, and 163 of these isolates harbored SCC*mec* type IVh. The combination of PFGE, *spa*, and *dru* typing methods significantly improved discrimination of the 168 PFG-01 isolates, yielding 65 type combinations with a Simpson's index of diversity (SID) of 96.53, compared to (i) pairwise combinations of s*pa* and *dru* typing, *spa* and PFGE typing, and *dru* and PFGE typing, which yielded 37, 44, and 43 type combinations with SIDs of 90.84, 91.00, and 93.57, respectively, or (ii) individual *spa*, *dru*, and PFGE typing methods, which yielded 17, 17, and 21 types with SIDs of 66.9, 77.83, and 81.34, respectively. Analysis of epidemiological information for a subset of PFG-01 isolates validated the relationships inferred using combined PFGE, *spa*, and *dru* typing data. This approach significantly enhances discrimination of ST22-MRSA-IV isolates and could be applied to epidemiological investigations of other highly clonal MRSA strains.

Staphylococcus aureus is an important human pathogen, due largely to its ability to express a wide variety of virulence factors and antimicrobial resistance determinants which are often encoded by mobile genetic elements (7, 10, 21, 24, 25, 39, 56). Methicillin-resistant *S. aureus* (MRSA) infections are a major public health problem worldwide, both in hospitals and in the community, although the incidence varies. Ireland has one of the highest prevalence rates of nosocomial MRSA infection in Europe and also has an emerging problem with community-acquired MRSA (CA-MRSA) infections (http://www.rivm.nl/earss/result/Monitoring_reports/Annual_reports.jsp) (49).

MRSA first emerged in Irish hospitals in 1971 (22) and, following a major increase in prevalence in the late 1970s, 1980s, and 1990s, has now been endemic in Ireland for three decades (8, 9, 45–48). Molecular typing showed that each decade since the 1970s has been associated with a major shift in the predominant MRSA clonal type in Irish hospitals (51). The clone that predominated in the 1970s and early 1980s, ST250-MRSA-I (or staphylococcal cassette chromosome *mec* element I [SCC*mec* I variant]), was replaced by the ST239-MRSA-III (or SCC*mec* III variant) clone in the mid-1980s, and this clone was in turn displaced by the ST8-MRSA-II clone (harboring SCC*mec* IIA to IIE) in the 1990s (51). Since the late 1990s, a strain designated locally as antibiogram-resistogram type-pulsed field group (AR-PFG) 06-01, belonging to the international MRSA clone classification ST22-MRSA-IV, which is similar to the United Kingdom epidemic strain EMRSA-15, has predominated in Irish hospitals, and its incidence increased from 22% in 1999 to 80% in 2003 (46, 47).

EMRSA-15 (ST22-MRSA-IV) was first reported in England in 1991 (44) and has since been described as a pandemic MRSA strain due to the predominance of ST22-MRSA-IV among nosocomial MRSA strains in many countries (1, 17, 23, 26, 33, 35, 47, 50, 53). ST22-MRSA-IV has also been identified among patients with hospital-acquired (HA) MRSA infections

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(11, 42, 43, 60, 63) and CA-MRSA infections (5, 15, 32, 49) in several countries, among health care workers (2, 55), and among companion animals (3, 18, 36, 41).

Informative molecular typing is essential for investigating MRSA strains and populations in individual institutions, countries, and wider geographic areas. This approach permits the genetic relatedness of isolates to be determined, which in turn allows the spread of different MRSA strains to be monitored both locally and globally. However, differentiating among isolates of some MRSA strains is very difficult, particularly in a setting where a single strain is endemic, due to the limited genetic diversity exhibited by MRSA strains such as ST22-MRSA-IV (17, 58). ST22-MRSA-IV isolates yield indistinguishable or closely related pulsed-field gel electrophoresis (PFGE) patterns (17). In Ireland, ST22-MRSA-IV isolates belong to a PFGE group described as pulsed-field group 01 (PFG-01) and exhibit the non-multiantibiotic-resistant antibiogram-resistogram (AR) type AR06 or closely related AR patterns (47). ST22-MRSA-IV isolates also yield a limited number of *spa* types following DNA sequencing of the protein A (*spa*) gene (27, 55).

Sequencing of the SCC*mec*-associated direct repeat unit (*dru*) of MRSA isolates has shown potential for differentiating MRSA isolates exhibiting limited diversity in PFGE analyses, including EMRSA-15 isolates from Scotland (17, 62). The *dru* region is a noncoding DNA segment consisting of imperfect 40-bp variable-number tandem repeats (VNTRs) located in the hypervariable region between *mecA* and IS*431mec* of SCC*mec* (17, 38). The majority of MRSA isolates investigated harbor the *dru* region, which ranges in size from 1 to 15 repeat units (17, 38, 58, 59, 62) (http://www.dru-typing.org). The *dru* region has been shown to be stable over time by *dru* typing of individual MRSA isolates following repeated subculture (59), and there is now an internationally agreed-upon *dru* typing nomenclature and a Web-based *dru* database (17) (http://www.dru-typing.org).

Currently, there is no effective method for subtyping of ST22-MRSA-IV isolates. The objective of the present study was to investigate the efficacy of *dru* typing in combination with PFGE and *spa* typing to discriminate among the highly clonal ST22-MRSA-IV (PFG-01) isolates in an Irish hospital where ST22-MRSA-IV is endemic and to investigate the potential of the combined integrated typing approach to facilitate epidemiological tracking of this MRSA strain.

MATERIALS AND METHODS

Isolates and experimental design. MRSA isolates ($n = 173$) from 90 patients and 83 environmental sites in four wards in a 700-bed acute care hospital in Dublin, Ireland, were investigated. The isolates were recovered over two 6-week study periods in each of the four wards between May 2007 and September 2008. Isolates recovered from individual patients and their immediate ward environments during the same 6-week study period are referred to as pairs or triplets of isolates. In the majority of cases, one isolate per patient or patient-associated environmental site was investigated.

The validity of inferences drawn from the typing data was confirmed with epidemiological evidence during a pilot study in one ward. Epidemiological data collected included the numbers for the bed and bed bay corresponding to the patient or the environmental site from whom/which the sample was taken and the sample date and source (i.e., a patient or an environmental site). For patient isolates only, the probable source of the patient's MRSA (whether it was HA or whether the patient was MRSA positive on admission or had a previously known MRSA-positive status) was also recorded. An isolate was deemed to be HA if the patient was negative for MRSA upon admission screening but upon subsequent screening was found to be positive for MRSA.

Isolates were identified as *S. aureus* and stored in bacterial preserver vials at −70°C and methicillin resistance was confirmed, all as described previously (49). All isolates were typed by AR typing against a panel of 23 antimicrobial agents as described previously (47, 49).

Molecular typing. All isolates were typed by DNA macrorestriction digestion analysis using SmaI and PFGE, *spa*, *dru*, and SCC*mec* typing. One representative isolate of each *spa* type identified among the 173 MRSA isolates investigated was typed by multilocus sequence typing (MLST). PFGE was performed as described previously (47). Each PFGE pattern was assigned a 5-digit pulsed-field type (PFT) to allow for future variation in PFGE patterns, and related 5-digit PFTs that differed by ≤6 bands were abbreviated to 2-digit PFGs (47). PFGs were combined with AR typing results to give AR-PFGs (47).

Genomic DNA for use in *spa*, *dru*, and SCC*mec* typing and MLST was extracted using a DNeasy kit according to the instructions of the manufacturer (Qiagen, Crawley, United Kingdom). *spa* typing was performed using the primers and thermal cycling conditions described by the European Network of Laboratories for Sequence Based Typing of Microbial Pathogens (SeqNet [http://www.seqnet.org]). Analysis of *spa* sequences and assignment of *spa* types were performed using the Spa typing plug-in tool of the BioNumerics software package (version 5.1; Applied Maths, Ghent, Belgium). For *dru* typing, the *dru* region was amplified and sequenced as described previously (17). The BioNumerics tandem-repeat sequence typing (TRST) plug-in tool was used for *dru* sequence analysis and assignment of *dru* types. *dru* types were assigned using an alphanumeric nomenclature (17). SCC*mec* typing was performed using four multiplex PCR assays to identify (i) the *mec* complex type (class A, B, or C) (28), (ii) the *ccr* complex type (*ccrAB1*, *ccrAB2*, *ccrAB3*, *ccrAB4*, or *ccrC*) (28), (iii) the various J regions and *mecI* (40), and (iv) the SCC*mec* IV subtype (34). Previously described MRSA control strains were used as positive controls for multiplex PCR assays i to iii (52). The following *S. aureus* reference strains and clinical isolates were used as positive controls for SCC*mec* IV subtyping: CA05 (SCC*mec* IV.1/IVa) (31), 8/63P (SCC*mec* IV.2/IVb) (31), JCSC4788 (SCC*mec* IV.3/IVc) (30), JCSC4469 (SCC*mec* IV.4/IVd) (30), M04/0177 (SCC*mec* IV.5/IVg) (52), and E1749 (SCC*mec* IV.6/IVh) (52). MLST was performed and sequences were analyzed as described previously (13, 52).

