An investigation of ST22-MRSA-IV hospital transmission events using whole-genome sequencing compared with a combination of *spa*, *dru* and pulsed-field gel electrophoresis typing and epidemiological data

Peter M. Kinnevey[1], Anna C. Shore[1,2], Micheál MacAogáin[2], Gráinne I. Brennan[1,3], Hilary Humphreys[4], Brian O' Connell[2,3], Thomas R. Rogers[2], David C. Coleman[1]

[1]Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Ireland. [2]Department of Clinical Microbiology, School of Medicine, Trinity College Dublin, St. James's Hospital, Dublin 8, Ireland, [3]National MRSA Reference Laboratory, St. James's Hospital, James's St., Dublin 8, Ireland, [4]Department of Clinical Microbiology, Royal College of Surgeons in Ireland Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland.

Introduction

The ST22-MRSA-IV clone predominates among nosocomial MRSA in several European countries and is endemic in Ireland, accounting for approximately 80% of MRSA isolates per year. ST22-MRSA-IV is highly clonal and tracking its spread is challenging. We previously identified potential transmission events among ST22-MRSA-IV isolates from patients and environmental sites in one Irish hospital using a combination of *spa*, *dru* and pulsed-field gel electrophoresis typing and epidemiological data (1, 2). In that study, five transmission events were identified in one ward (ward A) by molecular epidemiological typing (Table). The aim of the present study was to investigate the application of whole-genome re-sequencing to confirm or disprove these transmission events and to determine if other possible transmission events had been missed.

Materials and Methods

Isolates: ST22-MRSA-IV isolates recovered from patients (n = 19) and environmental sites (n = 20) in ward A over six weeks in 2007 (1).

Whole-genome sequencing (WGS) of all isolates was performed using a MiSeq desktop sequencer (Illumina, San Diego, CA, USA). Paired-end reads were imported as fastQ files into the BioNumerics (version 7.1) genome analysis tool (GAT) software plugin (Applied Maths, Belgium) and were assembled by re-sequencing using the ST22-MRSA-IV reference strain HO 5096 0412 as a scaffold (3). Single nucleotide variation (SNV) analysis was performed using the BioNumerics GAT plugin to investigate evolutionary relationships between all isolates including isolates involved in five transmission events previously identified by molecular epidemiological typing (Table). Based on previous studies, isolates considered to be involved in a transmission event differed by ≤ 40 SNVs (2,3).

Results

Previously identified transmission events

- 3/5 previously identified transmission events were supported by SNV analysis i.e. transmitted isolates within 3/5 transmission events differed from at least one of the putative source isolates by ≤ 40 SNVs (6-34 SNVs) (colored font in Table and matching colored circles/squares in Figure).
- Within the two remaining previously identified transmission events, transmitted isolates differed from source isolates by 54-211 SNVs (Table and Figure).

Previously unidentified transmission events

- A significantly greater number of isolates were deemed to be related to at least one other isolate by SNV analysis (37/39) compared to molecular epidemiological typing (19/39).
- When all isolates were compared by SNV analysis, 27.1% (201/741 pairwise comparisons) differed by ≤ 40 SNVs including:
  - 18.9% (38/201) from patients only
  - 29.4% (59/201) from environmental sites only
  - 51.7% (104/201) from patient and environmental sites
    - ➢ 97% (195/201) of potential transmission events identified by SNV analysis were missed by conventional molecular epidemiological typing.

Conclusions

Whole-genome sequencing identified a significantly greater number of related isolates of the ST22-MRSA-IV clone circulating in a hospital than indicated by molecular epidemiological typing. These results reveal that SNV analysis is significantly more sensitive at identifying ST22-MRSA-IV transmission events in the hospital setting than conventional molecular epidemiological typing and indicate a high rate of nosocomial transmission particularly between patients and environmental sites. However, there is little in the literature regarding the mutation rates of MRSA clones, such as ST22-MRSA-IV, to inform SNV analysis transmission event determination. Further work is required to investigate this.

Table. Details of the five transmission events (TEs) identified previously among 39 MRSA isolates from patients and environmental sites in ward A in 2007 by molecular epidemiological typing[1] and details of SNV differences identified by whole-genome sequencing

TE no.	Putative source isolate(s)	Transmitted isolate	Possible/ probable event[1]	No. of SNVs
1	00667 (P)	00777	Probable	22
2	00871 (E)	00953	Probable	13
	00868 (E)		Probable	34
	00851 (P)		Possible	62
3	00795 (E)	00992	Probable	6
	00801 (E)		Probable	128
	00804 (E)		Probable	17
	00953 (P)		Possible	32
	00851 (P)		Possible	94
4	00878 (E)	01010	Probable	118
	00873 (E)		Probable	75
	00684 (P)		Possible	211
5	01027 (P)	01207	Possible	54

[1]Transmission events were previously determined using a combination of epidemiological data and combined pulsed-field gel electrophoresis, *spa* and *dru* typing data (1). Transmission events were assigned as probable (recovered within a 3 week time-period within the same ward bay) or possible (recovered within a 3 week time-period within the same ward) if isolates differed by ≤1 typing method only (1). Transmission events confirmed by SNV analysis i.e. differing by ≤40 SNVs, are indicated with colored font. Abbreviations: P, patient; E, environment; SNV, single nucleotide variation.

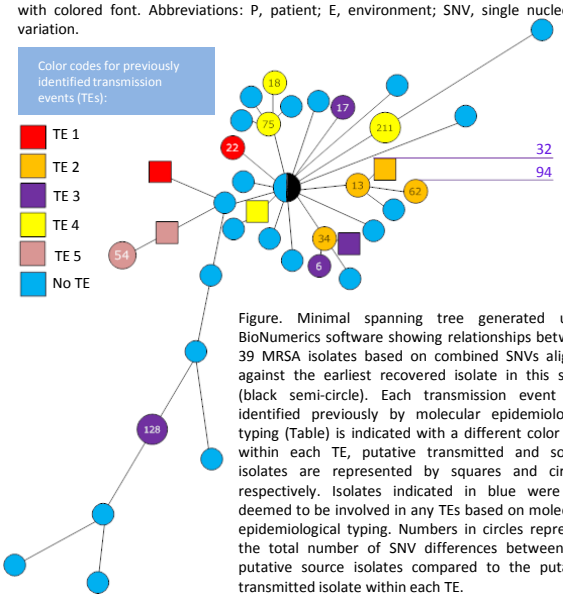

Figure. Minimal spanning tree generated using BioNumerics software showing relationships between 39 MRSA isolates based on combined SNVs aligned against the earliest recovered isolate in this study (black semi-circle). Each transmission event (TE) identified previously by molecular epidemiological typing (Table) is indicated with a different color and, within each TE, putative transmitted and source isolates are represented by squares and circles, respectively. Isolates indicated in blue were not deemed to be involved in any TEs based on molecular epidemiological typing. Numbers in circles represent the total number of SNV differences between the putative source isolates compared to the putative transmitted isolate within each TE.

- Transmission of isolates within TEs 1-3 were confirmed by SNV analysis: isolates exhibited ≤ 40 SNVs when compared to the putative transmitted isolate.
- Transmission of isolates within TEs 4 & 5 could not be confirmed as the putative transmitted isolates exhibited ≥ 40 SNVs.
- Two isolates in TE 2 (one putative transmitted isolate and one putative source isolate) were also putative source isolates in TE 3 (shown as purple lines; SNV differences of 32 & 94, respectively, from TE 3 transmitted isolated).

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References

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