Methicillin-resistant *Staphylococcus aureus* transmission among healthcare workers, patients and the environment in a large acute hospital under non-outbreak conditions investigated using whole-genome sequencing

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Running Title: Transmission of MRSA under non-outbreak conditions

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Abbreviations; CC; clonal complexes, HCW; healthcare worker, NPE; near patient environment, RIG; related isolated groups, TEs; transmission events, WGS, whole-genome sequencing; cgMLST, core-genome multilocus sequence typing

Summary

Background: The role of MRSA colonization of healthcare workers (HCWs), patients and the hospital environment in MRSA transmission in non-outbreak settings is poorly understood.

Aims: To investigate transmission events (TEs) involving HCWs, patients and the environment under non-outbreak conditions in a hospital with a history of endemic MRSA using whole-genome sequencing (WGS).

Methods: HCW (*N*=326) and patient (*N*=388) volunteers on nine wards were tested for nasal and oral MRSA colonization over two years. Near-patient environment (*N*=1,164), high-frequency touch sites (*N*=810) and air (*N*=445) samples were screened for MRSA. Representative MRSA and clinical isolates were analysed by WGS and core-genome multilocus sequence typing (cgMLST). Closely-related isolates (≤24 allelic differences) were segregated into related isolated groups (RIGs).

Findings: In total 155 MRSA were recovered: clinical isolates (*N*=41), HCWs (*N*=22), patients (*N*=37), environmental isolates (*N*=55). Nine clonal complexes (CCs) were identified among 110/155 MRSA sequenced with 77/110 assigned to CC22. Seventy-nine MRSA segregated into 17 RIGs. Numerous potential TEs were associated with CC22-MRSA (RIGs 1-15), CC45-MRSA (RIG-16) and CC8-MRSA (RIG-17). RIG-1, (the largest RIG) contained 24 ST22-MRSA-IVh from six HCWs, six patients, four clinical and eight environmental samples recovered over 17-months involving 7/9 wards. TEs involving HCW-to-patient, HCW-to-HCW, patient-to-patient and environmental contamination by HCW/patient isolates were evident. HCW, patient, clinical and environmental isolates were identified in four, nine, seven and 13 RIGs, respectively, with 12 /13 of these containing isolates closely-related to HCW and/or patient isolates.

Conclusions: WGS detected numerous potential hospital MRSA TEs involving HCWs, patients and the environment under non-outbreak conditions.

Keywords: MRSA, hospital transmission, whole-genome sequencing, MRSA colonisation, environmental contamination, non-outbreak conditions.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) have been endemic in Irish hospitals for several decades with ST22-MRSA-IVh currently being predominant, accounting for 73.7% of MRSA bloodstream infections (BSIs) in 2019[1,2]. The European antimicrobial-resistance surveillance network (EARS-Net) reported a 10% increase in *S. aureus* BSIs between 2014-2018. This included a 30% decrease associated with MRSA BSIs and a 20% increase associated with methicillin-susceptible *S. aureus* (MSSA)[3]. However, despite the decreases in MRSA BSIs it remains a significant clinical issue. The introduction of various MRSA clones into Irish hospitals is poorly understood, with no universal MRSA admission screening[4]. Patient colonisation with MRSA predisposes to increased opportunities for infection, leading to increased antibiotic consumption, associated hospitalisation costs and mortality[5,6]. The importance of hand hygiene compliance by healthcare workers (HCWs) for reducing MRSA spread is widely accepted[7–9]. However, the contribution of MRSA shedding by colonized HCWs and patients to transmission dynamics among HCWs, patients and the hospital environment is poorly understood. Significant proportions of humans harbour MRSA, primarily in the nasal and oral cavities but also in the perineum, axillae and hands[4,10]. It is well documented that approximately one third of healthy people are colonized nasally with *S. aureus*[11] either persistently or intermittently[12]. However, the frequency of HCW colonisation by MRSA and their relatedness to MRSA recovered from patient and hospital environmental sources requires further investigation[13]. Routine screening of HCWs for MRSA is uncommon in most countries without a suspected epidemiological link between patient infections and HCWs[4]. Environmental MRSA are not routinely investigated in hospitals[4,9].