Investigating the stability of *dru* types. The stability of the *dru* region was investigated using three MRSA isolates that had previously been subjected to *dru* typing. These comprised two Irish ST8-MRSA-IV isolates, M05/0028 (49) and M06/0376, both of which exhibited *dru* type dt9g, and one EMRSA-15 isolate from the Harmony collection with *dru* type dt10h (17, 37). Each isolate was cultured on brain heart infusion (BHI) agar (Becton Dickinson and Company, Sparks, MD) and incubated at 37°C for 24 to 48 h. Several colonies from each isolate were subsequently subcultured on fresh BHI agar plates and incubated at 37°C for 24 to 48 h. This procedure was repeated for a minimum of 10 subcultures over a 14-day period. For each isolate, several colonies from the original and final subculture plates were analyzed by *dru* typing as described above.

Cluster analyses of *spa* and *dru* types. The BioNumerics Spa typing and TRST plug-in tools were used for cluster analyses of *spa* and *dru* types, respectively. With both of these plug-ins, sequences are compared and aligned using an algorithm based on the DSI (duplication, substitution, and indels) model for pairwise alignment of repeats, which considers that modification of sequences can occur through duplication of tandem repeats, substitutions, insertions, and deletions (the latter two events are collectively termed indels) (4). A similarity matrix is generated based on the DSI model and used to construct a minimum spanning tree (MST); the type with the greatest number of related types is assigned as the root node, and the other types derive from this node. In the present study, the default parameters were used for alignment of sequences. The software creates groups of certain distance intervals or similarity values (which BioNumerics terms bins) and converts the data into distance units. Because of the highly clonal nature of the MRSA isolates investigated in the present study, the bin distance was set to 0.5%, i.e., the distance between two entries with >99.5% similarity was 0 (a distance interval of 99.5 to 100% similarity equals a distance of 0) on the MST, and the distance between two entries with 99 to 99.5% similarity was 1 (a distance interval of 99 to 99.5% similarity equals a distance of 1). Using the MSTs, the following criteria were established for clustering of *dru* types and for clustering of *spa* types: *spa* types and *dru* types were deemed to belong to different clusters if they were separated by an MST distance of >2 (i.e., if they showed <98.5% similarity). Therefore, if two *spa* types or two *dru* types were at an MST distance of ≤2, they were considered to be closely related (i.e., they formed a subgroup).

Clustering of isolates. Each isolate was assigned a 3-digit cluster code with the first number representing the *spa* type, the second representing the *dru* type, and

TABLE 1. Cluster code nomenclature used to describe clusters identified by *spa*, *dru*, and PFGE typing[a]

Method	Type	Repeat succession	Type cluster code	Type subcluster code
spa typing	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	01	Founder
	t022	26-23-13-23-31-29-17-31-29-17-25-17-25-16-28	01	01a
	t557[b]	26-23-23-13-23-31-31-29-17-31-29-17-25-17-25-16-28	01	01b[c]
	t628	26-23-23-13-23-31-29-17-31-29-17-31-29-17-25-17-25-16-28	01	01c
	t1214	26-23-23-13-23-31-29-17-31-29-17-25-16-28	01	01d
	t515	26-23-23-13-23-31-29-17-31-29-17-25-16-16-28	01	01da
	t4622	26-23-23-13-23-31-31-29-17-31-29-17-25-16-16-28	01	01daa
	t018	15-12-16-02-16-02-25-17-24-24-24	02	NSC
	t1802	26-16-16-28	03	NSC
	t025	26-23-23-13-23-29-17-31-29-17-25-17-25-16-28	04	NSC
	t578	26-23-23-13-23-31-29-17-31-29-17-25-17-25-28	05	NSC
	t4623	26-23-13-23-31-29-132-17-31-29-17-25-17-25-16-28	06	NSC
	t1865	26-23-23-17-31-29-17-25-17-25-16-28	07	NSC
	t2951	26-16-31-29-17-25-17-25-16-28	08	NSC
	t2978	04-20-17-20-17-31-16-16-34	09	NSC
	t3185	26-23-23-20-31-29-17-31-29-17-25-17-25-16-28	10	NSC
	t3213	26-23-23-13-23-31-29-17-31	11	NSC
	t4122	26-23-23-13-23-31-29-23-31-29-17-25-17-16-28	12	NSC
	t4267	26-23-13-23-31-36-25-28	13	NSC
	t4765	26-23-23-13-23-31-29-17-31-17-25-16-28	14	NSC
	t190	11-17-34-24-34-22-25	15	NSC
dru typing	dt10a	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	01	NSC
	dt10j	5a-2d-4a-0-2d-7a-3a-2g-3b-4e	01	01a
	dt10af	5a-2d-4a-0-2d-2c-3a-2g-3b-4e	01	01b
	dt10n	5a-2d-4a-0-2d-3b-3a-2g-3b-4e	01	01c
	dt10i	5a-2d-4a-0-2d-4f-3a-2g-3b-4e	01	01d
	dt10o	5a-2d-4a-0-2d-4f-3a-2g-2c-4e	01	01da
	dt10p	5a-2d-4a-1b-2d-7a-3a-2g-3b-4e	01	01aa
	dt11a	5a-2d-4a-0-2d-5b-3a-2g-3b-4e-3e	02	NSC
	dt11o	5a-2d-4a-0-2d-5b-3a-2g-3b-4e-4e	02	02a
	dt11j	5a-2d-2d-4a-0-2d-5b-3a-2g-3b-4e	02	02aa
	dt5b	5a-2d-4a-5b-3a	03	NSC
	dt6e	5a-7a-3a-2g-3b-4e	04	NSC
	dt7c	5a-2d-2d-4a-0-3e-3e	05	NSC
	dt7g	5a-2d-7a-3a-2g-3b-4e	06	NSC
	dt7i	5a-2d-4a-0-2d-3b-4e	07	NSC
	dt8a	5a-2d-4a-0-2d-2g-3b-4e	08	NSC
	dt8p	6d-0-2d-7a-3a-2g-3b-4e	09	NSC
	dt9j	5a-2d-4a-0-2d-5b-3a-2g-3b	10	NSC
	dt9p	5a-2d-4a-0-7a-3a-2g-3b-4e	11	NSC
PFGE	01018		1	Founder
	01002		1	1a
	01006		1	1b
	01022		1	1c
	01024		1	1d
	01030		1	1e
	01032		1	1f
	01039		1	1g
	01042		1	1h
	01047		1	1i
	01049		1	1j
	01063		1	1k
	01075		1	1l
	01077		1	1m
	01088		1	1n
	01114		1	1o
	01126		1	1p
	01146		1	1q
	01151		1	1r
	01154		1	1s
	01156		1	1t
	00041		2	Founder
	00080		2	2a
	00216		2	2b
	02017		3	NSC
	99083		4	NSC

[a] *spa* and *dru* types at a distance of >2 on the MSTs (i.e., types that showed <98.5% similarity) were assigned distinct cluster codes (Fig. 1). *spa* and *dru* types that showed >98.5% similarity on the MSTs (i.e., types at an MST distance of ≤2) were assigned *spa* subcluster codes. Subclusters were assigned alphabetic suffixes following the relevant numerical element of the cluster code. *spa* types and *dru* types that were identified as subgroups of *spa*/*dru* types that were already assigned to *spa*/*dru* subclusters were assigned additional alphabetic suffixes, e.g., *spa* type t515 was assigned the *spa* cluster code 01da, as it is a subgroup of t1214 (*spa* cluster code 01d), which is a subgroup of *spa* type t032 (*spa* cluster code 01). For each cluster that consisted of more than one *spa* or *dru* type, the type that was assigned as the founder by using the MSTs was not assigned a subcluster code but retained the original cluster code designation. PFTs were designated with distinct cluster codes if they differed by >6 bands. PFTs that differed by ≤6 bands were assigned subcluster codes. For PFT clusters that were represented by more than one PFT, the most frequently occurring PFT was assigned the numerical value for that cluster code and all other PFTs were assigned alphabetic suffixes. NSC, no subcluster code.

[b] Isolates exhibiting *spa* type t557 were recovered from staff members only during a wider investigation and were assigned *spa* cluster code 01b but were not included in the present study.

