Enhanced tracking of the nosocomial transmission of endemic ST22-MRSA-IV among patients and environmental sites using whole-genome sequencing

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ABSTRACT

Whole-genome sequencing (WGS) of 41 patient and environmental ST22-MRSA-IV isolates recovered over six-weeks on one acute hospital ward in Dublin, Ireland, where ST22-MRSA IV is endemic, revealed 228 pairwise combinations differing by <40 single nucleotide variants corresponding to potential cross transmission events (CTEs). In contrast, 15 pairwise combinations of isolates representing five CTEs were previously identified by conventional molecular epidemiological typing. WGS enhanced ST22-MRSA-IV tracking and highlighted potential transmission of MRSA via the hospital environment.

ST22-MRSA-IV is endemic in hospitals in Ireland and the UK and predominates in several other European countries, Asia and Australia (1-6). ST22-MRSA-IV is highly clonal and tracking its spread is difficult (6). We previously reported enhanced discrimination of ST22-MRSA-IV from patients and hospital environmental sites using a combination of *spa*, *dru* and pulsed-field gel electrophoresis (PFGE) typing in combination with key epidemiological data (6-8). Several studies have demonstrated the usefulness of whole-genome sequencing (WGS) for differentiating and tracking MRSA in long-term and global studies and in outbreak settings (2, 9-11). However, no studies have investigated WGS for tracking the spread of ST22-MRSA-IV in an endemic setting. Price *et al.* investigated the transmission of *Staphylococcus aureus* in an intensive care unit using WGS over 14 months and reported a low rate of patient-to-patient transmission (12). However, they concluded that important transmission events were probably not identified because environmental sites were not investigated (12). We investigated the usefulness of WGS for tracking ST22-MRSA-IV between patients and environmental sites in a endemic hospital setting and to confirm or disprove cross-transmission events (CTEs) previously identified using conventional-molecular epidemiological (CME) typing.

Forty-one ST22-MRSA-IVh isolates recovered from 22 patients (one per patient) and 19 environmental sites (mattresses, bedrails, pillows and air) in one surgical ward of a 700-bed acute care hospital in Dublin, Ireland, during a 6-week period in 2007 were investigated (6). The 35-bed ward included 6-, 4- and 2-bed bays and five single rooms as detailed previously by Creamer *et al.* (8). The 41 isolates were previously characterized using SCC*mec*-, *spa*- and *dru* typing and pulsed-field gel electrophoresis (PFGE) with some isolates undergoing multilocus-sequence typing (6).

Among these isolates CTEs were previously identified using epidemiological information and molecular typing (7). Isolates were deemed to be part of a CTE if they were

recovered from ≥2 patients or from a patient and an environmental site within a three-week period on the same ward bay (a "probable" CTE) or on the same ward but not on the same ward bay (a "possible" CTE) (7). The MRSA status of the patient on admission, the probable source of the patient's MRSA, dates of admission and discharge and when MRSA was first detected, were also considered (7). Isolates were only included in CTEs if they were deemed to be hospital-acquired (HA) or if the patient's MRSA status was determined 72 h after ward admission. The CTEs identified using the epidemiological information were confirmed if the isolates differed by ≤1 typing method i.e. *spa*, *dru* type or PFGE typing. Using these criteria five CTEs were identified (7) and included five transmitted isolates from patients with HA-MRSA and 14 source isolates, seven each from patients and environmental sites; two isolates (M07/0339 & M07/0348, CTEs 2 & 3) were each implicated in two CTEs (Fig. 1).

Five pairs of isolates, each consisting of one patient isolate and one immediate ward environment isolate, were also previously identified among the 41 isolates (Fig. 1) (6, 7). This included four isolates (M07/0333, M07/0329, M07/0339 & M07/0334) also implicated in CTEs. Isolates associated with 2/5 pairs (pairs 2 & 3) exhibited indistinguishable *spa*, *dru* and PFGE types but were not included in CTEs as the patients concerned were MRSA-positive on ward admission. All previously reported molecular epidemiological data for these 41 isolates is summarized is Supplemental Table S1.

Genomic DNA was extracted from isolates using the Qiagen DNeasy Kit according to the manufacturer's instructions (Crawley, United Kingdom). Nextera XT library preparation reagents were used according to the manufacturer's instruction (Illumina, Eindhoven, The Netherlands). Libraries were sequenced on an Illumina MiSeq. Ridom SeqSphere+ software (Munster, Germany), which incorporates the Burrows-Wheeler aligner, was used for assembly with trimmed reads mapped against a previously described ST22-MRSA-IV genome, HO 5096 0412 (Genbank accession number HE681097), recovered in a UK acute

care hospital (2, 12). Assembled genomes were further analyzed against each other using the BioNumerics genome analysis tool (GAT) (version 7.5; Applied Maths, Ghent, Belgium) using the earliest recovered isolate (M07/0319) as a reference genome. Single nucleotide variants (SNVs) were identified and confirmed if they exhibited ≥ 40x coverage i.e. each SNV was covered by at least 40 reads, thereby avoiding ambiguous SNVs and increasing confidence in SNV validity. In fact >50% of all SNVs exhibited ≥ 100x coverage. All synonymous and non-synonymous mutations were included. Insertions and deletions (indels) and repetitive regions were excluded. Genomic SNV data per isolate was compared to the other 40 genomes yielding 861 pairwise comparisons.

