Multiple distinct outbreaks of Panton-Valentine leukocidin (PVL)-positive community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Ireland investigated by whole-genome sequencing

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Running Title: PVL-+ve CA-MRSA outbreaks investigated by WGS

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Abbreviations; WGS, whole-genome sequencing; wgMLST, whole-genome multilocus sequence typing
Summary

**Background:** Panton-Valentine leukocidin (PVL)-positive community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are increasingly associated with infection outbreaks.

**Aim:** To investigate multiple suspected PVL-positive CA-MRSA outbreaks using whole-genome sequencing (WGS).

**Methods:** Forty-six suspected outbreak-associated isolates from 36 individuals at three separate Irish hospitals (H1-H3) and from separate incidents involving separate families associated with H2 were investigated by whole-genome multilocus sequence typing (wgMLST).

**Findings:** Two clusters (CH1 and CH2) consisting of 8/10- and 6/6- PVL-positive t008 ST8-MRSA-IVa isolates from H1 and H2, respectively, were identified. Within each cluster, neighbouring isolates were separated by $\leq 5$ allelic differences; however $\geq 73$ allelic differences were identified between the clusters, indicative of two independent outbreaks. Isolates from the H3 maternity unit formed two clusters (CH3-SCI and CH3-SCII) composed of four PVL-negative t4667 ST5-MRSA-V and 14 PVL-positive t002 ST5-MRSA-IVc isolates, respectively. Within clusters, neighbouring isolates were separated by $\leq 24$ allelic differences, whereas both clusters were separated by 1822 allelic differences, indicative of two distinct H3 outbreaks. Eight PVL-positive t127 ST1-MRSA-V+*fus* and three PVL-negative t267 ST97-MRSA-V+*fus* isolates from two distinct H2-associated families FC1 ($N=4$) and FC2 ($N=7$), formed three separate clusters (FC1 [t127], FC2 [t127] and FC2 [t267]). Neighbouring isolates within clusters were closely related and exhibited $\leq 7$ allelic differences. Intra-familial transmission was apparent, but the detection of $\geq 48$ allelic differences between clusters indicated no interfamilial transmission.

**Conclusion:** The frequent importation of PVL-positive CA-MRSA into healthcare settings, transmission and association with outbreaks is a serious ongoing concern. WGS is a highly discriminatory, informative method for deciphering such outbreaks conclusively.

**Keywords**

Community-associated MRSA, Panton-Valentine leukocidin, PVL toxin, healthcare-associated infection outbreaks, whole-genome sequencing
INTRODUCTION

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections were originally defined as those occurring in otherwise healthy populations without traditional healthcare-associated MRSA (HA-MRSA) risk factors[1]. CA-MRSA infections can range from superficial skin and soft tissue infections (SSTIs) to life-threatening illnesses[2].

Several genotypic characteristics of CA-MRSA strains previously indicated an evolutionary trajectory independent to that of HA-MRSA clones, which was subsequently confirmed by whole-genome sequence (WGS)-based phylogenetic studies [1,2]. CA-MRSA lineages typically carry smaller staphylococcal chromosomal cassette elements harbouring *mec* (SCCmec) such as SCCmec types IV and V, fewer antimicrobial resistance determinants and larger arsenals of virulence factor-encoding genes than HA-MRSA. These are considered contributory factors in the ability of CA-MRSA to infect otherwise healthy individuals. The boundaries between CA-MRSA and HA-MRSA have increasingly blurred as CA-MRSA lineages have diversified and become increasingly prevalent in hospitals and other healthcare settings[1].

The expression of the Panton-Valentine leukocidin (PVL) was originally considered one of the hallmark traits of CA-MRSA, however reports of PVL-negative CA-MRSA have been increasing[3]. PVL is encoded by the *lukF-PV* and *lukS-PV* genes located in the genomes of a range of lysogenic bacteriophages. SSTIs are considered the classic presentation of PVL-positive CA-MRSA strains, although more serious infections such as necrotizing pneumonia can ensue.

Methicillin-susceptible *S. aureus* (MSSA) isolates belonging to clonal complex (CC)8 and multilocus sequence typing (MLST) sequence type (ST) 8 emerged from Central Europe and spread to the United States of America approximately 160 years ago[4]. Here it developed into the well-documented CA-MRSA clone USA300, following the acquisition of SCCmec IVa, the *pvl* genes and the arginine catabolic mobile element (ACME)[4], the latter of which is thought to enhance its ability to persist on human skin[5]. The USA300 clone has since achieved global spread although it has yet to become endemic outside of North America, probably due to competition by native CA-MRSA clones in these regions[4,6]. Whilst the CA-MRSA population in Europe is diverse[7], the prevalence of USA300 is increasing[8].