TABLE 2. DGs, cluster codes, data from *spa*, *dru*, PFGE, SCC*mec* typing, MLST, and AR-PFGs for 173 MRSA isolates

DG	Cluster code[a]	*spa* type	*dru* type	PFT	AR-PFG[b] (no. of isolates)	SCC*mec* type	ST[c]
1a	01.01.1	t032	dt10a	01018	06-01 (8)	IVh	ST22
1a	01.01c.1	t032	dt10n	01018	06-01 (14)	IVh	ND
1a	01.01a.1	t032	dt10j	01018	06-01 (10)	IVh	ND
1a	01.01a.1	t032	dt10j	01018	Unf-01 (5)	IVh	ND
1a	01da.01a.1	t515	dt10j	01018	06-01 (1)	IVh	ND
1a	04.01a.1	t025	dt10j	01018	06-01 (1)	IVh	ST22
1a	01.10.1	t032	dt9j	01018	06-01 (1)	IVh	ND
1b	01a.01a.1	t022	dt10j	01018	06-01 (1)	IVh	ND
2	01c.01.1	t628	dt10a	01018	06-01 (9)	IVh	ST22
3	01.06.1	t032	dt7g	01018	06-01 (2)	IVh	ND
4a	01d.01.1g	t1214	dt10a	01039	06-01 (6)	IVh	ST22
4a	01daa.01.1g	t4622	dt10a	01039	06-01 (1)	IVh	ST22
4a	01da.01.1g	t515	dt10a	01039	06-01 (4)	IVh	ST22
4a	01da.01.1g	t515	dt10a	01039	NT-01 (1)	IVh	ND
4a	01d.02a.1g	t1214	dt11o	01039	06-01 (1)	IVh	ND
4a	01da.02a.1g	t515	dt11o	01039	06-01 (1)	IVh	ND
4b	01.01a.1g	t032	dt10j	01039	06-01 (1)	IVh	ND
4b	01.01a.1g	t032	dt10j	01039	NT-01 (1)	IVh	ND
4b	01.01.1g	t032	dt10a	01039	06-01 (3)	IVh	ND
4b	01.01.1g	t032	dt10a	01039	NT-01 (1)	IVh	ND
4b	01.01b.1g	t032	dt10af	01039	06-01 (6)	IVh	ND
4b	01.01da.1g	t032	dt10o	01039	06-01 (1)	IVh without *dcs*	ND
4b	12.01a.1g	t4122	dt10j	01039	06-01 (1)	IVh	ST22
5	01da.01.1k	t515	dt10a	01063	06-01 (1)	IVh	ND
6	01a.01.1g	t022	dt10a	01039	06-01 (4)	IVh	ST22
6	01a.01aa.1g	t022	dt10p	01039	06-01 (2)	IVh	ND
7	01da.07.1g	t515	dt7i	01039	06-01 (1)	IVh	ND
8	01da.08.1g	t515	dt8a	01039	06-01 (1)	IVh	ND
9	07.01a.1	t1865	dt10j	01018	06-01 (3)	IVh	ST22
10	07.01a.1a	t1865	dt10j	01002	06-01 (1)	IVh	ND
11	01.01c.1i	t032	dt10n	01047	06-01 (2)	IVh	ND
12	06.01.1 m	t4623	dt10a	01077	06-01 (1)	IVh	ST22
13	01.01.1b	t032	dt10a	01006	Unf-01 (1)	IVh	ND
13	01.01c.1b	t032	dt10n	01006	06-01 (1)	IVh	ND
13	01.01a.1b	t032	dt10j	01006	Unf-01 (1)	IVh	ND
14	01d.01.1b	t1214	dt10a	01006	06-01 (1)	IVh	ND
15	01.01a.1e	t032	dt10j	01030	06-01 (1)	IVh	ND
16	01.01a.1h	t032	dt10j	01042	NT-01 (1)	IVh	ND
16	01da.01.1h	t515	dt10a	01042	06-01 (3)	IVh	ND
16	01.01.1h	t032	dt10a	01042	06-01 (1)	IVh	ND
16	01.01c.1h	t032	dt10n	01042	06-01 (1)	IVh	ND
16	01d.01.1h	t1214	dt10a	01042	06-01 (1)	IVh	ND
17	01.01a.1d	t032	dt10j	01024	06-01 (2)	IVh	ND
17	01.01a.1d	t032	dt10j	01024	NT-01 (1)	IVh	ND
17	01.01a.1d	t032	dt10j	01024	Unf-01 (7)	IVh	ND
17	01.01c.1d	t032	dt10n	01024	06-01 (4)	IVh	ND
18	01.01a.1q	t032	dt10j	01146	Unf-01 (1)	IVh	ND
19	01da.01.1c	t515	dt10a	01022	06-01 (2)	IVh ($n = 1$)	ND
						IV, nonsubtypeable ($n = 1$)	ND
20	01.01.1l	t032	dt10a	01075	06-01 (1)	IVh	ND
20	01.02aa.1l	t032	dt11j	01075	06-01 (1)	IVh	ND
21	01.03.1	t032	dt5b	01018	06-01 (2)	IVh	ND
22	10.01da.1s	t3185	dt10o	01154	06-01 (2)	IVh	ND
22	10.01da.1s	t3185	dt10o	01154	Unf-01 (13)	IVh	ST22
23	01.01.1j	t032	dt10a	01049	06-01 (2)	IVh	ND
23	05.01.1j	t578	dt10a	01049	06-01 (1)	IVh	ST22
23	01da.02aa.1j	t515	dt11j	01049	06-01 (1)	IVh	ND
24	01.01a.1a	t032	dt10j	01002	06-01 (3)	IVh	ND
24	01da.01.1a	t515	dt10a	01002	06-01 (1)	IVh	ND
25	01.11.1j	t032	dt9p	01049	06-01 (1)	IVh	ND
26a	01a.09.1j	t022	dt8p	01049	06-01 (3)	IVh	ND
26b	01.09.1j	t032	dt8p	01049	06-01 (1)	IVh	ND
27	11.01.1g	t3213	dt10a	01039	06-01 (1)	IVh	ST22
28	11.01.1h	t3213	dt10a	01042	06-01 (1)	IVh	ND
29	13.01.1g	t4267	dt10a	01039	06-01 (1)	IVa	ST22
30	14.01.1k	t4765	dt10a	01063	06-01 (1)	IVh	ST22

Continued on following page

TABLE 2—*Continued*

DG	Cluster code[a]	*spa* type	*dru* type	PFT	AR-PFG[b] (no. of isolates)	SCC*mec* type	ST[c]
31	01.01.1f	t032	dt10a	01032	06-01 (1)	IVh	ND
32	01.01.1r	t032	dt10a	01151	06-01 (1)	IVh	ND
33	01.01a.1t	t032	dt10j	01156	06-01 (1)	IVh with *ccrAB4*	ND
34	01.02.1p	t032	dt11a	01126	06-01 (1)	IVh with *ccrC* and Tn*554* to *orfX*	ND
35	03.01.1g	t1802	dt10a	01039	06-01 (1)	IVh	ST22
36	01.01.1o	t032	dt10a	01114	06-01 (1)	IVh	ND
37	08.04.1n	t2951	dt6e	01088	06-01 (1)	IVh	ST22
38	02.05.3	t018	dt7c	02017	NT-02 (1)	II without p*UB110*	ST36
39	15.01a.2	t190	dt10j	00041	New03-00 (1)	Characterized by *ccrAB4*, class A *mec*, *mecI*, and *dcs*	ST8
40	15.01.02a	t190	dt10a	00080	14-00 (1)	Characterized by *ccrAB2*, *ccrAB4*, J1 type IVb, *dcs*, and novel *mec* complex	ND
41	15.01.2b	t190	dt10j	00216	13-00 (1)	IIE	ND
42	09.01d.4	t2978	dt10i	99083	Unf-99 (1)	IVb	ST87

[a] Based on the nomenclature presented in Table 1, each isolate was assigned a 3-digit cluster code in which the first number represents the *spa* type, the second represents the *dru* type, and the third represents the PFT.

[b] Unf, unfamiliar (these isolates exhibited a hitherto unfamiliar AR pattern); NT, nontypeable (these isolates exhibited AR patterns that differed from the AR06 group of patterns only with regard to resistance to lincomycin [see Table S1 in the supplemental material for further details]).

[c] One isolate representative of each *spa* type identified in the present study underwent MLST. ND, not determined.

the third representing the PFT (Table 1). For example, *spa* type t032, *dru* type dt10a, and PFT 01018 were assigned the codes 01, 01 and 1, respectively, and isolates with this *spa*, *dru*, and PFGE type combination were assigned the 3-digit cluster code 01.01.1. Subtypes recognized by each typing method were designated by alphabetic suffixes after the relevant numerical element of the cluster code (Table 1). To investigate the overall relatedness of isolates, a composite dendrogram for all PFG-01 (ST22-MRSA-IV) isolates identified during the present study was constructed in BioNumerics by using the averages of the similarity matrices from the individual experiments (PFGE, *spa*, and *dru* typing) and clustering by the unweighted-pair group method using average linkages (UPGMA).

Discriminatory powers of and concordance of data from *spa*, *dru*, and PFGE typing methods. The abilities of PFGE, *spa*, and *dru* typing methods alone and in every combination to discriminate among the PFG-01 (ST22-MRSA-IV) isolates investigated were assessed quantitatively by calculating Simpson's indices of diversity (SIDs) with 95% confidence intervals (CI) using an online tool developed by Faria et al. (14) (available at http://www.comparingpartitions.info). SID provides an objective assessment of the discriminatory power of a typing method (14, 20).

The concordance among the data from the typing methods was determined by calculating the adjusted Rand index (ARI) using the online tool mentioned above (14). The ARI indicates the overall concordance between data from two typing methods and includes a correction factor to take into account the possibility that concordance may have arisen by chance. The online tool was also used to calculate the Wallace (W) coefficient (14), which indicates the probability that two isolates classified as the same type by one method will also be classified as the same type by another method. Hence, the W coefficient gives a quantitative estimate of the value of including additional typing methods. A high W coefficient suggests that including a particular additional method does not yield further information. The W coefficient also provides directional information about the concordance of data from typing methods in that it quantifies the probability that isolates clustered by one typing method (e.g., PFGE) will be assigned to the same cluster by a second typing method (e.g., *spa* typing) and vice versa (14). Where the value of the W coefficient is low when comparing one method to another and results are similar in both directions (e.g., *spa* to *dru* versus *dru* to spa), the inference is that isolates clustered by one typing method may be subdivided by the other typing method (6).