The purpose of this study was to use whole-genome sequencing (WGS) to investigate the role of HCWs, patients and the environment in potential MRSA transmission events (TEs) in multiple wards of a large-acute hospital with a history of endemic MRSA over a two-year period under non-outbreak conditions. Detailed information regarding such TEs may inform measures targeted at reducing potential outbreaks.

Methods

Study design and participants

This study was undertaken in nine wards (A-I) of a large-acute hospital in Dublin, Ireland. The hospital has an active infection prevention and control team that undertakes surveillance of MRSA to detect and quickly control outbreaks. Beaumont Hospital Medical Research Ethics Committee approved the study (reference number, 17/01). HCWs and ward patients were invited to participate subject to previously-described selection and inclusion criteria and informed consent[4]. Nasal MRSA colonisation status of participants was unknown to the researchers. Participant sampling was undertaken in the wards during three phases (I-III): phase I, May 2017 to mid-October 2017; phase II, late October 2017 to May 2018; phase III, August 2018 to March 2019. Seven of the nine wards consisted of four 6-bed bays, one 4-bed bay, and one 2-bed bay. Two remaining smaller wards consisted of two 4-bed bays and one 2-bed bay. Single-occupancy rooms were excluded from the study. Environmental sampling included near-patient environments (NPEs) including bedside lockers, bedrails, and mattresses. High-frequency hand-touch sites were also sampled including ward-corridor handrails, ward-bay curtains, window curtains, windowsills, commodes and nurse desks (Table SI). Clinical MRSA isolates (e.g. from surgical site infections) from patients in the same wards during the study period were also investigated.

Participant and environmental sampling

Isolate recovery was described previously[4]. Briefly, participant samples were taken from the anterior nares using sterile cotton-tipped transport swabs (Venturi, Transystem, Copan, Italy), and from the oral cavity using a phosphate-buffered saline oral rinse (PBS)[14]. Environmental sampling was undertaken to provide a snapshot of the MRSA environmental burden during periods when patients and HCWs were sampled for MRSA. For all study patients, surface samples were collected from their mattress, bedside locker and bedframe at the time of nasal and oropharyngeal sampling. Air sampling was undertaken once per sampling phase using an EM0100A air sampler (Oxoid/Thermo Scientific, Fannin Healthcare, Dublin, Ireland) configured to collect 500 L air samples by placing the sampler vertically on the floor in the central part of each bay, the ward corridor, the ward kitchen, the ward treatment room, and the sluice room. Environmental-surface samples were taken using contact plates[15,16].

Microbiological methods

MRSAselect chromogenic agar (Colorex, E&O laboratories, Bonnybridge, United Kingdom) was used to culture all samples and for air and contact plate sampling. PBS samples were concentrated by centrifugation before plating on MRSASelect agar as described[14]. Inoculated plates were incubated for 24 h at 37°C, followed by examination for mauve/pink colonies indicative of MRSA. Colonies were definitively identified as *S. aureus* using the tube-coagulase test[17,18] and the Pastorex StaphPlus latex-agglutination kit (BioRad, Marnes la Coquette, France). PCR amplification targeting the *mecA* gene was undertaken to confirm MRSA as previously described[19]. Antimicrobial-susceptibility profiling including methicillin-resistance confirmation using 30-μg cefoxitin disks (Oxoid, Basingstoke, United Kingdom) was undertaken as previously described using a panel of 23 antimicrobial agents and heavy metals by disk diffusion using the European Committee on Antimicrobial Susceptibility testing (EUCAST) methodology and interpretive criteria[17,20]. Isolates were deemed multidrug resistant (MDR) if they exhibited resistance to three or more antibiotic classes other than beta-lactams. A participant was considered colonized by MRSA if either nasal or oral samples yielded MRSA.

