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Letter to the Editor

Improved understanding of an outbreak of meticillin-resistant *Staphylococcus aureus* in a regional burns centre via whole-genome sequencing

Sir,

The recent commentary by Coia discussed the evolution of meticillin-resistant *Staphylococcus aureus* (MRSA) and how whole-genome sequencing (WGS) has transformed our understanding of the phylogeny and epidemiology of this important nosocomial pathogen.[1] Whereas various methods are used globally to study the molecular epidemiology of MRSA [e.g. *spa* typing, multi-locus variable-number tandem repeat analysis (MLVA), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and pulsed-field gel electrophoresis (PFGE)], recent reports highlight the advantages of whole-genome sequencing (WGS) in improving our understanding of the transmission dynamics of MRSA in healthcare settings.[2–7] Herein we describe our experience of using WGS to guide epidemiological investigations and management of a nosocomial outbreak of MRSA where conventional molecular techniques lacked sufficient discrimination.

The infection control team was alerted to the possibility of an outbreak when two patients acquired MRSA 25 days apart on the regional burns unit, University Hospitals Birmingham (UHB) NHS Foundation Trust (Figure 1A); both were MRSA screen-negative on admission. Detailed phenotypic and genotypic testing showed that the MRSA lineage involved was EMRSA-15 (MLST-CC22; the dominant healthcare-associated MRSA seen in the UK) and that both isolates appeared indistinguishable (Table I). Multiple infection prevention and control measures were implemented. Both MRSA-colonized patients were isolated and received suppression therapy, specifically: 2% mupirocin nasal ointment applied three times a day to both nostrils for five days and daily octenidine bodywash for the duration of the patient stay. In addition, MRSA screening of all staff within the burns unit was undertaken by the occupational health department, enhanced hand hygiene teaching awareness sessions were delivered, and weekly MRSA screening of all inpatients was performed. During the following five months, a further four patients acquired MRSA on the same unit (Figure 1A). On day 45 environmental sampling of macroscopically clean touch-points was undertaken throughout the unit using the protocol described by Garvey *et al.*[8] Of the 40 samples taken, 19 were positive for MRSA in areas near colonized patients. Deep cleaning of all rooms including bays was implemented as described previously.[8] After cleaning, further environmental sampling revealed that six out of 40 samples were positive for MRSA (Figure 1A).

Conventional phenotypic and genotypic methodologies [susceptibility testing (Vitek, bioMérieux, Marcy l'Etoile, France), *spa* typing, and PFGE] showed that all six MRSA from patients plus two representative environmental isolates taken from both sets of sampling were closely related, differing only in fusidic acid susceptibility and *spa* type (where minor differences could reflect a simple mutational, insertion, deletion and/or duplication genetic event), making it difficult to assess whether the cluster represented (i) a point source, single strain outbreak or (ii) a pseudo-outbreak associated with independent MRSA acquisition events occurring during a five-month period. Isolates were therefore subjected to WGS as described previously.[5,6] An additional two MRSAs with phenotypic and genotypic characteristics similar to those of the cluster (data not shown) collected contemporaneously from different wards in the same hospital were included as epidemiologically unrelated comparator strains.

WGS data confirmed that all isolates belonged to the EMRSA-15 lineage (ST22-SCC*mec*IV). The resistome (acquired genes and chromosomal mutations) correlated with the phenotypic data (Table I). Phylogenetic analyses showed that the MRSA strains from patients 1, 2, 3, and 6 (belonging to two different but related *spa* types) clustered together with the environmental isolates (clade A) and were highly related, showing five or fewer single nucleotide polymorphism (SNP) differences (Figure 1B). Similarly, the MRSA strains from patients 4 and 5 clustered together (two SNP differences between them) but belonged to a separate clade (clade B) distant by >100 SNPs from clade A (Figure 1B). The background comparator strains were unrelated to these two clades by >100 SNPs.