RESULTS

MRSA isolates ($n = 173$) were recovered from one Dublin hospital during two 6-week study periods in four different wards. The PFTs and AR-PFGs as well as the *spa*, *dru*, and SCC*mec* typing and MLST results for the 173 MRSA isolates are shown in Table 2.

PFGE and AR typing. Twenty-six PFTs representing four PFGs were identified among the 173 isolates (Table 2). PFG-01 predominated, accounting for 97% of isolates (168 of 173). The 168 PFG-01 isolates exhibited 21 highly similar PFTs, with the two most predominant patterns (PFT 01018 [$n = 57$] and PFT 01039 [$n = 39$]), which accounted for 57.1% of all PFG-01 isolates (96 of 168), differing by only a single band.

The majority of PFG-01 isolates (135 of 168 [80.4%]) exhibited AR type AR06 and were assigned to AR-PFG 06-01 (Table 2). The AR types and subtypes and the antimicrobial resistance patterns for all isolates investigated are shown in Table S1 in the supplemental material.

***spa* typing.** Seventeen *spa* types were identified among the 168 isolates classified into PFG-01, but 55.4% of these isolates (93 of 168) belonged to *spa* type t032. The proportions of isolates of other *spa* types among the PFG-01 isolates were as follows: t515, 16 of 168 (9.5%); t3185, 15 of 168 (8.9%); t1214, 10 of 168 (6%); t022, 10 of 168 (6%); t628, 9 of 168 (5.4%); t1865, 4 of 168 (20.4%); and t3213, 2 of 168 (1.2%). *spa* types t025, t578, t1802, t2951, t4122, t4267, t4622, t4623, and t4765 were exhibited by single isolates only (Table 2).

***dru* typing.** The stability of the *dru* region in three MRSA isolates was confirmed by the finding that several colonies from original cultures of each isolate on BHI agar plates and from growth following a minimum of 10 sequential subcultures exhibited the same *dru* types originally assigned in earlier studies (i.e., dt9g for M06/0376 and M05/0028 and dt10h for the Harmony EMRSA-15 isolate).

Seventeen *dru* types were identified among the 168 PFG-01 isolates, with dt10a isolates (61 of 168 [36.3%]) predominating. Proportions of isolates of other types were as follows: dt10j, 43 of 168 (25.6%); dt10n, 22 of 168 (13%); dt10o, 16 of 168 (9.5%); dt10af, 6 of 168 (3.6%); dt8p, 4 of 168 (2.4%); dt5b, 2

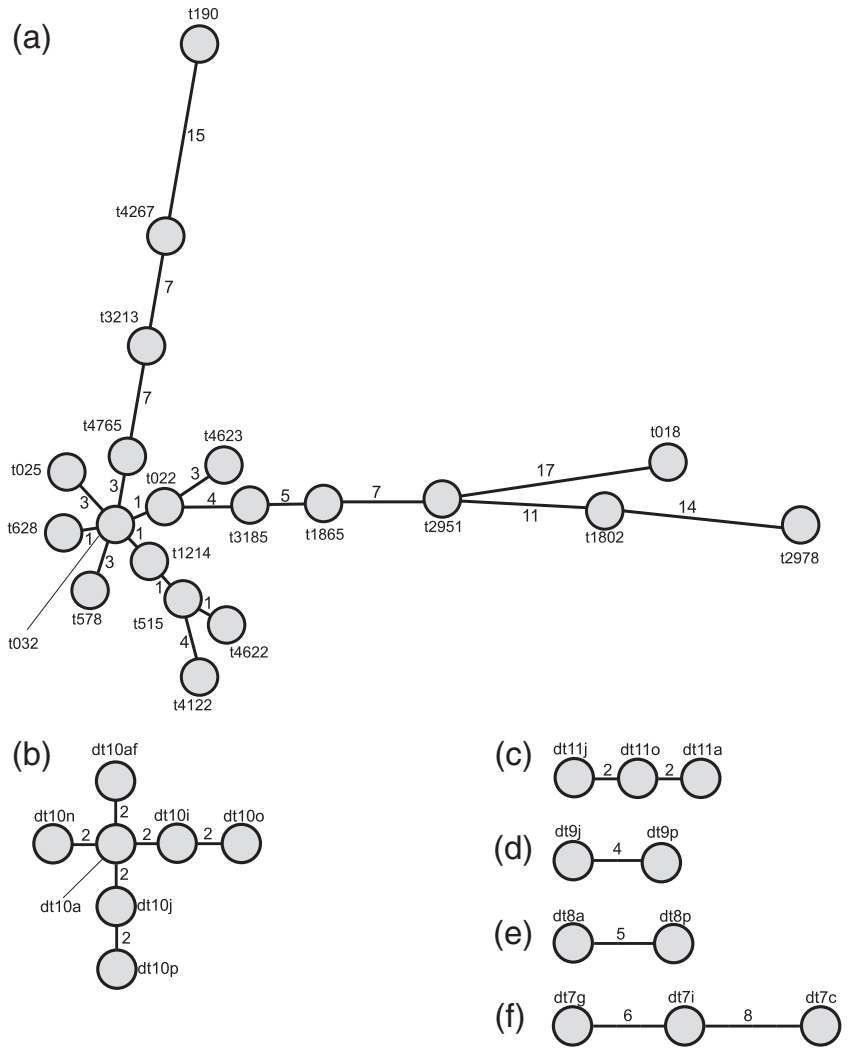


FIG. 1. MSTs generated using the BioNumerics software program representing the 20 *spa* types (a) and the *dru* types with 10 (b), 11 (c), 9 (d), 8 (e), and 7 (f) repeat units identified among the 173 MRSA isolates investigated. Each individual circle represents a different *spa* or *dru* type, and the numerical values on the branches represent the similarity (expressed as the MST distance) between two *spa* or two *dru* types. The BioNumerics software creates groups of certain distance intervals or similarity values (termed bins) and converts these data into distance units. The bin distance was set to 0.5% (i.e., two entries at a distance of 1 on the MST have between 99 and 99.5% similarity, and two entries at a distance of 2 have between 98.5 and 99% similarity, etc.). *spa* types and *dru* types were assigned the same cluster code if they were separated by an MST distance of <2 (i.e., if they showed >98.5% similarity) (Table 1).

of 168 (1.2%); dt7g, 2 of 168 (1.2%); dt10p, 2 of 168 (1.2%); dt11j, 2 of 168 (1.2%); and dt11o, 2 of 168 (1.2%). The *dru* types dt6e, dt7i, dt8a, dt9j, dt9p, and dt11a were exhibited by single isolates among the remaining PFG-01 isolates (Table 2).

MLST and SCC*mec* typing. By MLST, four distinct sequence types (STs) were detected among 20 isolates representative of each of the 20 *spa* types identified (Table 2). Isolates exhibiting the 17 *spa* types found among the 168 PFG-01 isolates were all identified as ST22 (i.e., MLST allelic profile 7-6-1-5-8-8-6) and belonged to clonal complex 22 (i.e., CC22).

SCC*mec* typing revealed that the majority of PFG-01 isolates (163 of 168 [97%]) harbored SCC*mec* IVh (with *ccrAB2*, class B *mec*, *dcs*, and J1 region type IVh). Of the remaining five PFG-01 isolates, one harbored SCC*mec* IVa (with *ccrAB2*, class B *mec*, *dcs*, and J1 region type IVa) and four harbored novel SCC*mec* IV variants (Table 2).

Clustering of isolates. The 173 MRSA isolates were divided into clusters based on *spa*, *dru*, and PFGE typing data. Each isolate was assigned a 3-digit cluster code with the first number representing the *spa* type, the second representing the *dru* type, and the third representing the PFT (Tables 1 and 2). Subgrouping among *spa* and *dru* types was investigated by constructing MSTs (Fig. 1), and subgrouping among PFGE types was based on the numbers of band differences, as described below.

Cluster analysis of *spa* types. An MST constructed from all *spa* types identified is shown in Fig. 1a. *spa* types were deemed to be distinct if they differed from all others identified by an

TABLE 3. Discriminatory powers of *spa*, *dru*, and PFGE typing methods[a]

Typing method(s)	No. of types	SID	95% CI
spa typing	17	66.90	59.36–74.44
dru typing	17	77.83	73.86–81.80
PFGE	21	81.34	77.38–85.31
spa and *dru* typing	37	90.84	88.66–93.02
spa typing and PFGE	44	91.00	88.12–93.89
dru typing and PFGE	43	93.57	92.08–95.66
spa, *dru*, and PFGE typing	65	96.53	95.53–97.52

[a] Discriminatory powers of the methods used individually and in combination for the 168 PFG-01 (ST22-MRSA-IV) isolates investigated were measured by using SID (with 95% CIs).

MST distance of >2 (corresponding to <98.5% similarity) and were assigned different *spa* cluster codes with numerical values ranging from 01 to 15 (Fig. 1a and Table 1). *spa* types that showed >98.5% similarity on the MST (i.e., those at an MST distance of ≤2) were assigned *spa* subcluster codes (Table 1). Each *spa* type within a subcluster, apart from the founder of a subgroup, was assigned an additional alphabetic suffix (Table 1). The MST showed that some *spa* type subgroups contained further subgroups, so additional alphabetic suffixes were added to the alphanumeric *spa* subcluster codes (Table 1).

