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

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Running title: Tracking ST22-MRSA-IV spread by 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.

Journal of Clinical Microbiology

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

Journal of Clinical Microbiology

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

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

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

Journal of Clinical Microbiology

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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Journal of Clinical Microbiology

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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.