Potential CTEs were defined as two isolates recovered at any time during the 6-week period differing by ≤ 40 SNVs based on reports of up to 40 SNVs among related *S. aureus* isolates from outbreaks or among multiple isolates from an individual and studies that used a cut-off of ≤40 SNVs for determining CTEs (12-14).

Whole-genome sequencing of the 41 isolates yielded an average coverage of 189x per genome (range 100-425x) and a total of 20,848 SNVs. Pairwise comparisons across the 41 genomes identified 228/861 pairwise comparisons, involving all 41 genomes in at least one pairwise comparison, where two isolates differed by ≤ 40 SNVs (range 0-40 SNVs). This included (i) 110 instances, involving 40/41 isolates, where one isolate was recovered from a patient and the other from an environmental source (shaded in supplemental Fig. S1(A)), (ii) 97 instances, involving 26/41 isolates, where both isolates were recovered from patient sources (shaded in supplemental Fig. S1(B)) and (iii) 21 instances, involving 11/41 isolates, where both isolates were recovered from an environmental source (shaded in supplemental Fig. S1(C)). There was no correlation between isolates within pairwise comparisons differing by ≤40 SNVs or >40 SNVs and the CME typing. Isolates differing by one, two or three of the conventional-molecular typing methods were identified among isolates within pairwise

126 comparisons differing by ≤40 and >40 SNVs as were isolates with a range of epidemiological
127 characteristics (Table S2). This may be due to low correlation between SNV analysis, which
128 detects mutations within the core genome and PFGE, which is affected by mobile genetic
129 elements (15). Additionally, SNV accumulation within the *spa* and *dru* regions may not
130 correlate with the entire genome.

131 In contrast to the 228 pairwise comparisons implicated as CTEs by SNV analysis, just
132 15/861 pairwise comparisons were associated with five CTEs using CME typing. The SNV
133 analysis confirmed 4/5 CTEs (CTEs 2, 3, 4 and 5) involving just 5/15 pairwise comparisons
134 as they differed by ≤ 40 SNVs (Fig 1). The transmitted and 2/3 source isolates within CTE 2
135 were indistinguishable based on *spa, dru* and PFGE typing but one isolate (M07/0340)
136 exhibited a different *dru* type (Fig. 1). However, only 1/3 source isolates (M07/0341)
137 exhibited ≤ 40 SNVs compared to the transmitted isolate (M07/0348) (Fig. 1). Five source
138 isolates within CTE 3 were indistinguishable from the transmitted isolate by *spa* and PFGE
139 typing but two isolates (M07/0339 & M07/0348) exhibited a different *dru* type. However,
140 only two of these isolates (M07/0334 & M07/0339) exhibited ≤ 40 SNVs when compared to
141 the transmitted isolate (M07/0350), one of which exhibited the different *dru* type (Fig. 1).
142 The four CTE 4 source isolates exhibited the same dru and PFGE type but a different *spa*
143 type to the transmitted isolate and only one of these (M07/0353) exhibited ≤ 40 SNV
144 differences compared to the transmitted isolate (Fig. 1). The one source isolate within CTE 5
145 differed in *dru* type only to the transmitted isolate and differed by 20 SNVs only (Fig. 1). The
146 transmitted and source isolates within CTE 1 differed in *dru* type only and exhibited 43 and
147 86 SNVs compared to the transmitted isolate (Fig. 1).

148 In relation to the five pairs of patient and environmental isolates, SNV analysis
149 indicated that 3/5 pairs of isolates i.e. pairs 1, 2 & 5, differed by ≤ 40 SNVs compared to 0/5
150 pairs which were assigned to CTEs by CME typing (Fig. 1). Among those that differed by ≤

40 SNVs, different molecular typing results were detected in pairs 1 (differences in *spa*, *dru* and PFGE) and 5 (differences in *dru* and PFGE) only (Fig. 1). Among the two pairs of isolates that differed by > 40 SNVs, one pair exhibited identical *spa*, *dru* and PFGE types (Pair 3) and one pair differed in *spa* and PFGE type (pair 4) (Fig. 1).

This study highlights the increased sensitivity of WGS over CME typing for tracking the highly clonal ST22-MRSA-IV in an endemic setting. The involvement of all isolates in at least one potential CTE using WGS and the identification of 228 pairwise comparisons differing by ≤40 SNVs compared to 15 pairwise comparisons representing CTEs by CME typing highlights ST22-MRSA-IVh transmissibility and how MRSA transmission may be significantly underestimated or incorrectly designated by CME approaches. The hospital environment had a significant role in ST22-MRSA-IV transmission with the identification of 110 instances of isolates from a patient and their immediate ward environment differing by ≤40 SNVs, a further 21 instances involving environmental sites only and 3/5 pairs of isolates from patients and their surrounding environment that were potential CTEs. However, healthcare workers should also be considered as a reservoir for nosocomial MRSA transmission. Further *in-vivo* and *in-vitro* investigations are required with SNV accumulation rates in particular MRSA clones to enable accurate inference of CTEs. This will allow more accurate assignment of SNV thresholds for defining strain relatedness as other studies used different thresholds (15). Indels were excluded from SNV analysis and could be considered in future investigations.