Cluster analysis of *dru* types. For *dru* typing, individual MSTs were generated for all groups of distinct *dru* types harboring the same number of *dru* repeat units. MSTs for *dru* types with 11 (dt11a, dt11j, and dt11o), 10 (dt10a, dt10i, dt10j, dt10n, dt10o, dt10p, and dt10af), 9 (dt9j and dt9p), 8 (dt8a and dt8p), and 7 (dt7c, dt7g, and dt7i) repeat units are shown in Fig. 1c, b, d, e, and f, respectively.

dru type cluster codes were based on the number of *dru* repeat units present (e.g., *dru* types with 10 repeats were assigned *dru* cluster code 01) (Table 1). Thereafter, subgroups of closely related *dru* types were identified using the same criteria used for subgrouping of *spa* types (Table 1).

Cluster analysis of PFTs. For cluster analysis, each of the four PFGs was assigned a PFGE cluster code consisting of a number ranging from 1 to 4. Where PFGs were represented by more than one PFT, the most frequently occurring PFT was assigned the numerical value for that PFG and all other PFTs were assigned additional alphabetic suffixes (Table 1).

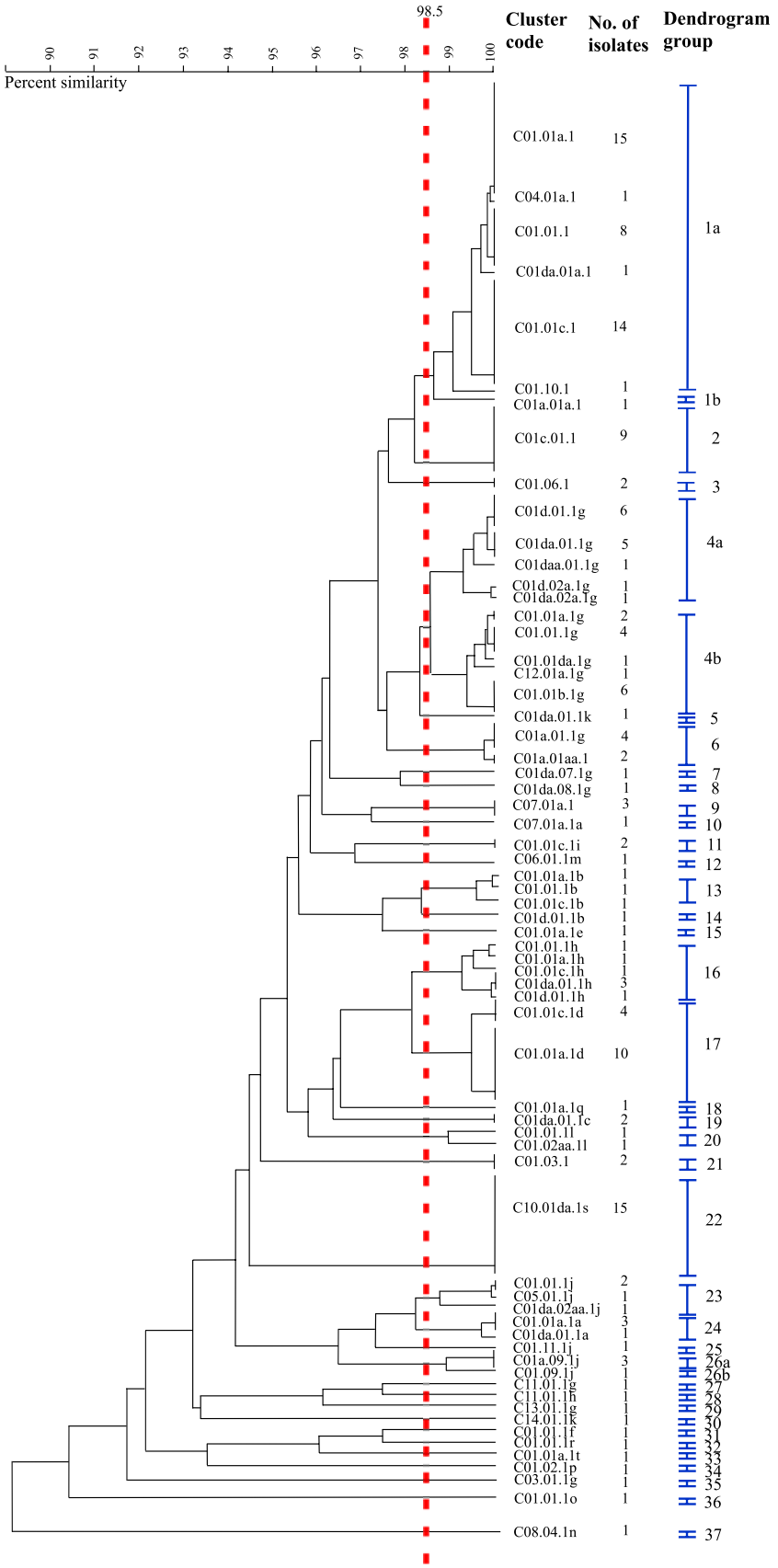
Discriminatory powers of and concordance of data from *spa*, *dru*, and PFGE typing methods. The abilities of PFGE, *spa*, and *dru* typing methods to discriminate among the 168 PFG-01 (ST22-MRSA-IV) isolates were determined quantitatively using SID for each individual typing method and for all combinations of the three methods (Table 3). The combination of *spa*, *dru*, and PFGE typing yielded the largest number of type combinations (65 types) and the greatest discriminatory power (SID, 96.53) with the narrowest 95% CI (Table 3). Of the three individual methods, PFGE was the most discriminatory (SID, 81.34) (Table 3).

The enhanced discrimination obtained by combining all three typing methods was confirmed by the ARI and W coefficient values (Table 4). Based on the ARI, the probability that the isolate clustering patterns obtained using the combination of *spa*, *dru*, and PFGE typing methods would be similar to those obtained using any one of the typing methods individually or pairwise combinations of the methods was <69% (range, ca. 13 to 69%) (Table 4). In addition, the low W coefficients obtained for the comparison of individual methods suggest that no method is redundant and that each method contributes additional information. The highest value for the comparison between a pair of individual methods was obtained for PFGE and *spa* typing (W coefficient, 0.482), but the value for the comparison between *spa* typing and PFGE was much lower (W coefficient, 0.272) (Table 4). Hence, the PFGE type could predict the *spa* type with 48% probability whereas the probability with which the *spa* type predicted the PFGE type was only 27%. High W coefficients were obtained for comparisons between a combination of two or three methods and one of the methods individually (e.g., the combination of *spa* typing and PFGE and *spa* typing alone) (Table 4).

Cluster analysis of isolates based on the combination of *spa*, *dru*, and PFGE typing results. Seventy cluster codes representative of each different combination of *spa*, *dru*, and PFGE types were identified for the 173 isolates investigated (Table 2). For the 168 PFG-01 isolates, 65 cluster codes were identified (Table 2). Many of the isolates with different cluster codes exhibited only minor differences by combinations of *spa*, *dru*, and/or PFGE typing methods. To further investigate the relationship among PFG-01 isolates, a dendrogram was generated from the averages of the similarity matrices for *spa*, *dru*, and PFGE typing data for all PFG-01 isolates (Fig. 2). Isolates that

TABLE 4. Concordance of data from *spa*, *dru*, and PFGE typing methods used individually and in combination for the 168 PFG-01 (ST22 MRSA-IV) isolates

Typing method(s)	ARI for comparison with:						W coefficient for comparison with:						
	spa typing	*dru* typing	PFGE	*spa* and *dru* typing	*spa* typing and PFGE	*dru* typing and PFGE	*spa* typing	*dru* typing	PFGE	*spa* and *dru* typing	*spa* typing and PFGE	*dru* typing and PFGE	*spa*, *dru*, and PFGE typing
spa typing								0.277	0.272	0.277	0.272	0.105	0.105
dru typing	0.090						0.413		0.290	0.413	0.157	0.290	0.157
PFGE	0.143	0.141					0.482	0.345		0.186	0.482	0.345	0.186
spa and *dru* typing	0.339	0.523	0.144				1.000	1.000	0.379		0.379	0.379	0.379
spa typing and PFGE	0.333	0.109	0.602	0.321			1.000	0.386	1.000	0.386		0.386	0.386
dru typing and PFGE	0.076	0.389	0.461	0.400	0.406		0.540	1.000	1.000	0.540	0.540		0.540
spa, *dru*, and PFGE typing	0.136	0.224	0.271	0.526	0.534	0.687	1.000	1.000	1.000	1.000	1.000	1.000	