Whole-genome sequencing isolate selection

One isolate per HCW, patient and sampled environmental site per sampling phase was selected for WGS unless antimicrobial-susceptibility profiles from multiple isolates from the same participant/site were different.

Whole-genome sequencing

Genomic DNA was extracted using the *S. aureus* Genotyping Kit 2.0 (Abbott [Alere Technologies GmbH], Jena, Germany) and the Qiagen DNeasy blood and tissue kit (Qiagen, West Sussex, United Kingdom)[21]. DNA quality was assessed as previously described[21]. Sequencing libraries were prepared using the Nextera DNA Flex Library Preparation Kit (Illumina, Eindhoven, The Netherlands), which underwent paired-end Illumina MiSeq sequencing using the 500-cycle MiSeq Reagent Kit v2 (Illumina). Libraries were scaled to exhibit an average coverage of 100× and the quality of sequencing runs was assured following cluster density and Q30 assessment. Resulting fastq files were uploaded from Illumina BaseSpace to the BioNumerics cloud-based calculation engine for assembly with the SPAdes de novo assembly algorithm (version 3.13.1) using

BioNumerics software, version 8 (Applied Maths, BioMerieux, Belgium). Assembled genomes and associated fastq files were submitted to the BioNumerics cgMLST scheme (1861 loci) for assembly-free and assembly-based allele calling, respectively. Variation between isolate cgMLST profiles of each clonal complex (CC) was investigated using the categorical differences algorithm and the UPGMA method in BioNumerics to generate a best fit, circularised UPGMA tree. Isolates within each CC exhibiting ≤24 cgMLST allelic differences were deemed closely-related based on previously proposed relatedness thresholds[22,23] and clustered into related isolates groups. All read datasets have been submitted to the NCBI Sequence Read Archive (BioProject-No. PRJNA744773).

Results

Healthcare workers

In total 326 HCWs were enrolled in the study as described previously [4], of which 15/326 (4.6%) were colonized with MRSA. MRSA was detected from 2/149, 9/145 and 5/83 HCWs during phases I-III, respectively. These included HCW-0036 who yielded nasal and oral MRSA over two sampling phases and HCWs-194, -214, -406 and -460 who yielded nasal and oral MRSA during one sampling phase. The remaining 10 HCWs yielded MRSA during one sampling phase: nasal (*N*=1), oral (*N*=9) (Table SI).

Patients, environmental sites, and clinical isolates

In total 388 patients were enrolled in the study as described previously (Table SI) [4]. Of these, 31/388 (8%) yielded 37 MRSA isolates. Seven patients harboured MRSA both nasally and orally, 10 patients harboured nasal MRSA only and 14 patients harboured oral MRSA only. MRSA (*N*=28) were recovered from 9/1164 (0.77%) NPE samples (five bedframes, two bedside lockers and two mattresses) and 18/810 (2.2%) high-frequency touch sites (nine handrails, and one each of the following: window curtain, desk, computer mouse, armchair, countertop, bathroom door handle, commode, windowsill, and a patient folder. Air sampling yielded 32 MRSA isolates from 445 (7.2%) samples. Forty-one clinical MRSA isolates were recovered on the nine wards during the study (Table SI).

Isolates and antimicrobial susceptibility

In total 155 MRSA were recovered from 15 HCWs (*N*=22), 31 patients (*N*=37), patient infections (*N*=41) and 2419 environmental samples (i.e. air and surfaces) (*N*=55). Forty

distinct antimicrobial-susceptibility profiles were detected among the 155 MRSA, of which 110 (71%) were MDR. Eight of the 110 MDR MRSA exhibited cefoxitin/oxacillin-susceptible phenotypes but were *mecA*-positive. Similar isolates have been reported previously by others (Table SI)[24,25].