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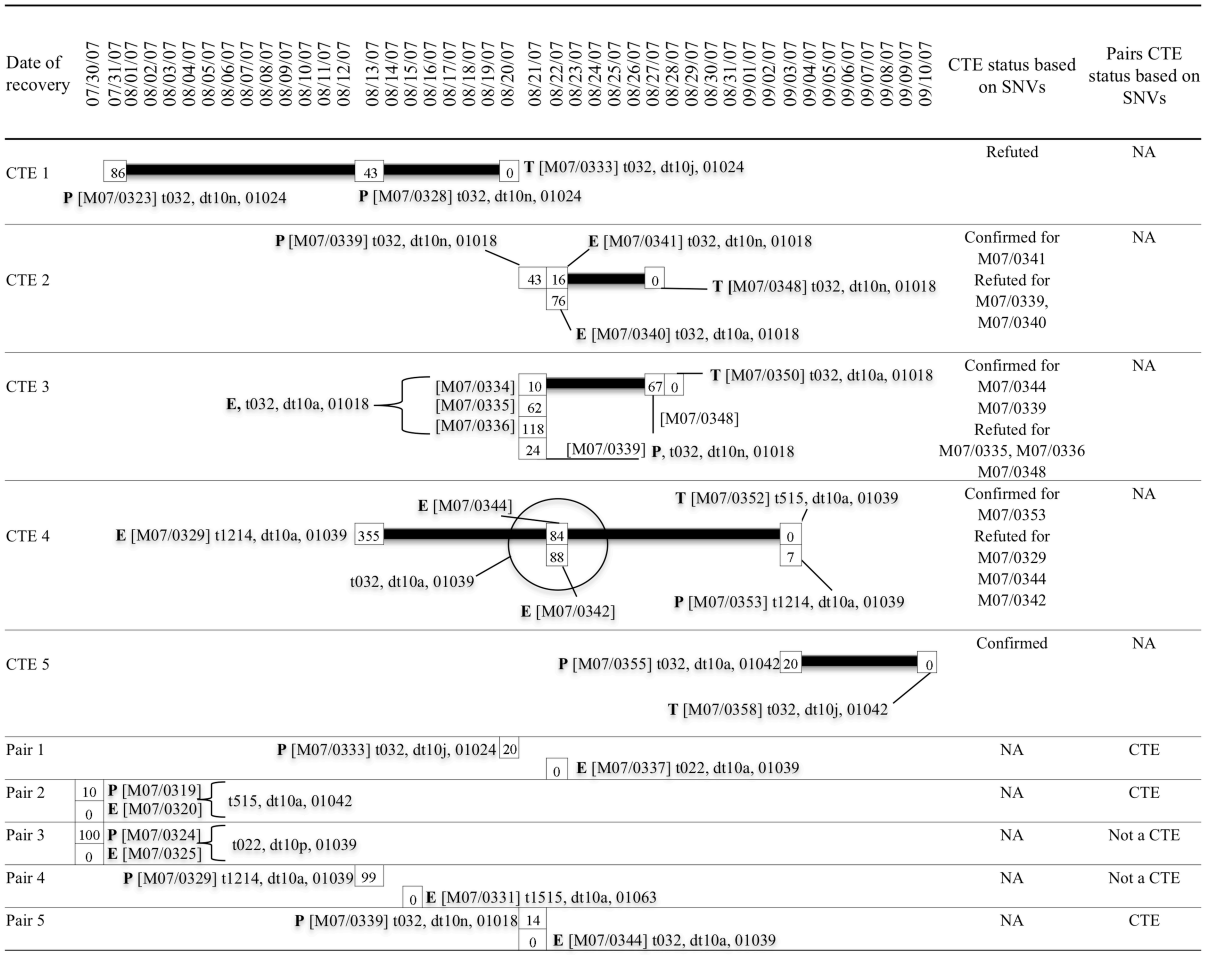
Figure Legend

Figure 1. Timeline showing dates of recovery of ST22-MRSA-IV isolates involved in cross-transmission events (CTEs) previously identified by conventional molecular epidemiological (CME) typing or identified as a pair of isolates recovered from a patient and their immediate ward environment. For each CTE, putative source isolates recovered from patients (P) and the environment (E) as well as putative transmitted isolates (T) are shown and for each pair of isolates, the patient (P) and environmental (E) isolate are also indicated. Isolate numbers are shown in square brackets followed by the *spa* type, *dru* type and PFGE type. The single nucleotide variant (SNV) comparison between each of the source isolates compared with the transmitted isolates within a CTE or between each pair of isolates is indicated by numerals within a square with the transmitted isolate SNV value denoted by 0 (0 SNVs resulting from self-comparison). CTEs were confirmed by SNV analysis if one or more of the source isolates differed from the transmitted isolate by ≤ 40 SNVs. For CTEs consisting of multiple source isolates and where some were confirmed and some refuted as CTEs by SNV analysis, the isolate numbers for the CTEs either confirmed or refuted are indicated in the second last column to the right of the figure. Pairs of isolates were confirmed as a CTE if the patient and environmental isolate differed by ≤ 40 SNVs. Further molecular epidemiological details of isolates implicated in each of the CTEs or as a pair of isolates are provided in supplemental Table S1 and have been published previously (6, 7). Abbreviation: NA, Not applicable.