98.5
90
91
92
93
94
95
96
97
98
99
100
Percent similarity
Cluster code
No. of isolates
Dendrogram group
C01.01a.1 15
C04.01a.1 1
C01.01.1 8
C01da.01a.1 1
C01.01c.1 14
1a
C01.10.1 1
C01a.01a.1 1
1b
C01c.01.1 9
2
C01.06.1 2
3
C01d.01.1g 6
C01da.01.1g 5
C01daa.01.1g 1
C01d.02a.1g 1
C01da.02a.1g 1
4a
C01.01a.1g 2
C01.01.1g 4
C01.01da.1g 1
C12.01a.1g 1
C01.01b.1g 6
4b
C01da.01.1k 1
5
C01a.01.1g 4
6
C01a.01aa.1 2
C01da.07.1g 1
7
C01da.08.1g 1
8
C07.01a.1 3
9
C07.01a.1a 1
10
C01.01c.1i 2
11
C06.01.1m 1
12
C01.01a.1b 1
C01.01.1b 1
C01.01c.1b 1
13
C01d.01.1b 1
14
C01.01a.1e 1
15
C01.01.1h 1
C01.01a.1h 1
C01.01c.1h 1
C01da.01.1h 3
C01d.01.1h 1
16
C01.01c.1d 4
C01.01a.1d 10
17
C01.01a.1q 1
18
C01da.01.1c 2
19
C01.01.1l 1
20
C01.02aa.1l 1
C01.03.1 2
21
C10.01da.1s 15
22
C01.01.1j 2
C05.01.1j 1
C01da.02aa.1j 1
23
C01.01a.1a 3
C01da.01.1a 1
24
C01.11.1j 1
25
C01a.09.1j 3
26a
C01.09.1j 1
26b
C11.01.1g 1
27
C11.01.1n 1
28
C13.01.1g 1
29
C14.01.1k 1
30
C01.01.1f 1
31
C01.01.1r 1
32
C01.01a.1t 1
33
C01.02.1p 1
34
C03.01.1g 1
35
C01.01.1o 1
36
C08.04.1n 1
37

showed ≥98.5% similarity on the dendrogram were deemed to be very closely related and were assigned to the same dendrogram group (DG), while those with <98.5% similarity were deemed to be distinguishable and were assigned to different DGs (Fig. 2). By using these criteria, a total of 37 DGs were identified among the 168 PFG-01 isolates (Table 2 and Fig. 2). Three of these DGs (DG-1, DG-4, and DG-26) were divided into subgroups because they included isolates that showed between 98.5 and 99% similarity (Fig. 2). DG-1 and DG-4 were the largest groups identified and consisted of 41 of 168 isolates (24.4%) and 28 of 168 isolates (16.7%), respectively (Fig. 2).

Five distinct cluster codes were identified among the five non-PFG-01 isolates; these isolates showed <90% similarity to one another and to all other isolates investigated according to a dendrogram generated from the averages of the similarity matrices for *spa*, *dru*, and PFGE typing data for all isolates investigated in the present study (data not shown). Therefore, these five isolates were deemed to be distinguishable and were assigned to distinct DGs (DG-38 to DG-42) (Table 2).

Combining the dendrogram groupings with epidemiological evidence. The dendrogram groupings for one hospital ward during two 6-week study periods were analyzed in the context of available epidemiological data. Dendrograms were generated from the averages of the similarity matrices for *spa*, *dru*, and PFGE typing data for all PFG-01 isolates recovered from patients and environmental sites in one ward (ward 1) from (i) July to September 2007, with one additional isolate recovered in November 2007 (study period I; $n = 38$) (Fig. 3, top), and (ii) April to May 2008 (study period II; $n = 22$) (Fig. 3, bottom).

Twelve DGs were identified among the 38 PFG-01 isolates recovered during study period I (Fig. 3, top). The largest DG recognized was DG-1a, consisting of 11 isolates. The earliest DG-1a isolate was recovered from a patient who was MRSA positive upon admission to the ward (Fig. 3, top). Over the next 10 days, DG-1a isolates were recovered from three patients and seven environmental sites; MRSA isolates from two of these patients were deemed to have been HA (Fig. 3, top).

The second largest DG (DG-4) consisted of nine isolates belonging to DG-4a ($n = 6$) or DG-4b ($n = 3$) (Fig. 3, top). The earliest DG-4 isolate was recovered from a bed mattress and belonged to DG-4a (Fig. 3, top). Two weeks later, a second DG-4a isolate was recovered from a patient whose MRSA infection was considered to have been HA. Subsequently, DG-4a and DG-4b isolates were recovered from additional patients and environmental sites, but none of the MRSA isolates from the patients were deemed to have been HA (Fig. 3, top). Four pairs of isolates were recovered during study period I, but isolates from one pair only (pair 02) were assigned to the same DG (DG-16) (Fig. 3, top).

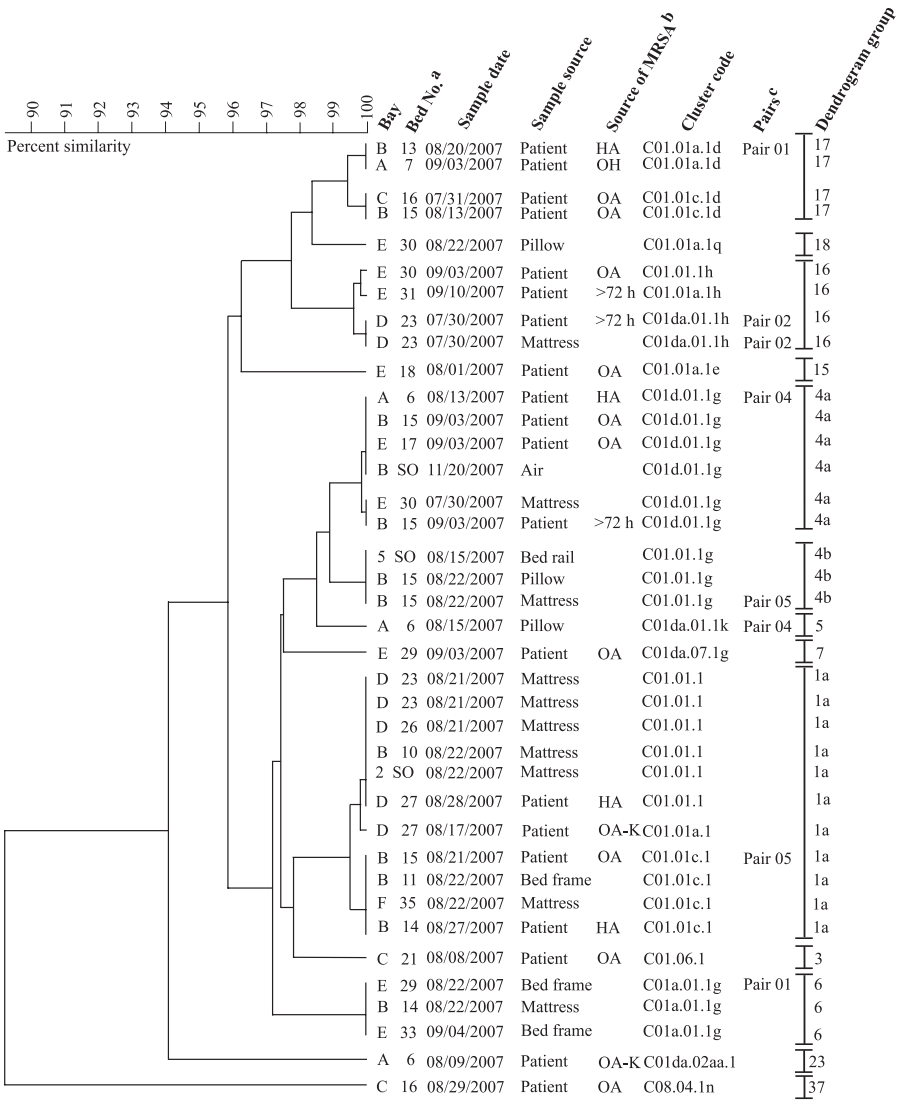
During study period II, eight DGs were identified among the 22 PFG-01 isolates recovered, with DG-2 isolates (9 of 22 [40.9%]) predominating (Fig. 3, bottom). The earliest DG-2 isolate came from a bed mattress. Subsequently, DG-2 isolates were recovered from six environmental sites and from two patients, both of whom were MRSA positive upon admission (Fig. 3, bottom). Four DG-26 isolates, including a triplet of isolates (triplet 01) recovered from a patient and the patient's mattress and bed rail, were identified during study period II. Two DG-1a, two DG-4a, and two DG-17 isolates were also identified during study period II (Fig. 3, bottom). The two DG-1a isolates (pair 03) were recovered from a patient and, 8 days later, from that patient's locker, while the DG-4a isolates were recovered from two patients within 24 h of each other. The first DG-17 isolate was from a patient, and 1 day later, the second isolate was recovered from a bed rail of a different bed (bed 10) in the same bed bay occupied by the patient (Fig. 3, bottom). A DG-16 isolate had been recovered the previous day from the patient in bed 10. This DG-16 isolate and the DG-17 isolate recovered from the rail of bed 10 (pair 06) differ by one PFGE band only, and while they showed 98.1% similarity on the dendrogram generated for all PFG-01 isolates (Fig. 2), they showed ca. 98.4% similarity on the dendrogram for PFG-01 isolates recovered in ward 1 during study period II (Fig. 3, bottom).