The collective data show that the environmental strains belonged to the same clade as the MRSAs from patients 1, 2, 3 and 6. Moreover, the same strain of MRSA was found in the environment after 70 days; this is consistent with previous reports of MRSA surviving for more 175 days on a ward.[8]

In this outbreak, conventional strain characterization techniques were unable to differentiate robustly between the

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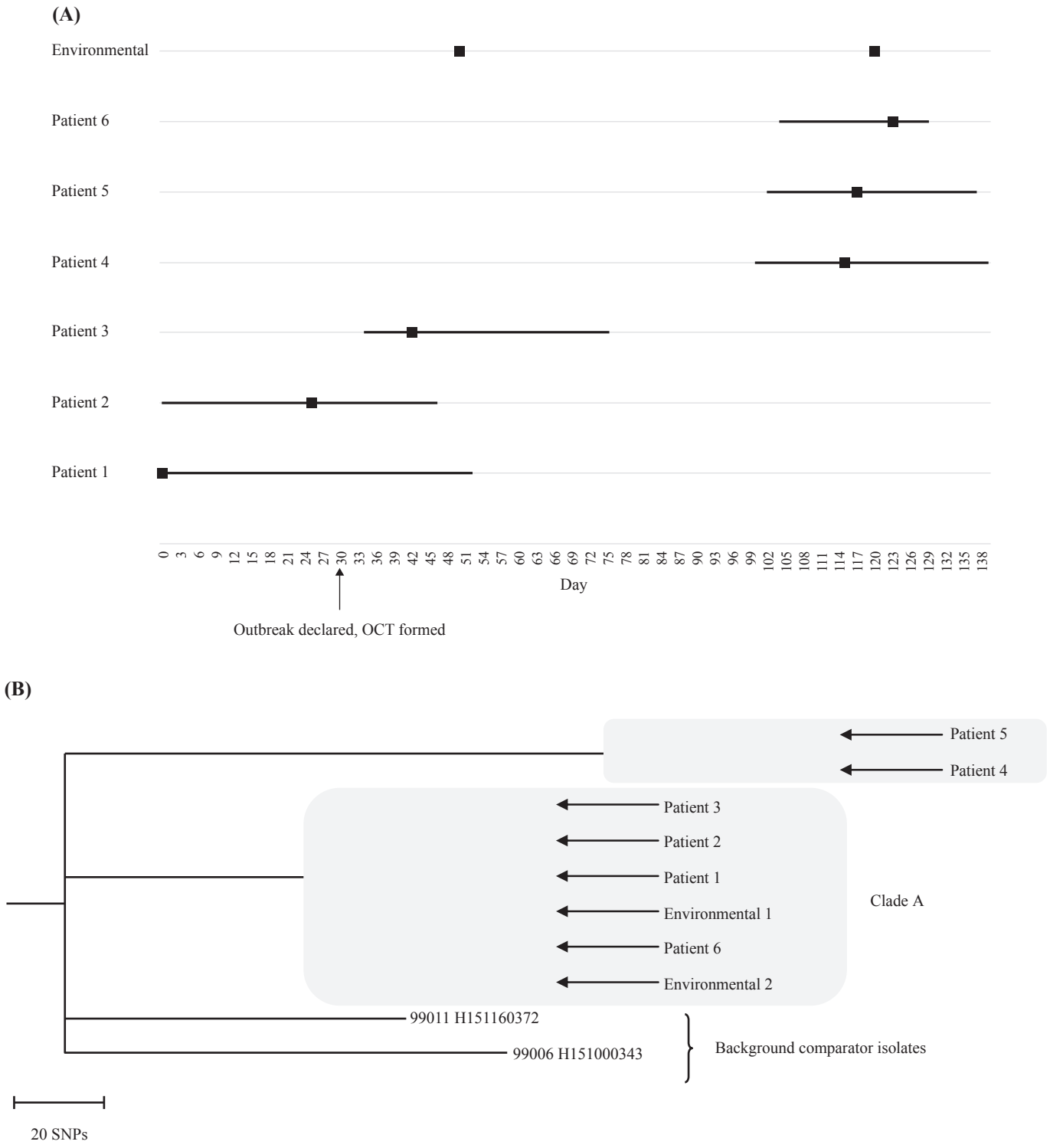