The PVL-positive ST5-MRSA-IV USA800 clone is well dispersed globally[9,10] and predominantly associated with CA- and HA-SSTIs. Recent WGS studies revealed a closely
related PVL-positive CC5-MRSA-IVc clade of the USA800 clone (known as the Sri Lankan clade), comprising isolates from Sri Lanka, the United Kingdom and Australia[10], and the association of the USA300 clone with infection outbreaks in neonatal intensive care units[11].

In Ireland, PVL-negative ST22-MRSA-IV remains the predominant cause of MRSA bloodstream infection (BSI), and the prevalence of PVL-positive MSSA is also low (0.8%)[12]. The proportion of PVL-positive MRSA isolates submitted to the Irish National MRSA Reference Laboratory (NMRSARL) has gradually increased since 2002 (Figure S1)[13,14]. Between 2011 and 2017, the PVL genes were detected in an average of 9.5% of MRSA isolates sent to the NMRSARL. These PVL-positive isolates harboured SCCmec types IV or V and predominantly belonged to CC5 (14.7%), CC8 (31.4%) or CC30 (17.6%) [13–15]. Outbreaks caused by PVL-positive ST772-MRSA-V (known as the pandemic Bengal Bay clone)[16] and PVL-negative ST78-MRSA-IVa and ST1-MRSA-IV[17] CA-MRSA lineages have been reported in Irish hospitals also. Earls et al. recently demonstrated the transmission of multidrug-resistant PVL-negative ST1-MRSA-IV isolates, originally considered a CA-MRSA lineage, within and between hospitals in Ireland and the involvement of healthcare workers (HCWs) in transmission events[17]. The advent of WGS has revolutionised the epidemiological investigation of microbial pathogens over the last decade. In laboratories where WGS is the most cost-effective method for highly informative molecular typing and the required bioinformatic tools are available, conventional molecular typing approaches have largely been replaced by WGS-based techniques, although isolate lineages are still described according to conventional MLST. WGS has facilitated the expansion of the traditional S. aureus MLST scheme based on seven loci to a core genome (cg) MLST scheme based on 1,861 loci[18], providing a globally-available, standardised and highly-discriminatory method for strain comparison and population structure investigations. Strain discrimination resolution can be further enhanced using whole-genome (wg) MLST, which analyses a total of 3,904 loci including the cgMLST loci[19], or single nucleotide variation (SNV) analysis for the investigation of infection outbreaks or transmission studies. Isolates exhibiting ≤24 wgMLST or cgMLST allelic differences or ≤15 SNVs are deemed closely related and indicative of recent transmission[20].

The purpose of the present study was to use WGS to investigate the genetic relatedness of isolates recovered during distinct outbreaks caused by ST8, ST5 and ST1 PVL-positive MRSA within healthcare and community settings in Ireland.
METHODS

Bacterial isolates. Forty-six MRSA isolates from 36 individuals suspected of being involved in several distinct infection outbreaks between 2011 and 2020 were submitted to the NMRSARL for routine analyses and investigated here (Table I). Thirty-five isolates were from patients or HCWs who had previously attended or worked at one of three Irish hospitals (H1-H3). Eleven isolates were recovered from members of two separate families, each of which had at least one member that attended the emergency department or was hospitalised in H2 during 2011, 2012 or 2018 due to an MRSA infection (Table SI).

Twenty MRSA isolates submitted to the NMRSARL were included as comparator reference isolates (CRFs) including PVL-positive MRSA identified as spa types t002, t008 and t127 recovered between 2014 and 2019 from community general practice clinics, regional Irish hospitals, or Dublin-based teaching hospitals other than those included in the present study (Table SI).

Seven international CRFs were selected following comparison of the DNA microarray profiling patterns (see below) of isolates recovered from each H2-associated family cluster (FC) to a S. aureus DNA microarray profile database. This database comprises approximately 25,000 microarray profiles of human and animal strains recovered worldwide[21]. The seven CRFs selected exhibited highly similar array patterns to those of the FC isolates and consisted of two PVL-positive ST5 MRSA from humans, two PVL-positive ST1 MRSA from human (N=1) and bovine (N=1) hosts and three PVL-negative ST97 MRSA from humans (N=2) and poultry meat (N=1) in three countries in the Middle East (Table SI).