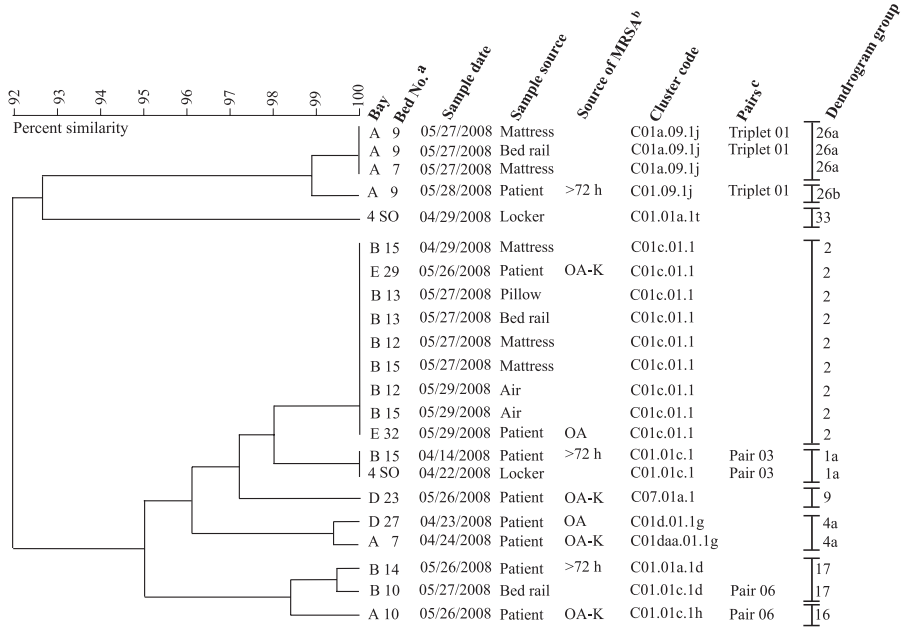
DISCUSSION

Epidemiological tracking of ST22-MRSA-IV isolates is a major challenge, as they exhibit limited diversity by PFGE and *spa* typing, the most frequently used epidemiological typing methods available for MRSA. The present study investigated whether integration of PFGE, *spa*, and *dru* typing data would provide improved discrimination among ST22-MRSA-IV isolates recovered in a large tertiary-referral hospital in Ireland.

A total of 168 ST22-MRSA-IV isolates were investigated using the three typing methods. The combined use of the PFGE, *spa*, and *dru* typing data yielded the highest number of type combinations (65 types) and the greatest discriminatory power (SID, 96.53) with the narrowest 95% CI. Faria et al. (14) compared the abilities of several typing methods, including PFGE and *spa* typing, to discriminate among a diverse collection of MRSA and methicillin-susceptible *S. aureus* isolates. They reported that *spa* typing and PFGE differentiated their MRSA isolates with SIDs of 95.85 and 94.27, respectively. In contrast, the SIDs for *spa* and PFGE typing of the ST22-MRSA-IV isolates obtained in the present study were 66.9 and 81.34, respectively. Faria et al. (14) also found that the combination of PFGE and *spa* typing had a discriminatory power yielding a SID of 98.32, whereas the SID for this combination of typing methods in the present study was 91.00. These findings indicate that while the combination of PFGE and *spa*

FIG. 2. Composite dendrogram generated using UPGMA clustering and the averages of the similarity matrices from *spa*, *dru*, and PFGE typing data for the 168 PFG-01 MRSA (ST22 MRSA-IV) isolates investigated during the present study. Isolates were assigned 3-digit cluster codes (C) with the first number representing the *spa* type, the second representing the *dru* type, and the third representing the PFT. Isolate cluster codes were then assigned to DGs as follows: isolates with cluster codes that showed ≥98.5% similarity on the dendrogram were deemed to be very closely related and were assigned to the same DG. Those isolates with cluster codes showing <98.5% similarity were deemed to be distinguishable and were assigned to different DGs. The dendrogram demonstrates that the PFG-01 isolates were assigned to 65 cluster codes that were divided into 37 DGs. The red, dashed vertical line marks 98.5% similarity.


90 91 92 93 94 95 96 97 98 99 100
Percent similarity
Bay
Bed No. a
Sample date
Sample source
Source of MRSA b
Cluster code
Pairs c
Dendrogram group
B 13 08/20/2007 Patient HA C01.01a.1d Pair 01 17
A 7 09/03/2007 Patient OH C01.01a.1d 17
C 16 07/31/2007 Patient OA C01.01c.1d 17
B 15 08/13/2007 Patient OA C01.01c.1d 17
E 30 08/22/2007 Pillow C01.01a.1q 18
E 30 09/03/2007 Patient OA C01.01.1h 16
E 31 09/10/2007 Patient >72 h C01.01a.1h 16
D 23 07/30/2007 Patient >72 h C01da.01.1h Pair 02 16
D 23 07/30/2007 Mattress C01da.01.1h Pair 02 16
E 18 08/01/2007 Patient OA C01.01a.1e 15
A 6 08/13/2007 Patient HA C01d.01.1g Pair 04 4a
B 15 09/03/2007 Patient OA C01d.01.1g 4a
E 17 09/03/2007 Patient OA C01d.01.1g 4a
B SO 11/20/2007 Air C01d.01.1g 4a
E 30 07/30/2007 Mattress C01d.01.1g 4a
B 15 09/03/2007 Patient >72 h C01d.01.1g 4a
5 SO 08/15/2007 Bed rail C01.01.1g 4b
B 15 08/22/2007 Pillow C01.01.1g 4b
B 15 08/22/2007 Mattress C01.01.1g Pair 05 4b
A 6 08/15/2007 Pillow C01da.01.1k Pair 04 5
E 29 09/03/2007 Patient OA C01da.07.1g 7
D 23 08/21/2007 Mattress C01.01.1 1a
D 23 08/21/2007 Mattress C01.01.1 1a
D 26 08/21/2007 Mattress C01.01.1 1a
B 10 08/22/2007 Mattress C01.01.1 1a
2 SO 08/22/2007 Mattress C01.01.1 1a
D 27 08/28/2007 Patient HA C01.01.1 1a
D 27 08/17/2007 Patient OA-K C01.01a.1 1a
B 15 08/21/2007 Patient OA C01.01c.1 Pair 05 1a
B 11 08/22/2007 Bed frame C01.01c.1 1a
F 35 08/22/2007 Mattress C01.01c.1 1a
B 14 08/27/2007 Patient HA C01.01c.1 1a
C 21 08/08/2007 Patient OA C01.06.1 3
E 29 08/22/2007 Bed frame C01a.01.1g Pair 01 6
B 14 08/22/2007 Mattress C01a.01.1g 6
E 33 09/04/2007 Bed frame C01a.01.1g 6
A 6 08/09/2007 Patient OA-K C01da.02aa.1 23
C 16 08/29/2007 Patient OA C08.04.1n 37


92 93 94 95 96 97 98 99 100
Percent similarity
Bay
Bed No. a
Sample date
Sample source
Source of MRSA b
Cluster code
Pairs c
Dendrogram group
A 9 05/27/2008 Mattress C01a.09.1j Triplet 01 26a
A 9 05/27/2008 Bed rail C01a.09.1j Triplet 01 26a
A 7 05/27/2008 Mattress C01a.09.1j 26a
A 9 05/28/2008 Patient >72 h C01.09.1j Triplet 01 26b
4 SO 04/29/2008 Locker C01.01a.1t 33
B 15 04/29/2008 Mattress C01c.01.1 2
E 29 05/26/2008 Patient OA-K C01c.01.1 2
B 13 05/27/2008 Pillow C01c.01.1 2
B 13 05/27/2008 Bed rail C01c.01.1 2
B 12 05/27/2008 Mattress C01c.01.1 2
B 15 05/27/2008 Mattress C01c.01.1 2
B 12 05/29/2008 Air C01c.01.1 2
B 15 05/29/2008 Air C01c.01.1 2
E 32 05/29/2008 Patient OA C01c.01.1 2
B 15 04/14/2008 Patient >72 h C01.01c.1 Pair 03 1a
4 SO 04/22/2008 Locker C01.01c.1 Pair 03 1a
D 23 05/26/2008 Patient OA-K C07.01a.1 9
D 27 04/23/2008 Patient OA C01d.01.1g 4a
A 7 04/24/2008 Patient OA-K C01daa.01.1g 4a
B 14 05/26/2008 Patient >72 h C01.01a.1d 17
B 10 05/27/2008 Bed rail C01.01c.1d Pair 06 17
A 10 05/26/2008 Patient OA-K C01.01c.1h Pair 06 16

typing is highly discriminatory for collections of diverse MRSA isolates, it is inadequate in local epidemiological studies where strain diversity is limited, as with ST22-MRSA-IV. While the 168 ST22-MRSA-IV isolates investigated here were differentiated into 65 type combinations by integrating *spa*, *dru*, and PFGE typing data, some of the types identified using each individual method exhibited only minor differences and were assigned to subgroups. All 21 PFTs identified among the 168 ST22-MRSA-IV isolates were assigned to a single group (PFG-01) and were deemed to be possibly related according to the criteria of Tenover et al. (57), as they all differed by ≤6 bands. These criteria were originally devised for a range of bacterial species, including *S. aureus*, but can present problems with clonal populations of MRSA exhibiting limited genetic diversity (16). A cutoff of 80% similarity for grouping clusters of MRSA isolates, with a cutoff of 95% similarity for recognition of subtypes, has been proposed previously (14, 37). In the present study, dendrogram clustering of PFGE data showed >80% similarity among all 168 ST22-MRSA-IV isolates. The most frequently occurring PFTs (PFT 01018 and PFT 01039) differed from each other by a single band and showed 98% similarity to each other (data not shown). With PFGE, undue weight cannot be placed on a single band difference, so for the majority of the isolates in the study population, PFGE alone could not provide reliable differentiation. Unlike PFGE, for which there are agreed-upon interpretive criteria (57), *spa* and *dru* typing currently have no criteria defined by international consensus for interpreting the significance of differences in results. Consequently, isolates with different *spa* types or *dru* types are deemed to be distinct even though they may be closely related if the types differ by changes consistent with a single genetic event, such as duplication of a tandem repeat, a point mutation, or a base insertion or deletion. To investigate the significance of such differences objectively, *spa* and *dru* types were assigned as subgroups by using MSTs if they showed ≥98.5% similarity. This cutoff value grouped 7 of the 17 *spa* types identified among the 168 ST22-MRSA-IV isolates investigated into six *spa* subgroups (Table 1). Each *spa* type within each subgroup differed from the other members of the subgroup by the presence or absence of one to three tandem repeats. With *dru* typing, 10 of the 17 *dru* types identified were grouped into eight subgroups (Table 1). The *dru* types within each subgroup differed by nucleotide changes in one or two repeat units only.