Figure 1


Date of recovery
07/30/07
07/31/07
08/01/07
08/02/07
08/03/07
08/04/07
08/05/07
08/06/07
08/07/07
08/08/07
08/09/07
08/10/07
08/11/07
08/12/07
08/13/07
08/14/07
08/15/07
08/16/07
08/17/07
08/18/07
08/19/07
08/20/07
08/21/07
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08/23/07
08/24/07
08/25/07
08/26/07
08/27/07
08/28/07
08/29/07
08/30/07
08/31/07
09/01/07
09/02/07
09/03/07
09/04/07
09/05/07
09/06/07
09/07/07
09/08/07
09/09/07
09/10/07
CTE status based on SNVs
Pairs CTE status based on SNVs
CTE 1
86
43
0
T [M07/0333] t032, dt10j, 01024
P [M07/0323] t032, dt10n, 01024
P [M07/0328] t032, dt10n, 01024
Refuted
NA
CTE 2
P [M07/0339] t032, dt10n, 01018
E [M07/0341] t032, dt10n, 01018
43
16
0
T [M07/0348] t032, dt10n, 01018
76
E [M07/0340] t032, dt10a, 01018
Confirmed for M07/0341
Refuted for M07/0339, M07/0340
NA
CTE 3
E, t032, dt10a, 01018
[M07/0334]
[M07/0335]
[M07/0336]
10
62
118
24
67
0
T [M07/0350] t032, dt10a, 01018
[M07/0348]
[M07/0339]
P, t032, dt10n, 01018
Confirmed for M07/0344 M07/0339
Refuted for M07/0335, M07/0336 M07/0348
NA
CTE 4
E [M07/0344]
T [M07/0352] t515, dt10a, 01039
E [M07/0329] t1214, dt10a, 01039
355
84
88
0
7
t032, dt10a, 01039
E [M07/0342]
P [M07/0353] t1214, dt10a, 01039
Confirmed for M07/0353
Refuted for M07/0329 M07/0344 M07/0342
NA
CTE 5
P [M07/0355] t032, dt10a, 01042
20
0
T [M07/0358] t032, dt10j, 01042
Confirmed
NA
Pair 1
P [M07/0333] t032, dt10j, 01024
20
0
E [M07/0337] t022, dt10a, 01039
NA
CTE
Pair 2
10
0
P [M07/0319]
E [M07/0320]
t515, dt10a, 01042
NA
CTE
Pair 3
100
0
P [M07/0324]
E [M07/0325]
t022, dt10p, 01039
NA
Not a CTE
Pair 4
P [M07/0329] t1214, dt10a, 01039
99
0
E [M07/0331] t1515, dt10a, 01063
NA
Not a CTE
Pair 5
P [M07/0339] t032, dt10n, 01018
14
0
E [M07/0344] t032, dt10a, 01039
NA
CTE

Supplemental Table S1. Molecular epidemiological details of 41 ST22-MRSA-IVh isolates recovered from one ward of a Dublin hospital during a six-week period in 2007[a]