Identification, molecular characterisation and antimicrobial susceptibility testing. Isolates were confirmed as S. aureus using the tube coagulase test and as MRSA using 30 µg cefoxitin disks (Oxoid Ltd, Basingstoke, UK). Isolates underwent antimicrobial susceptibility testing against 15 antimicrobial agents (Table S2) as described previously[22,23]. The PVL-encoding lukF-PV and lukS-PV genes were detected by PCR [24], spa typing was performed as previously described[25] and DNA microarray profiling was performed using the S. aureus Genotyping Kit 2.0 [Abbott (Alere Technologies GmbH), Jena, Germany] according to the manufacturer’s instructions[26]. Isolate STs and SCCmec types were inferred based on spa types and/or DNA microarray profiles prior to WGS-based confirmation.
**Passaged isolates.** To investigate the genomic stability of each lineage investigated in this study, serial passaging of representative MRSA (CC1:M18/0051, CC5:M18/1033, CC8:M18/0227 & CC97:M18/0578) was undertaken. Isolates were reactivated from storage at -80°C on Microbank cryogenic bead vials (Pro-Lab Diagnostics, Cheshire, UK) and single colony sub-cultured on fresh Colombia blood agar plates every 24 h for a total of 10 days. On days 2, 4, 8 and 10, a single colony was randomly selected and subjected to WGS.

**Whole-genome sequencing.** WGS was carried out on all isolates investigated using DNA extracted as described previously[25]. Libraries were prepared using the Nextera DNA Flex Library Preparation kit (Illumina, Eindhoven, The Netherlands) according to the manufacturer’s instructions. Prepared libraries were subjected to paired-end sequencing using the MiSeq instrument (Illumina, The Netherlands) using the 500-cycle MiSeq Reagent kit v2 (Illumina). Libraries were scaled to yield a minimum of 70x coverage per isolate and the quality of each sequencing run was determined according to cluster density and Q30 values according to the manufacturer’s instructions. All read datasets are available in the NCBI Sequence Read Archive as BioProject PRJNA638834 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA638834?reviewer=r1ti4589pbk67a8badrb1jftki).

**Bioinformatic analyses.**

The Bionumerics software package v7.7 and incorporated SPAdes assembly software v3.7.1 (Applied Maths, Belgium) was used to perform wgMLST, pairwise SNV analyses and construct minimum spanning trees (MSTs) as previously described[25]. Both assembly-based and assembly-free methods were used to detect and identify MLST alleles as described previously[25]. Sequence reads were also assembled using the VELVET assembler incorporated within SeqSphere+ software version 7 (Ridom, GmBH, Germany). Previously identified spa types and inferred STs were confirmed based on these assemblies using the web-based SCCmecFinder tool ([https://cge.cbs.dtu.dk/services/SCCmecFinder/][27]). The resistance and virulence task templates within SeqSphere+ were used to identify antimicrobial resistance genes and virulence factor genes, respectively.
RESULTS

Investigation of ST8-MRSA-IV outbreaks at hospitals H1 and H2

Ten isolates recovered from eight patients at the outpatient (N=3), gynaecology (N=1), emergency department (N=1) and neonatal intensive care unit (N=5) of a maternity hospital in Dublin (H1) during a four-month period spanning 2017 and 2018 were investigated. All 10 isolates were identified as ACME- and PVL-positive ST8-MRSA-IVa, features characteristic of the USA300 clone (Table SI). Six additional isolates recovered from two separate patients at a separate Dublin hospital (H2) during one week within the same period of the H1 outbreak were also identified as ACME- and PVL-positive ST8-MRSA-IVa. One H1 isolate was identified as spa type t723, whereas the remaining 15 H1 and H2 isolates were t008 (Table I). To investigate possible transmission events within and between hospitals H1 and H2, all isolates underwent WGS.

A wgMLST-based MST tree was constructed for all 16 isolates, the USA300 type strain FPR3757 (GenBank accession number CP000255.1) and 10 t008 PVL-positive MRSA-IV CRFs. This MST revealed two discrete clusters (CH1 [8/10 H1 isolates] and CH2 [all 6 H2 isolates]) within which the majority of H1 and H2 isolates clustered, respectively (Figure 1a).