While *spa* typing and PFGE are well established methods for typing of MRSA isolates, few studies have investigated the usefulness of *dru* typing for MRSA. Smyth et al. (54) identified 42 *dru* types among 111 isolates of the pandemic nosocomial strain ST239-MRSA-III. Goering et al. (17) identified 13 and 12 *dru* types among 47 EMRSA-15 (ST22-MRSA-IV) and 57 EMRSA-16 (ST36-MRSA-II) isolates, respectively. In contrast, the majority of CA-MRSA USA300 (ST8-MRSA-IV) and CC80 isolates from patients from various geographical locations exhibited dt9g and dt10a, respectively (58, 29). The results of these studies indicate that CA-MRSA strains exhibit less genetic diversity within the *dru* region than nosocomial MRSA strains. This difference may reflect the fact that the nosocomial strains have been extant longer than CA-MRSA strains (12, 44, 54, 61). In the present study, 17 *spa* types were identified among 168 ST22-MRSA-IV isolates, of which 4, t032, t515, t022, and t1214, were further differentiated into 12, 6, 4, and 2 *dru* types, respectively. Nevertheless, *dru* typing cannot be used as a stand-alone method for typing MRSA isolates, as the two predominant types recognized among the ST22-MRSA-IV isolates (dt10a and dt10j) were also identified among the three ST8-MRSA-II variant isolates in the present study and among CC80-MRSA-IV isolates described in a previous study (29). Three *dru* types identified among ST22-MRSA-IV isolates in the present study (dt10a, dt11a, and dt8a) were also identified previously among ST239-MRSA-III isolates (54), while dt10i has been identified in unrelated MRSA lineages, including EMRSA-15 (17) and the ST87-MRSA-IV isolate in the present study. These data indicate that an isolate's *dru* type is not lineage or SCC*mec* type specific. However, unrelated MRSA lineages sharing indistinguishable *dru* types may reflect the presence of related SCC*mec* elements in diverse genetic backgrounds.

The composite dendrogram generated from the combined *spa*, *dru*, and PFGE typing data for all 168 ST22-MRSA-IV isolates provides a visual representation of the overall relatedness of isolates. Using a cutoff of 98.5% similarity, isolates were differentiated into 37 DGs, 17 (46%) of which contained more than one isolate and 10 of which contained isolates representing more than one cluster code, further indicating the close relatedness of the isolates. Of the 10 DGs consisting of isolates belonging to different cluster codes, 4 contained isolates with *spa* and/or *dru* types that were not assigned as subgroups (Fig. 2 and Table 2), including DG-1a (t025 and dt9j), DG-4 (for DG-4b, t4122, and for DG-4a, dt11o), DG-23 (t578 and dt11j), and DG-20 (dt11j). In each case, the PFGE patterns were indistinguishable from those of other isolates within that DG. In addition, where the *spa* type was distinct, the *dru* type was indistinguishable from or closely related to that of other isolates within that DG; where the *dru* type was distinct, the *spa* type was indistinguishable from or closely related to that of other isolates within that DG (Table 2). These findings

FIG. 3. Composite dendrogram generated using UPGMA clustering and the averages of the similarity matrices from *spa*, *dru*, and PFGE typing data for 38 PFG-01 MRSA isolates investigated during study period I (top) and 22 PFG-01 MRSA isolates investigated during study period II (bottom). Available epidemiological information for each isolate, as well as the cluster code and DG, is indicated. DGs were assigned to the different cluster codes determined from the dendrogram depicting all PFG-01 isolates identified in the present study (Fig. 2). The dendrogram shows that the PFG-01 isolates from study period I ($n = 38$) and study period II ($n = 22$) were differentiated into 12 and 8 DGs, respectively.[a], the abbreviation SO in place of a bed number indicates a single-occupancy room.[b], OA, the patient was MRSA positive on admission; OA-K, the patient's MRSA-positive status was known at the time of admission to the ward; >72 h, the patient's MRSA status was determined 72 h after admission to the ward.[c], the pairs or triplets of isolates recovered during study periods I and II are indicated and include isolates recovered from patients and from their immediate environments during the same study period. Each pair or triplet consists of one isolate from a patient and at least one environmental isolate.

highlight the need for caution when interpreting data from individual typing methods and show how combining data from the three typing methods permits a more informative evaluation of the relationship among isolates.

In the present study, analysis of available epidemiological information for a selected subset of ST22-MRSA-IV isolates was used to confirm the validity of the relationships inferred from the combined PFGE, *spa*, and *dru* typing data. Six pairs and one triplet of isolates were recovered from individual patients and their immediate ward environments during the same time periods, and by using the combination of all three typing methods, isolates in four of these pairs (pairs 01, 04, 05, and 06) were differentiated into distinct DGs. Isolates in pair 01 differed by all three typing methods and exhibited <96% similarity on the composite dendrogram (Fig. 3, top). Interestingly, the environmental isolate in pair 01 was recovered from a bed bay 2 days after the patient isolate was obtained while the patient was in a different bed bay. Pair 04 isolates belonged to DG-4a and DG-5 and differed in their *spa* type and PFT, and the composite dendrogram showed that these isolates had <98.5% similarity (Fig. 3, top). Pair 05 isolates belonged to DG-1a and DG-4b, and although they shared the same *spa* type and belonged to *dru* types and PFTs that were assigned as subtypes, the composite dendrogram showed that they had <98% similarity (Fig. 3, top), suggesting that they are distinguishable. However, pair 06 isolates belonged to DG-16 and DG-17, differed only with regard to the PFGE patterns (exhibiting a one-band difference), and showed ca. 98.4% similarity (Fig. 3, bottom), suggesting that these isolates should be considered to be very closely related. Isolates in each of the two remaining pairs (pair 02, consisting of DG-16 isolates, and pair 03, consisting of DG-1a isolates) and the triplet (triplet 01, consisting of DG-26 isolates) were indistinguishable from each other (Fig. 3). This analysis revealed that certain patient and environmental ST22-MRSA-IV isolates could be differentiated while others remained indistinguishable and showed that the combination of typing methods used in the present study significantly improves isolate discrimination and therefore can be used for epidemiological tracking of isolates of this highly clonal strain.

Different DGs predominated among isolates from the two study periods (DG-1a in study period I and DG-2 in study period II). Particular isolates within DG-1a and DG-2 differed in the *spa* type only, with DG-1a isolates exhibiting *spa* type t032 while DG-2 isolates belonged to *spa* type t628. While these *spa* types were assigned to the same subgroup, only nine isolates exhibiting *spa* type t628 were recovered during the study and all nine were recovered from ward 1 during study period II, suggesting that the difference between t032 and t628 is significant (Fig. 3).

Based on the results of this study, it is recommended that in performing epidemiological investigations of a highly clonal MRSA strain, such as ST22-MRSA-IV, in a hospital setting where the strain is endemic, optimal tracking can be achieved by combining *spa* and PFGE typing data with *dru* typing data. This approach has revealed a previously unrecognized level of diversity among ST22-MRSA-IV isolates that can be used to provide data fundamental to epidemiological tracking of isolates of this pandemic MRSA strain. PFGE and *spa* typing are routinely used for typing of MRSA isolates (19, 37), and while *dru* typing may not be as well established or widely used for typing of MRSA isolates, it involves the same techniques and principles as *spa* typing (DNA sequencing of a VNTR unit). Therefore, use of *dru* typing by a laboratory that currently uses DNA-based sequencing methods for routine epidemiological typing of MRSA isolates should not require additional expertise or result in a major increase in costs. In addition, all *spa*, *dru*, and PFGE data analyses can be performed with a commonly used software package (i.e., BioNumerics), and if required, statistical analysis of results can be readily and easily attained using a previously published free online tool (http://www.comparingpartitions.info). However, the ability of *dru* typing in combination with PFGE and *spa* typing to discriminate among isolates of highly clonal strains of MRSA other than ST22-MRSA-IV remains to be determined.

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