Isolate number	Date of isolation	Bay	Bed	Source	Pairs[b]	CTEs[c]	*spa* type	*dru* type	PFGE type	MRSA acquisition
M07/0319	07/30/2007	D	23	Patient	Pair 2	NA	t515	dt10a	01042	OA
M07/0320	07/30/2007	D	23	Mattress	Pair 2	NA	t515	dt10a	01042	NA
M07/0321	07/30/2007	E	30	Mattress	NA	NA	t515	dt10a	01039	NA
M07/0322	08/01/2007	E	18	Patient	NA	NA	t032	dt10j	01030	OA
M07/0323	07/31/2007	C	16	Patient	NA	CTE 1	t032	dt10n	01024	OA
M07/0324	07/30/2007	A	6	Patient	Pair 3	NA	t022	dt10p	01039	OA
M07/0325	07/30/2007	A	6	Mattress	Pair 3	NA	t022	dt10p	01039	NA
M07/0326	08/08/2007	C	21	Patient	NA	NA	t032	dt7g	01018	OA
M07/0327	08/09/2007	A	6	Patient	NA	NA	t515	dt11j	01049	OA-K
M07/0328	08/13/2007	B	15	Patient	NA	CTE 1	t032	dt10n	01024	OA
M07/0329	08/13/2007	E	31	Patient	Pair 4	CTE	t1214	dt10a	01039	HA
M07/0330	08/15/2007	5	SO	Bedrail	NA	NA	t032	dt10a	01039	NA
M07/0331	08/15/2007	E	31	Pillow	Pair 4	NA	t515	dt10a	01063	NA
M07/0332	08/17/2007	D	27	Patient	NA	NA	t032	dt10j	01018	OA-K
M07/0333	08/20/2007	B	13	Patient	Pair 1[d]	CTE 1	t032	dt10j	01024	HA
M07/0334	08/21/2007	D	23	Mattress	NA	CTE 3	t032	dt10a	01018	NA
M07/0335	08/21/2007	D	23	Mattress	NA	CTE 3	t032	dt10a	01018	NA
M07/0336	08/21/2007	D	26	Mattress	NA	CTE 3	t032	dt10a	01018	NA
M07/0337	08/22/2007	E	29	Bedframe	Pair 1[d]	NA	t022	dt10a	01039	NA
M07/0338	08/22/2007	E	30	Pillow	NA	NA	t032	dt10j	01146	NA
M07/0339	08/21/2007	B	15	Patient	Pair 5	CTE 2&3	t032	dt10n	01018	OA
M07/0340	08/22/2007	B	10	Bedframe	NA	CTE 2	t032	dt10a	01018	NA
M07/0341	08/22/2007	B	11	Bedframe	NA	CTE 2	t032	dt10n	01018	NA
M07/0342	08/22/2007	B	15	Pillow	NA	CTE 4	t032	dt10a	01039	NA
M07/0343	08/22/2007	B	14	Mattress	NA	NA	t022	dt10a	01039	NA
M07/0344	08/22/2007	B	15	Mattress	Pair 5	CTE 4	t032	dt10a	01039	NA
M07/0345	08/22/2007	B	35	Mattress	NA	NA	t032	dt10n	01018	NA
M07/0346	08/22/2007	2	SO	Bedframe	NA	NA	t032	dt10a	01018	NA
M07/0348	08/27/2007	B	14	Patient	NA	CTE 2&3	t032	dt10n	01018	HA
M07/0350	08/28/2007	D	27	Patient	NA	CTE 3	t032	dt10a	01018	HA
M07/0351	09/03/2007	A	7	Patient	NA	NA	t032	dt10j	01024	OA
M07/0352	09/03/2007	B	15	Patient	NA	CTE 4	t515	dt10a	01039	OA
M07/0353	09/03/2007	B	15	Patient	NA	CTE 4	t1214	dt10a	01039	OA
M07/0354	09/03/2007	E	17	Patient	NA	NA	t1214	dt10a	01039	OA
M07/0355	09/03/2007	E	30	Patient	NA	CTE 5	t032	dt10a	01042	OA
M07/0356	09/03/2007	E	29	Patient	NA	NA	t515	dt7i	01039	OA
M07/0357	09/04/2007	E	33	Bedframe	NA	NA	t022	dt10a	01039	NA
M07/0358	09/10/2007	E	31	Patient	NA	CTE 5	t032	dt10j	01042	>72 h
M07/0359	08/29/2007	C	16	Patient	NA	NA	t2951	dt6e	01088	OA
M07/0415	10/04/2007	A	9	Patient	NA	NA	t032	dt10j	01024	HA
M07/0475	11/20/2007	B	SO	Air	NA	NA	t1214	dt10a	01039	NA

[a]Molecular epidemiological data for isolates was determined as part of previous studies (1, 2).

[b]Each pair of isolates included one isolate recovered from a patient and one recovered from their immediate ward environment. Four of the 10 isolates in these pairs were also previously implicated in the cross transmission events (CTEs).

[c]CTEs were previously determined by conventional molecular epidemiological typing.

[d]Isolates associated with pair 1 were recovered from a patient and environmental site in two separate bays. The patient was found to be positive for MRSA while in bay B but was subsequently moved to bay E where the environmental isolate was recovered allowing these two isolates to be classified as a pair.

Abbreviations: SO, single occupancy; NA, not applicable; PFGE, pulsed-field gel electrophoresis; >72 h, the patient's MRSA status was determined 72 h after admission to the ward; OA; the patient was positive for MRSA on admission to the ward; OA-K; the patients' MRSA positive status was known at the time of admission to the ward; HA, hospital-acquired MRSA.

Supplemental Table S2. Comparison of the conventional molecular epidemiological typing data and single-nucleotide variant (SNV) differences identified among isolates within 10 arbitrarily selected pairwise comparisons (PCs)[a] exhibiting ≤40 SNVs and >40 SNVs