A separate SNV-based MST constructed for the same isolates but excluding the FPR3757 type strain also revealed the same discrete clusters (Figure S2a). Isolates within CH1 and CH2 were differentiated from the USA300 type strain FPR3757 by 73 and 80 allelic differences, respectively (Figure 1a) and each cluster was separated by ≥127 SNVs (Figure S2a). Isolates within CH1 were all closely related with a median of 0 (average 1.5, range 0-4) allelic differences or 0 (average 1.1, range 0-6) SNVs. The two remaining H1 isolates differed from CH1 isolates by ≥73 allelic differences (Figure 1a) and ≥120 SNVs (Figure S2a). Isolates within CH2 were also closely related with a median of 0.5 (average 0.5, range 0-5) allelic differences (Figure 1a) or 0 (average 0.8, range 0-5) SNVs (Figure S2a).

In contrast, the 10 ST8 CRFs and the FPR3757 reference exhibited an average of 74 allelic differences between neighbouring isolates and none clustered with CH1 or CH2 (Figure 1a). These findings indicated the occurrence of two separate outbreaks caused by genetically distinct strains, one in H1 involving the 8/10 CH1 isolates and the other in H2 involving the six CH2 isolates.

Whilst all 16 isolates recovered from H1 and H2 exhibited ciprofloxacin and ampicillin resistance, two distinct phenotypic antibiotic resistance patterns were identified. In contrast, the 10 CRFs exhibited five distinct antibiotic susceptibility patterns (Table I).
Investigation of ST5-MRSA outbreak at hospital H3

Nineteen isolates from 17 separate patients and one HCW associated with the maternity unit of a regional Irish hospital (H3) recovered between 2018 and 2020 were sent to the NMRSARL for epidemiological analysis. Fifteen isolates identified as t002 ST5-MRSA-IVc (commonly referred to as USA800) were recovered over a 15 month period during 2018 and four t4667 ST5-MRSA-V isolates were recovered during one month in 2019 (Table SI). Eighteen were PVL-positive and one t002 isolate was PVL-negative. These isolates were recovered from patients in the maternity (N=10), special care baby (N=5), outpatient (N=1), emergency (N=1) and paediatric (N=1) units, and from a HCW (N=1) and subjected to WGS to determine if the isolates were part of a protracted outbreak or two separate outbreaks.

Both wgMLST-based (Figure 1b) and SNV-based (Figure S2b) MSTs were constructed based on all 19 isolates, five Irish t002 CRFs and two international PVL-positive CC5-MRSA-IV CRFs (125-318618 and 37-158951; Table SI) which were selected on the basis of having highly similar DNA array profiles to the t002 isolates investigated here. These MSTs revealed two distinct sub-clusters consisting of either t4667 (sub-cluster CH3-SCI, N=4 isolates) or t002 (sub-cluster CH3-SCII; N=14) isolates. The PVL-negative t002 ST5-MRSA-IVc isolate from H3 (M18/1063) did not cluster with any other isolates and exhibited 324 allelic differences to the CH3-SCII sub-cluster (Figure 1b). All four t4667 ST5-MRSA-V isolates within CH3-SCI were closely related and exhibited a median of 1 (average 1, range 1-2) allelic difference (Figure 1b) and 0 (average 0.25, range 0-1) SNVs (Figure S2b). These four isolates were recovered from three patients and one HCW in H3 during a one month period in 2019 (Table SI). All four yielded identical phenotypic antibiotic susceptibility profiles, and exhibited resistance to ≥3 classes of antibiotics (Table I). According to the wgMLST-based MST, the 14 t002 ST5-MRSA-IVc CH3-SCII isolates exhibited a median of 1 (average 3.6, range 0-24) allelic difference (Figure 1b). Four isolates within CH3-SCII were genetically indistinguishable (M18/033, M19/0889, M19/0930 and M19/1191) despite being recovered from four separate patients at intervals of 11, one and two months apart, respectively (Table SI). The seven CRFs (Table SI) exhibited ≥80 allelic differences to the closest relative isolate within CH3-SCII (Figure 1b). The SNV-based MST sub-clusters correlated with those of the wgMLST-based tree with the exception of isolate M19/0811, which was separated by its nearest CH3-SCII neighbour by 24 allelic differences (Figure 1b) or 28 SNVs (Figure S2b), respectively.