Clonal complex groups identified using WGS

In total 110/155 MRSA isolates were selected for WGS based on the criteria of unique antimicrobial-susceptibility profiles and only one isolate per patient/HCW (per sampling phase) and 20 clinical isolates (Table SI). Analysis of WGS data identified nine CC groups based on cgMLST (Table SI) of which CC22 (*N*=76) was the largest, followed by CC8 (*N*=11), CC5 (*N*=8), CC45 (*N*=6), CC1 (*N*=3), CC15 (*N*=2) and CC30 (*N*=2). The two remaining isolates belonged to sequence type (ST) ST96 and ST2250 (Table SI). The majority of MRSA sequenced (79/110, 72%) segregated into 17 related isolate groups (RIGs). Isolates within each RIG were very closely-related (≤24 cgMLST allelic differences). The pairwise allelic differences between the 79 MRSA in RIGs are shown in Table SII. Isolates in RIGs 1-15, RIG-16 and RIG-17 belonged to CC22, CC45 and CC8, respectively (Figure 1 & Table SI). Thirty-one MRSA not assigned to RIGs were deemed unrelated and for convenience were assigned to RIG-0 (Table SI). RIGs 1-17 are described in detail below and the timeline for recovery of isolates in each RIG is shown in Figure 2. Epidemiological information relating to the 79 MRSA isolates in RIGs is summarised in Table SIII.

CC22 MRSA in RIGs 1-15

Of the 76 CC22-MRSA isolates sequenced, 65/76 (85.5%) grouped into RIGs 1-15. RIG-1 was the largest consisting of 24 ST22-MRSA-IVh (two phenotypically cefoxitin/oxacillin-susceptible) recovered from seven wards over 17-months (Figure 2, RIG-1). The earliest isolate (ADA0022.1) was recovered from a Ward D air sample in June 2017 followed 54-days later by a clinical isolate (C104066) from a Ward H patient (Table SI and Figure 2, RIG-1). Other RIG-1 isolates were recovered from five HCWs on Wards A (*N*=1), B (*N*=1) and F (*N*=4) between November 2017 and November 2018, from six patients on wards A (*N*=2), E (*N*= 2), G (*N*=1) and H (*N*=1) between November 2017 and August 2018 and from four clinical isolates from patients on Wards E (*N*=2), F (*N*=1) and H (*N*=1) between August 2017 and March 2019 (Table SI and Figure 2, RIG-1).

In addition to RIG-1, ST22-MRSA-IVh isolates from HCWs clustered in RIG-5, RIG-14 and RIG-15. RIG-5 contained three isolates, the first of which (HN0036.1) was recovered from HCW-0036 in July 2017 in Ward A, followed by a Ward H air-sample isolate a month later (AAB0042.1A) and a second isolate (HN0036.2) from the same Ward A HCW recovered five-months later in November 2017 (Figure 2, RIG-5).

RIG-14 consisted of five isolates, the first (HN0214.1) of which was recovered from HCW-0214 in Ward I in August 2017, followed by an isolate (EB0267) from a patient's bedframe in Ward D in September 2017. The third isolate (PO0283) was recovered from a Ward D patient a week afterwards and a day later the fourth isolate (APA0096.1), was recovered by air sampling in Ward I. The remaining RIG-14 isolate (PO0289) was recovered from a Ward C patient at the start of November 2017 (Table SI and Figure 2, RIG-14). RIG-15 contained four ST22-MRSA-IVh, including an oxacillin-susceptible isolate (HN0084.2) from HCW-0084 in Ward B on the 5th of May 2018. Three days later two further Ward I ST22-MRSA-IVh (PN0385 and EL0385) were recovered from a patient and the patient's NPE. A week later, the remaining isolate (HN0390.1) was recovered from HCW-0390 in ward H (Figure 2, RIG-15).