PCs	Compared isolates[b]	Date of isolation	Bay	Bed	Source	MRSA acquisition	*spa* type	*dru* type	PFGE type	No of conventional typing method differences	SNVs
	≤40 SNV differences										
1	M07/0325	07/30/2007	A	6	mattress	NA	t022	dt10p	01039	3	0
	M07/0328	08/13/2007	B	15	patient	OA	t032	dt10n	01024		
2	M07/0326	08/08/2007	C	21	patient	OA	t032	dt7g	01018	2	4
	M07/0328	08/13/2007	B	15	patient	OA	t032	dt10n	01024		
3	M07/0319	07/30/2007	D	23	patient	OA	t515	dt10a	01042	0	10
	M07/0320	07/30/2007	D	23	mattress	NA	t515	dt10a	01042		
4	M07/0321	07/30/2007	E	30	mattress	NA	t515	dt10a	01039	1	1
	M07/0329	08/13/2007	E	31	patient	HA	t1214	dt10a	01039		
5	M07/0324	07/30/2007	A	6	patient	OA	t022	dt10p	01039	3	15
	M07/0336	08/21/2007	D	26	mattress	NA	t032	dt10a	01018		
6	M07/0355	09/03/2007	E	30	patient	OA	t032	dt10a	01042	1	20
	M07/0358	09/10/2007	E	31	patient	>72h	t032	dt10j	01042		
7	M07/0337	08/22/2007	E	29	bedframe	NA	t022	dt10a	01039	0	27
	M07/0359	08/29/2007	C	16	patient	OA	t2951	dt6e	01088		
8	M07/0333	08/20/2007	B	13	patient	HA	t032	dt10j	01024	2	33
	M07/0342	08/22/2007	B	15	pillow	NA	t032	dt10a	01039		
9	M07/0341	08/22/2007	B	11	bedframe	NA	t032	dt10n	01018	2	38
	M07/0355	09/03/2007	E	30	patient	OA	t032	dt10a	01042		
10	M07/0337	08/22/2007	E	29	bedframe	NA	t022	dt10a	01039	3	39
	M07/0415	10/04/2007	A	9	patient	HA	t032	dt10j	01024		

	> 40 SNV differences										
1	M07/0335	08/21/2007	D	23	mattress	NA	t032	dt10a	01018	1	43
	M07/0415	10/04/2007	A	9	patient	HA	t032	dt10j	01024		
2	M07/0319	30/07/2007	D	23	patient	OA	t515	dt10a	01042	2	54
	M07/0475	11/20/2007	B	SO	air	NA	t1214	dt10a	01039		
3	M07/0328	08/13/2007	B	15	patient	OA	t032	dt10n	01024	3	63
	M07/0337	08/22/2007	E	29	bedframe	NA	t022	dt10a	01039		
4	M07/0320	07/30/2007	D	23	mattress	NA	t515	dt10a	01042	2	78
	M07/0346	08/22/2007	2	SO	bedframe	NA	t032	dt10a	01018		
5	M07/0355	09/03/2007	E	30	patient	OA	t032	dt10a	01042	1	75
	M07/0351	09/03/2007	A	7	patient	OA	t032	dt10j	01024		
	M07/0336	08/21/207	D	26	mattress	NA	t032	dt10a	01018		
6	M07/0328	08/13/2007	B	15	patient	OA	t032	dt10n	01024	2	96
	M07/0338	08/22/2007	E	30	pillow	NA	t032	dt10j	01146		
7	M07/0339	08/21/2007	B	15	patient	OA	t032	dt10n	01018	3	99
	M07/0352	09/03/2007	B	15	patient	OA	t515	dt10a	01039		
8	M07/0321	07/30/2007	E	30	mattress	NA	t515	dt10a	01039	0	354
	M07/0352	09/03/2007	B	15	patient	OA	t515	dt10a	01039		
	M07/0334	08/21/2007	D	23	mattress	NA	t032	dt10a	01018		
9	M07/0323	07/31/2007	C	16	patient	NA	t032	dt10n	01024	2	209
	M07/0332	08/17/2007	D	27	patient	OA-K	t032	dt10j	01018		
10	M07/0324	07/30/2007	A	6	patient	OA	t022	dt10p	01039	3	112
	M07/0340	08/22/2007	B	10	bedframe	NA	t032	dt10a	01018		

[a]Pairwise comparisons were randomly selected from those differing by ≤ 40 SNV and > 40 SNV for comparison to previously reported conventional molecular epidemiological typing data (1, 2).

Abbreviations: NA, not applicable; PFGE, pulsed-field gel electrophoresis; OA; the patient was positive for MRSA on admission to the ward; >72 h, the patient's MRSA status was determined 72 h after admission to the ward; HA, hospital-acquired MRSA; OA-K, the patients' MRSA positive status was known at the time of admission to the ward

1. **Shore AC, Rossney AS, Kinnevey PM, Brennan OM, Creamer E, Sherlock O, Dolan A, Cunney R, Sullivan DJ, Goering RV, Humphreys H, Coleman DC.** 2010. Enhanced discrimination of highly clonal ST22-methicillin-resistant *Staphylococcus aureus* IV isolates achieved by combining *spa*, *dru*, and pulsed-field gel electrophoresis typing data. J Clin Microbiol **48:**1839-1852.

2. **Creamer E, Shore AC, Rossney AS, Dolan A, Sherlock O, Fitzgerald-Hughes D, Sullivan DJ, Kinnevey PM, O'Lorcain P, Cunney R, Coleman DC, Humphreys H.** 2012. Transmission of endemic ST22-MRSA-IV on four acute hospital wards investigated using a combination of *spa*, *dru* and pulsed-field gel electrophoresis typing. Eur J Clin Microbiol Infect Dis **31:**3151-3161.