All 15 t002 isolates exhibited phenotypic ampicillin resistance and one also exhibited ciprofloxacin resistance (Table I).
Collectively, these findings revealed the occurrence of two separate outbreaks in H3, one involving ST5-MRSA-V (CH3-SCI) and the other involving ST5-MRSA-IVc (CH3-SCII).

Investigation of ST1-MRSA and ST97-MRSA isolates recovered from two separate families associated with H4.

These isolates were recovered from two separate, unrelated families; referred to as FC1 and FC2. Four isolates were recovered from three FC1 members during a three month period spanning 2011 and 2012. Of these four isolates, two were recovered from separate members who presented at the H2 emergency department with SSTIs. Two were from screening samples; one from an individual previously treated for a SSTI, and one from a third family member (Table SI). All four isolates were identified as PVL-positive t127 ST1-MRSA and exhibited phenotypic resistance to ≥3 classes of antibiotics (Table I).

In an unrelated episode, seven MRSA isolates were recovered from five FC2 members during a seven month period spanning 2017 and 2018. Two isolates were recovered from infections in two family members (one of whom was a H2 inpatient) and the remaining five isolates were collected from screening samples of one of these patients and three other family members (Table SI). All seven isolates were identified as PVL-positive t127 ST1-MRSA (N=4) or PVL-negative t267 ST97-MRSA (N=3) and exhibited phenotypic resistance to ≥3 classes of antibiotics (Table I).

To investigate the potential transmission of the isolates within FC2 and explore potential relatedness of the t127 isolates to those from FC1, all 11 FC1 and FC2 isolates underwent WGS. Three international PVL-negative ST97 CRFs, two international and five Irish PVL-positive ST1 CRFs were also included. Separate wgMLST and SNV-based MSTs revealed that within each distinct family cluster and spa type, isolates were separated from their nearest neighbour by ≤7 allelic differences or ≤8 SNVs. Isolates belonging to FC1 (t127) were separated from their nearest neighbouring isolate by a median of 4 (average 4.6, range 3-7) allelic differences (Figure 1c) or 1 (average 3, range 1-7) SNV (Figure S2c).

Isolates belonging to FC2 (t127) were separated from their nearest neighbour by a median of 6 (average of 3.8, range 2-7) allelic differences (Figure 1b), 5 (average 4.7, range 1-8) SNVs (Figure S2c) and by 48 allelic differences or 52 SNVs to the FC1 (t127) isolates. Each of the t127 and t267 CRFs investigated were separated from their neighbouring isolate by ≥44 allelic differences (Fig. 1c). The three spa type t267 isolates from FC2 were separated from each other by a median of 4 (average of 4, range 4-4) allelic differences (Figure 1c) and
4 (average 4, range 4-4) SNVs (Figure S2c). The three ST97 CRFs exhibited ≥72 allelic differences or ≥76 SNVs to the FC2 isolates.

The FC1, FC2 and CRF t127 ST1 isolates harboured genes indicative of SCC\textit{mec IV}, V or the SCC\textit{mec V} subtype (\textit{V+fus+tirS+ccrA1}) (Table SI) previously identified in sporadic PVL-positive CC1 isolates[28]. The FC2 and CRF t267 ST97 isolates harboured genes indicative of SCC\textit{mec V+fus} (Table SI) previously identified in sporadic CC97 isolates[28]. Collectively, these findings indicated the transmission of distinct MRSA strains amongst two separate families. One t127 strain was transmitted amongst FC1 members, whereas two distinct t127 and t267 strains were independently transmitted amongst several FC2 members. Direct transmission of the t127 isolates between the two families investigated was deemed unlikely due to the identification of 48 allelic differences between the two t127 clusters (Figure 1c).

\textbf{Serially Passaged Isolates}

The wgMLST analysis of colonies randomly selected from serial passage of the ST8-MRSA-IV\textit{a} isolate M18/0227, ST5-MRSA-IV\textit{c} isolate M18/1033, ST1-MRSA-\textit{V+fus+tirS+ccrA1} isolate M18/0051 and ST97-MRSA-\textit{V+fus} isolate M18/0578 revealed a maximum of two, zero, three and three allelic differences between each passage, respectively.
DISCUSSION

Despite the decreasing reports of MRSA infections in Ireland in recent years, the proportion of PVL-positive MRSA has continually increased since 2002 (Figure S1)[13,14]. The present investigation revealed the transmission of distinct PVL-positive CA-MRSA lineages within separate Irish hospitals (H1-H3). Three of these lineages (ST8-MRSA-IVa, ST5-MRSA-V and ST5-MRSA-IVc) were associated with outbreaks in neonatal care facilities (Table SI). Outbreaks of ST5 and ST8 CA-MRSA infections have been reported in neonatal and paediatric care units previously[11].