In addition to RIG-1, patient isolates were also clustered in RIG-14 and RIG-15, which have been described above, and with six additional RIGs (RIGs 4, 8-9, 11-13). RIG-4 and RIG-9 contained isolates from patients and from clinical samples: RIG-4 contained patient isolates from Ward C (PN0273, recovered in October 2017), Ward H (PN0469, recovered in April 2018) and a Ward B clinical isolate (C54910, recovered in October 2018) (Figure 2, RIG-4). RIG-9 contained two isolates including Ward B clinical isolate C12553 recovered in February 2018 and a Ward I patient isolate (PN0435) recovered in March 2018 (Figure 2, RIG-9). RIG-8 contained two ST22-MRSA-IVh isolates from two patients (PO0761 and PN0755) in Wards A and C, respectively, recovered on the same day (Figure 2, RIG-8). RIG-11 contained three isolates from two patients (PN0377 and PN0387) in Ward I recovered in January and February 2018, respectively, and a third isolate (EB0405) from a separate patient's NPE in Ward H in late February 2018 (Figure 2, RIG-11). RIG-12 involved seven ST22-MRSA-IVh isolates recovered over an 18-month period starting with a Ward E air sample isolate (AJE0111.1) in October 2017, followed by a Ward F patient isolate (PO0373) in January 2018 and a further five environmental isolates recovered between October 2018 and April 2019, including a Ward B handrail isolate (EPconPE0004.1A), a Ward I air-sample isolate (APA0009.3), a Ward G patient's bedframe (EB0661), a Ward E handrail (EPconJE0001.3) and a Ward E commode armrest

(EPconJE0005.3) (Table SI and Figure 2, RIG-12). The final patient-associated RIG (RIG-13) contained two ST22-MRSA-IVh isolates; the first isolate (EB0307) was recovered from a patient's NPE in Ward A, early November 2017 and the second isolate (PN0337) was recovered 21-days later in Ward B from a separate patient (Table SI and Figure 2, RIG-13).

The five remaining CC22 RIGs (RIG-2, RIG3, RIG-6, RIG-7, and RIG-10) all contained two isolates each, four of which contained clinical isolates: RIG-2 included clinical isolate C114756 recovered in August 2017 in Ward B and environmental isolate EPconJE0006.3 recovered from a pantry windowsill in Ward E in April 2019. RIG-3 consisted of two ST22-MRSA-IVh clinical isolates (C3843630 and C166287) recovered in November 2017 recovered one week apart in Wards A and H (Supplemental Table S1; Figure 2). RIG-6, contained two environmental isolates (AHA0006.2B and AHA0003.2) from air samples on Ward F in January 2018 (Supplemental Table S1; Figure 2). RIG-7 included a Ward E oxacillin-susceptible ST22-MRSA-IVh clinical isolate (C12210) recovered in January 2018 and a Ward H ST22-MRSA-IVh isolate (AAB0001.3) from an air sample in November 2018. The remaining ST22-MRSA-IVh RIG-10, consisted of a Ward C ST22-MRSA-IVh isolate (AMA0006.1B) from an air sample in May 2017 and a closely-related clinical isolate (C13322) recovered in Ward A in January 2018.

CC45 MRSA in RIG-16

Six CC45-MRSA isolates were sequenced of which four CC45-ST508-MRSA-IVc isolates clustered in RIG-16. Ward G isolates HN0406.1 and HO0172.2 were recovered from two HCWs (HCW-0406 and HCW-0172) on the 9th and the 22nd March 2018, respectively. The third isolate (EM0423) was recovered from a patient's mattress in Ward I on the 27th March 2017 and the fourth isolate (HN0460.2) also from HCW-0460 was recovered in Ward G two months later (May 2017) (Figure 2, RIG-16).

CC8 MRSA in RIG-17

Eleven CC8-MRSA isolates were sequenced, of which 10 CC8-MRSA-Vc clustered in RIG-17 including two HCW and eight environmental isolates. The first HCW isolate (HN0376.1) was recovered from HCW-0376 on the 26th January 2018 in Ward G. Four environmental isolates were recovered from air samples (N=3) and a nightstand in Ward D (N=1) on the 15th January 2019 followed by four isolates recovered from Ward C air

297 samples one day later. The second HCW (HCW-0578) yielded isolate HO0578.1 in Ward
298 G on the 23rd January 2019 (Figure 2, RIG-17).