Supplemental Fig. S1

(A)

Isolate no.		M07/0319	M07/0320	M07/0321	M07/0322	M07/0323	M07/0324	M07/0325	M07/0326	M07/0327	M07/0328	M07/0329	M07/0330	M07/0331	M07/0332	M07/0333	M07/0334	M07/0335	M07/0336	M07/0337	M07/0338	M07/0339	M07/0340	M07/0341	M07/0342	M07/0343	M07/0344	M07/0345	M07/0346	M07/0348	M07/0350	M07/0351	M07/0352	M07/0353	M07/0354	M07/0355	M07/0356	M07/0357	M07/0358	M07/0359	M07/0415
		P	E	E	P	P	P	E	P	P	P	P	P	E	P	P	P	E	E	E	E	P	E	E	E	E	E	P	P	P	P	P	P	P	P	P	E	P	P	P
M07/0319	P																																							
M07/0320	E	10																																						
M07/0321	E	384	374																																					
M07/0322	P	171	161	213																																				
M07/0323	P	65	55	319	106																																			
M07/0324	P	208	198	176	37	143																																		
M07/0325	E	108	98	276	63	43	100																																	
M07/0326	P	104	94	280	67	39	104	4																																
M07/0327	P	301	291	83	130	236	93	193	197																															
M07/0328	P	108	98	276	63	43	100	0	4	193																														
M07/0329	P	385	375	1	214	320	177	277	281	84	277																													
M07/0330	P	163	153	221	8	98	45	55	59	138	55	222																												
M07/0331	E	286	276	98	115	221	78	178	182	15	178	99	123																											
M07/0332	P	274	264	110	103	209	66	166	170	27	166	111	111	12																										
M07/0333	P	151	141	233	20	86	57	43	47	150	43	234	12	135	123																									
M07/0334	P	115	105	269	56	50	93	7	11	186	7	270	48	171	159	36																								
M07/0335	E	167	157	217	4	102	41	59	63	134	59	218	4	119	107	16	52																							
M07/0336	E	223	213	161	52	158	15	115	119	78	115	162	60	63	51	72	108	56																						
M07/0337	E	171	161	213	0	106	37	63	67	130	63	214	8	115	103	20	56	4	52																					
M07/0338	E	204	194	180	33	139	4	96	100	97	96	181	41	82	70	53	89	37	19	33																				
M07/0339	P	129	119	255	42	64	79	21	25	172	21	256	34	157	145	22	14	38	94	42	75																			
M07/0340	E	96	86	288	75	31	112	12	8	205	12	289	67	190	178	55	19	71	127	75	108	33																		
M07/0341	E	156	146	228	15	91	52	48	52	145	48	229	7	130	118	5	41	11	67	15	48	27	60																	
M07/0342	E	118	108	266	53	53	90	10	14	183	10	267	45	168	156	33	3	49	105	53	86	11	22	38																
M07/0343	E	171	161	213	0	106	37	63	67	130	63	214	8	115	103	20	56	4	52	0	33	42	75	15	53															
M07/0344	E	114	104	270	57	49	94	6	10	187	6	271	49	172	160	37	1	53	109	57	90	15	18	42	4	57														
M07/0345	P	146	136	238	25	81	62	38	42	155	38	239	17	140	128	5	31	21	77	25	58	17	50	10	28	25	32													
M07/0346	P	88	78	296	83	23	120	20	16	213	20	297	75	198	186	63	27	79	135	83	116	41	8	68	30	83	26	58												
M07/0348	P	172	162	212	1	107	36	64	68	129	64	213	9	114	102	21	57	5	51	1	32	43	76	16	54	1	58	26	84											
M07/0350	P	105	95	279	66	40	103	3	1	196	3	280	58	181	169	46	10	62	118	66	99	24	9	51	13	66	9	41	17	67										
M07/0351	P	92	82	292	79	27	116	16	12	209	16	293	71	194	182	59	23	75	131	79	112	37	4	64	26	79	22	54	4	80	13									
M07/0352	P	30	20	354	141	35	178	78	74	271	78	355	133	256	244	121	85	137	193	141	174	99	66	126	88	141	84	116	58	142	75	62								
M07/0353	P	23	13	361	148	42	185	85	81	278	85	362	140	263	251	128	92	144	200	148	181	106	73	133	95	148	91	123	65	149	82	69	7							
M07/0354	P	40	30	344	131	25	168	68	64	261	68	345	123	246	234	111	75	127	183	131	164	89	56	116	78	131	74	106	48	132	65	52	10	17						
M07/0355	P	118	108	266	53	53	90	10	14	183	10	267	45	168	156	33	3	49	105	53	86	11	22	38	0	53	4	28	30	54	13	26	88	95	78					
M07/0356	P	131	121	253	40	66	77	23	27	170	23	254	32	155	143	20	16	36	92	40	73	2	35	25	13	40	17	15	43	41	26	39	101	108	91	13				
M07/0357	E	114	104	270	57	49	94	6	10	187	6	271	49	172	160	37	1	53	109	57	90	15	18	42	4	57	0	32	26	58	9	22	84	91	74	4	17			
M07/0358	P	138	128	246	33	73	70	30	34	163	30	247	25	148	136	13	23	29	85	33	66	9	42	18	20	33	24	8	50	34	33	46	108	115	98	20	7	24		
M07/0359	P	198	188	186	27	133	10	90	94	103	90	187	35	88	76	47	83	31	25	27	6	69	102	42	80	27	84	52	110	26	93	106	168	175	158	80	67	84	60	
M07/0415	P	210	200	174	39	145	2	102	106	91	102	175	47	76	64	59	95	43	13	39	6	81	114	54	92	39	96	64	122	38	105	118	180	187	170	92	79	96	72	12