Multidrug resistance (resistance to ≥3 classes of clinically-relevant antibiotics) is not a common characteristic of CA-MRSA, but is increasingly reported amongst PVL-positive MRSA populations in Europe, North and South America, Asia and Australia[29]. In the present study, multidrug resistance was detected in 22/53 (41.5%) isolates, including those belonging to the ST1, ST5, ST8, and ST97 lineages (Table SI).

The USA300 isolates were recovered from neonatal, paediatric and adult patients in two separate hospitals. All were identified as PVL-positive t008/t723, ST8-MRSA-IVa and exhibited ampicillin and ciprofloxacin resistance (Table SI). Despite these similarities, wgMLST revealed the presence of distinct clusters of isolates within each hospital and no evidence of transmission between the two. HCW-associated CA-MRSA transmission between hospitals in Ireland has previously been suggested, as transfer of staff between distinct healthcare facilities is common[17]. Each cluster was separated from the USA300 type strain by a ≥73 allelic differences (Figure 1a) indicative of separate, unrelated outbreaks. Due to the identification of t008 spa type and carriage of the SCCmec type IVa, these isolates bear the closest similarity to the clone originating in North America[4], however no travel histories were available for the patients investigated.

Isolates identified as PVL-positive ST5-MRSA-IVc were recovered from H3-associated individuals over a 15 month period during 2018-20. These isolates were identified as spa types t002 and t4667, which correlated with the two distinct sub-clusters identified by wgMLST (Figure 1b). The t4667 isolates belonging to CH3-SCI were all recovered during the same month, harboured SCCmec V, exhibited multidrug resistance and were recovered from a HCW and patients. The PVL-positive t002 isolates were recovered between 2018 and 2020, harboured SCCmec IVc and exhibited ampicillin resistance (Table SI). The low average number of allelic differences (3.4) detected amongst the H3 isolates compared to the average 75.7 allelic differences among CRFs is indicative of a protracted ST5 MRSA outbreak in H3.
The PVL-positive t127 ST1-MRSA isolates were from two separate families (FC1 and FC2) with links to H2, however the FC1 t127 isolates were recovered during 2011 and 2012, whereas the FC2 t127 isolates were recovered during 2018 (Table SI). Based on the detection of 48 allelic differences between the FC1 and FC2 t127 isolates and the fact that these isolates were recovered more than five years apart (Table SI), it is likely that these isolates represent independent transmission networks. The \( \leq 24 \) allelic differences or \( \leq 15 \) SNVs thresholds for inferring epidemiological relationships between \textit{S. aureus} isolates[20] were deemed appropriate for all lineages investigated and were supported by the available epidemiological information.

Similar SCC\textit{mec} type \( V+fus+tirS+ccrA1 \) elements were detected in all FC1 and FC2 t127 isolates and SCC\textit{mec} \( V+fus \) elements were detected in the PVL-negative FC2 t267 ST97 isolates. Genes indicative of similar elements[28] have also been identified in both PVL-positive ST1 and PVL-negative ST97 isolates recovered in the Middle East[28,30], an area where some members of FC2 had travelled, suggesting possible importation of these strains from this geographical region and subsequent intra-familial transmission. Previous research has shown that MRSA transmission is common in the household and can result in 67% of household contacts of an index carrier becoming colonised with the same strain[31].

The present study demonstrated the high resolution offered by WGS for investigation of outbreaks and transmission of MRSA strains both within and outside healthcare settings. For each separate investigation, distinct wgMLST and SNV-based MSTs were congruent (Figure 1 and S2). The ST8 isolates recovered from H1 and H2 were deemed highly similar based on phenotypic antibiotic susceptibility patterns and \textit{spa} types, suggestive of possible transmission between the two Dublin-based hospitals. However, these isolates were separated into genotypically distinct clusters by wgMLST, indicating the independent transmission of two distinct USA300 strains within each hospital and highlighting the advantage of WGS over conventional molecular typing techniques. The unparalleled discriminatory power offered by WGS also demonstrated the possibility of outbreak-associated isolates being incorrectly excluded from outbreak investigations if based on \textit{spa} types alone. In the present investigation, isolate M18/0106 identified as t723 (repeat succession: 11-19-12-34-22-25) exhibited between four-six allelic differences (Figure 1a), and between six-eight SNPs (Figure S2a) to the seven t008 (repeat succession: 11-19-12-21-17-34-24-34-22-25) isolates in CH1.