Figure 1. (A) Timeline of patients who acquired meticillin-resistant *Staphylococcus aureus* (MRSA) on the burns unit. Black bars indicate the patients' admission. Squares represent the first MRSA-positive specimen. OCT, outbreak control team. (B) Phylogenetic tree of MRSA from the burns unit and other hospital wards. Sequence reads were mapped to the ST22 reference genome H050960412 (NC_017763) using BWA. Single nucleotide polymorphisms (SNPs) were called using GATK2. High-quality polymorphic sites were filtered based on SNP quality $\geq$40, AD ratio $\geq$0.9, depth $\geq$5; SNPs failing these metrics were called 'Ns'. SNPs from each genome were thereafter combined to generate a single multiple alignment file with the maximum proportion of Ns accepted at any position of the alignment set to 50% for maximum likelihood phylogenetic inference (RaXML: www.phylo.org). In all, 368 polymorphic sites were detected. The dendrogram was drawn using Molecular Evolutionary Genetics Analysis (MEGA6). Scale: number of SNPs. Bootstrap values indicated at nodes. Tree rooted on reference genome.

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Table I
Phenotypic and genotypic characteristics of MRSA isolates identified during the outbreak

Isolate source	Conventional test results			WGS-derived results				
	Resistance phenotype	*spa* type[a]	Pulsotype	In-silico MLST	SCC*mec* type	Clade	Genetic markers of antimicrobial resistance	
							Acquired genes	Chromosomal mutations
Patient 1	PEN, OXA, CIP, ERY, CLI, TRI	t022	A	22	IV	A	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T)
Patient 2	PEN, OXA, CIP, ERY, CLI, TRI	t022	A	22	IV	A	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T)
Patient 3	PEN, OXA, CIP, ERY, CLI, TRI	t022	A	22	IV	A	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T)
Patient 4	PEN, OXA, CIP, ERY, CLI, TRI, FUS	t1390	A	22	IV	B	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T); *fusA* (90:V-I)
Patient 5	PEN, OXA, CIP, ERY, CLI, TRI, FUS	t1390	A	22	IV	B	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T); *fusA* (90:V-I)
Patient 6	PEN, OXA, CIP, ERY, CLI, TRI	t032	A	22	IV	A	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T)
Environment 1	PEN, OXA, CIP, ERY, CLI, TRI	t032	A	22	IV	A	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T)
Environment 2	PEN, OXA, CIP, ERY, CLI, TRI	t032	A	22	IV	A	*blaZ*, *mecA*, *erm(C)*	*grlA* (80:S-F); *gyrA* (84:S-L); *dfrB* (99:F-Y;135:A-T)

MRSA, meticillin-resistant *Staphylococcus aureus*; WGS, whole-genome sequencing; MLST, multi-locus sequence type; SCCmec, staphylococcal cassette chromosome *mec*; PEN, penicillin; OXA, oxacillin; CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; TRI, trimethoprim; FUS, fusidic acid. All isolates were susceptible to tetracycline, gentamicin, rifampicin, daptomycin, teicoplanin, vancomycin, and mupirocin.

[a] *spa* types t022, t032 and t1390 are closely related; minor differences in repeat succession patterns (data not shown) could reflect an insertion, deletion and/or duplication genetic event.

eight MRSA strains recovered from humans and their environment, suggesting that they were likely to be part of a single incident. However, the improved granularity afforded by WGS pointed to two independent clusters and acquisition pathways. Review of these findings alongside the epidemiological information supported the notion that patient 4 acquired a different strain of MRSA from an unknown source following a 12-day admission to the intensive care unit (ICU) and that patient 5 subsequently acquired MRSA from patient 4 as they were located in adjacent rooms on the burns unit. Since the last positive MRSA patient involved in the outbreak was discharged from the burns unit, no further cases have been identified during a 12-month follow-up period.

The present study describes an MRSA outbreak on a burns unit and how WGS provided a greater understanding of the MRSA transmission pathways compared to more conventional molecular techniques. WGS revealed that two different strains were circulating on the unit, allowing us to map where patients acquired MRSA and focusing our control measures accordingly from staff practice to environmental cleaning where appropriate. Our experience adds to the growing body of evidence that WGS holds great promise for making an important contribution to outbreak investigations and better targeting of infection prevention and control interventions to reduce the burden of infectious disease.

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Conflict of interest statement
None declared.

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