299 **Discussion**

300 This is the first study combining high-resolution WGS analysis and epidemiological data to
301 highlight the complex roles colonized HCWs, patients and environmental contamination
302 contribute towards MRSA transmission in a large-acute hospital with a history of endemic
303 MRSA under non-outbreak conditions. In total 155 MRSA were recovered from 15 HCWs
304 (*N*=22), 31 patients (*N*=37), patient infections (*N*=41) and environmental samples (*N*=55).
305 A subset of 110/155 MRSA was selected for WGS based on unique antimicrobial-
306 susceptibility profiles and one isolate per patient or HCW (per sampling phase). The
307 majority of all sequenced MRSA (79/110 isolates) grouped into 17 RIGs, each consisting
308 of closely-related isolates (≤24 allelic differences) determined by cgMLST (Table SI).

309 Colonized HCWs that yielded ST22-MRSA-IVh isolates in RIGs 1, 5, 14 and 15,
310 were likely associated with TEs. However, it is possible that other colonized HCWs that
311 did not participate in the study also contributed to TEs. A HCW isolate was the earliest
312 recovered MRSA in RIGs-5, -14 and -15. In RIG-1, which contained 24 ST22-MRSA-IVh
313 recovered over 17-months spanning seven wards including isolates from several HCWs,
314 patients and environmental samples, an air sample isolate (ADA0022.1) was the earliest
315 recovered (Figures 1 and 2, RIG-1). The recovery of RIG-1 CC22-MRSA-IV clinical
316 isolates C104066, C166370, C30011 and C99331 from three wards between March 2017
317 and March 2018 indicate that the patients concerned likely acquired their infections in the
318 hospital. Six HCWs also yielded RIG-1 CC22-MRSA-IV isolates between November 2017
319 and November 2018 and were possibly involved in isolate transmission (Figure 2, RIG-1).
320 Evidence for potential undetected HCW carriers is evident in RIG-2 (isolates C114756 and
321 EPconJE0006.3), RIG-7 (isolates C12210 and AAB0001.3) and RIG-10 (isolates
322 AMA0006.1B and C13322): each of these RIGs contained one CC22-MRSA-IV clinical
323 isolate and one CC22-MRSA-IV environmental isolate recovered between 11-months to
324 two-years apart and it is likely a colonized HCW(s) was responsible for the persistence of
325 these closely-related hospital isolates (Table SI and Figure 2).

326 Potential evidence of a persistently colonized HCW shedding MRSA into the
327 hospital environment is evident in RIG-5: HCW-0036 initially yielded ST22-MRSA-IVh
328 isolate HN0036.1 in July 2017 and four-months later yielded the closely-related isolate
329 HN0036.2, with the closely-related environmental isolate AAB0042.1A detected in August

2017 (Figure 2, RIG-5). Possible TEs and environmental shedding were evident in RIG-14 involving HCW-0214 or another undetected HCW(s). HCW-0214 yielded an ST22-MRSA-IVh isolate (HN0214.1) in Ward I in September 2017. A closely-related Ward D isolate (EB0267) was recovered from a patient's bedframe in mid-October 2017 and a closely-related Ward D patient isolate (PO0283) was recovered one-week later on the 25th October 2017. A fourth closely-related Ward I isolate, APA0096.1, was recovered from air sampling on the 26th October and a fifth closely-related Ward C isolate (PO0289) was recovered from a patient on the 2nd November 2017 (Figure 2, RIG-14).

The potential direction of transmission of isolates in RIG-15 is less clear as all four closely-related isolates were recovered within 11 days. The earliest isolate (HN0084.2) was recovered from HCW-0084 in Ward B and related Ward I isolates (PN0385 and EL0385) were detected three-days later from a patient and the patients' immediate environment (Figure 2, RIG-15). A second HCW, (HCW-0390) yielded isolate HO0390.1 on Ward H 11-days after the recovery of HN0084.2, eight-days after the patient and environmental isolates.