As WGS becomes more routinely available to more clinical microbiology laboratories, the high resolution offered by the technology will help to inform and direct infection...
prevention and control strategies for outbreak management in real-time, both within healthcare facilities and/or private households. The high resolution offered by WGS can often reveal transmission events directly. This information would be highly beneficial in the implementation of routine strategies for (i) decontamination (e.g. improved cleaning and monitoring of most frequently outbreak-associated fomites within healthcare facilities and domestic settings), (ii) transmission risk minimisation (e.g. screening of HCWs, incoming patients and the identification of long-term carriers) and (iii) decolonisation (e.g. use of topical or systemic prophylactic therapies for management of endogenous infection risk prior to admittance to healthcare facilities, which may also reduce the risk of outbreak-associated infections in healthcare settings). In addition, the WGS data can be utilised to inform approaches for antimicrobial treatments.

In the present study, two PVL-positive ST8-MRSA-IVa MRSA infection outbreaks in two Dublin-based hospitals were shown to be unrelated, a protracted PVL-positive ST5-MRSA-IVc outbreak in a Dublin-based teaching hospital was revealed and the intra-familial transmission of PVL-positive ST1-MRSA-V+fus+\textit{tirS}+\textit{ccrA1} and PVL-negative ST97-MRSA-V+fus isolates in two separate families was described.

CONCLUSIONS

The prevalence of PVL-positive MRSA is continually increasing in Ireland. The frequent importation of CA-MRSA strains into healthcare settings and increasing association with HCWs and infection outbreaks is a serious concern. The application of WGS is highly advantageous in the ongoing surveillance of MRSA strains and investigation of suspected outbreaks.

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CONFLICT OF INTEREST STATEMENT

Declarations of interest: none

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Figure 1. Minimum spanning trees (MSTs) based on whole-genome multilocus sequencing typing (wgMLST) analysis of the 42 Panton-Valentine leukocidin (PVL) positive and four PVL-negative MRSA isolates investigated in addition to epidemiologically unrelated but genotypically similar comparator reference isolates (CRFs).

In each MST, MRSA isolates recovered from separate hospitals or families and identified as distinct spa types are indicated by separate colours. Genotypically similar but epidemiologically unrelated CRFs included for comparative purposes are indicated in each MST as red circles. Closely related clusters of isolates (≤24 wgMLST allelic differences [20]) are outlined with grey shadowing. A black spot in the centre of each circle is used to indicate PVL-negative isolates which were included in the study as they were identified as the same spa types/STs as the outbreak-associated PVL-positive MRSA and were recovered in the same hospital over the relevant time periods. The PVL-negative t267 isolates were included as they were also recovered from one of the families affected by the PVL-positive t127 MRSA lineage. The numbers on each branch indicate the numbers of wgMLST allelic differences detected between neighbouring isolates. The epidemiological information for each isolate and CRF investigated is provided in Table SI. (a) MST constructed from the t008, ST8-IVa MRSA isolates associated with hospitals 1 and 2 (H1 [N=10] & H2 [N=6]), CRFs (N=10) and the USA300 reference strain FPR3757. The two distinct clusters CH1 and CH2, refer to isolates recovered from hospitals H1 and H2, respectively. (b) MST constructed from the t002-ST5-IVc (N=15) and t4667-ST5-V (N=4) isolates associated with hospital 3 (H3) and CRFs (N=7). With the exception of the PVL-negative isolate M18/1063, all t002-ST5-IVc isolates formed a distinct sub-cluster (CH3-SCI), and all four t4667-ST5-V isolates formed a second sub-cluster (CH3-SCII). (c) MST constructed from the t127-ST1-V+fus+tirS+ccrA1 (N=8) and t267-ST97-V+fus (N=3) isolates recovered from multiple members of two distinct families (FC1 [N=4] and FC2 [N=7]), of which each family had at least one member that either attended the emergency department of, or was admitted to hospital 2 (H2), and 10 epidemiologically unrelated CRFs. Three distinct sub-clusters were apparent, FC1 (t127), FC2 (t127) and FC2 (t267) each of which consisted of all of the isolates identified as each distinct spa type and from each separate family.
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