(B)

Isolate no.		M07/0319	M07/0322	M07/0323	M07/0324	M07/0326	M07/0327	M07/0328	M07/0329	M07/0330	M07/0332	M07/0333	M07/0334	M07/0339	M07/0345	M07/0346	M07/0348	M07/0350	M07/0351	M07/0352	M07/0353	M07/0354	M07/0355	M07/0356	M07/0358	M07/0359	M07/0415	M07/0475
		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
M07/0319	P																											
M07/0322	P	171																										
M07/0323	P	65	106																									
M07/0324	P	208	37	143																								
M07/0326	P	104	67	39	104																							
M07/0327	P	301	130	236	93	197																						
M07/0328	P	108	63	43	100	4	193																					
M07/0329	P	385	214	320	177	281	84	277																				
M07/0330	P	163	8	98	45	59	138	55	222																			
M07/0332	P	274	103	209	66	170	27	166	111	111																		
M07/0333	P	151	20	86	57	47	150	43	234	12	123																	
M07/0334	P	115	56	50	93	11	186	7	270	48	159	36																
M07/0339	P	129	42	64	79	25	172	21	256	34	145	22	14															
M07/0345	P	146	25	81	62	42	155	38	239	17	128	5	31	17														
M07/0346	P	88	83	23	120	16	213	20	297	75	186	63	27	41	58													
M07/0348	P	172	1	107	36	68	129	64	213	9	102	21	57	43	26	84												
M07/0350	P	105	66	40	103	1	196	3	280	58	169	46	10	24	41	17	67											
M07/0351	P	92	79	27	116	12	209	16	293	71	182	59	23	37	54	4	80	13										
M07/0352	P	30	141	35	178	74	271	78	355	133	244	121	85	99	116	58	142	75	62									
M07/0353	P	23	148	42	185	81	278	85	362	140	251	128	92	106	123	65	149	82	69	7								
M07/0354	P	40	131	25	168	64	261	68	345	123	234	111	75	89	106	48	132	65	52	10	17							
M07/0355	P	118	53	53	90	14	183	10	267	45	156	33	3	11	28	30	54	13	26	88	95	78						
M07/0356	P	131	40	66	77	27	170	23	254	32	143	20	16	2	15	43	41	26	39	101	108	91	13					
M07/0358	P	138	33	73	70	34	163	30	247	25	136	13	23	9	8	50	34	33	46	108	115	98	20	7				
M07/0359	P	198	27	133	10	94	103	90	187	35	76	47	83	69	52	110	26	93	106	168	175	158	80	67	60			
M07/0415	P	210	39	145	2	106	91	102	175	47	64	59	95	81	64	122	38	105	118	180	187	170	92	79	72	12		
M07/0475	P	54	117	11	154	50	247	54	331	109	220	97	61	75	92	34	118	51	38	24	31	14	64	77	84	144	156	

(C)

Isolate no.		M07/0320	M07/0321	M07/0325	M07/0331	M07/0335	M07/0336	M07/0337	M07/0338	M07/0340	M07/0341	M07/0342	M07/0343	M07/0344	M07/0357
		E	E	E	E	E	E	E	E	E	E	E	E	E	E
M07/0320	E														
M07/0321	E	374													
M07/0325	E	98	276												
M07/0331	E	276	98	178											
M07/0335	E	157	217	59	119										
M07/0336	E	213	161	115	63	56									
M07/0337	E	161	213	63	115	4	52								
M07/0338	E	194	180	96	82	37	19	33							
M07/0340	E	86	288	12	190	71	127	75	108						
M07/0341	E	146	228	48	130	11	67	15	48	60					
M07/0342	E	108	266	10	168	49	105	53	86	22	38				
M07/0343	E	161	213	63	115	4	52	0	33	75	15	53			
M07/0344	E	104	270	6	172	53	109	57	90	18	42	4	57		
M07/0357	E	104	270	6	172	53	109	57	90	18	42	4	57	0	

Supplemental Fig. S1. Pairwise comparisons of single-nucleotide variant (SNV) differences identified between the 41 ST22-MRSA-IVh isolates based on alignment of all isolate whole-genome sequences to the earliest recovered isolate (M07/0319) with subsequent assignment of isolates exhibiting ≤ 40 SNVs as representing a cross-transmission event (CTE). (A) Isolates differing by ≤ 40 SNVs where one isolate was recovered from a patient and one was recovered from their immediate ward environment (indicated with grey shading). (B) Isolates differing by ≤ 40 SNVs where both isolates were recovered from separate patients (indicated with grey shading). (C) Isolates differing by ≤ 40 SNVs where both isolates were recovered from a different environmental source (indicated with grey shading). Abbreviations: Patient, P; Environment, E.