MRSA isolates from patients were associated with nine RIGs (RIGs 1, 4, 8, 9, 11, 12, 13, 14 and 15). Clinical isolates were associated with seven RIGs (RIGs 1, 2, 3, 4, 7, 9 and 10) and isolates from environmental sampling were associated with 13 RIGs (RIGs 1, 2, 5, 6, 7 and 10-17). RIG-17 contained ST8-MRSA-Vc isolates from two Ward G HCWs including HN0376.1 and one-year later HO0578.1. Eight closely-related environmental RIG-17 isolates were recovered in Wards C and D on the 15th and 16th January 2019, one-week before the second HCW isolate HO0578.1 was recovered. The extent to which either HCW was shedding ST8-MRSA-Vc into the environment would have otherwise gone undetected.

Several introductions of MRSA clones into the hospital were detected as evidenced by the nine CCs and 14 STs identified among the 110 sequenced isolates from HCWs, patients and the environment. The extent to which several ST22-MRSA-IVh strains were circulating throughout several wards and numerous HCWs, patients and the environment would have remained unknown without this study (Figures 1 and 2). Such introductions provide opportunities for the emergence of new MRSA clones within hospital environments as previously suggested by the authors[2]. The presence of cefoxitin- and oxacillin-susceptible MRSA presents a concern for laboratory detection of MRSA as genetic detection of *mecA* would be required for further differentiation of oxacillin-susceptible MRSA from MSSA [24].

Currently in Irish hospitals screening for MRSA focuses on high-risk patients, however screening regimens may change based on the requirements and policies for local infection-control and prevention (IPC) teams[4,26]. HCW screening is normally only considered upon identification of an epidemiological link with HCWs and MRSA recovered from patient clusters. In the present study, the role of environmental contamination due to shedding by HCWs and/or patients in potential TEs was shown to be a factor in 11/17 RIGs. This suggests that periodic screening of both HCWs and patients in non-outbreak settings should be considered, such as where MRSA is endemic in a defined clinical setting. Of the 15 HCWs and 31 patients colonized with MRSA, 9/15 (60%) and 14/31 (45%), respectively, exhibited oral carriage only.

This finding is in keeping with a study from this group, which identified the oral cavity as a significant reservoir for *S. aureus* carriage[4]. These findings indicate that oral screening should be implemented in routine screening procedures for MRSA. Currently, HCW and patient screening for MRSA usually does not involve testing the oral cavity. Monitoring indirect TEs involving the hospital environment is another consideration, possibly in conjunction with considerations for improved cleaning and decontamination (Table SI) [27-29].

Limitations

This was a single centre study confined to specific wards and excluding single rooms. Although extensive sampling was carried over three phases, it was not continuous for logistical reasons. Not all HCWs or patients on the hospital study wards provided consent. Therefore, it is likely a significant number of additional HCWs and patients in the study wards were colonized with MRSA, which could have contributed to the potential TEs identified in the RIGs.

Conclusions

The ability to detect potential TEs involving MRSA in a large-acute hospital under non-outbreak conditions can be readily facilitated using WGS and cgMLST, when combined with vital epidemiology data such as patient admission and location. The largest RIG in particular (RIG-1) provided numerous examples of potential TEs involving a range of HCWs (*N*=6), patients (*N*=6) and patients with MRSA infections (*N*=4) harbouring very closely-related ST22-MRSA-Ivh strains. These TEs exemplify the contributions that

unidentified MRSA carriage under non-outbreak settings may have, which may ultimately lead to outbreaks. Ideally, periodic surveillance and investigation of the dynamics of background MRSA colonization and TEs by HCWs and patients as well as environmental contamination should influence on-going review of IPC strategies.

Acknowledgements

The authors wish to acknowledge the support of both the patients and staff of Beaumont Hospital for facilitating this research and the staff of the Irish National MRSA Reference Laboratory (NMRSARL) for technical assistance.

Conflict of interest statement

Declarations of interest: HH has been in receipt of research funding from Astellas and Pfizer in recent years and has received a consultancy fee from Pfizer in the last three years. All other authors have no conflicts of interest to declare.

Funding source

This work was supported by a grant from the Irish Health Research Board (grant number: HRA-POR-2015-1051).

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Figure Legends

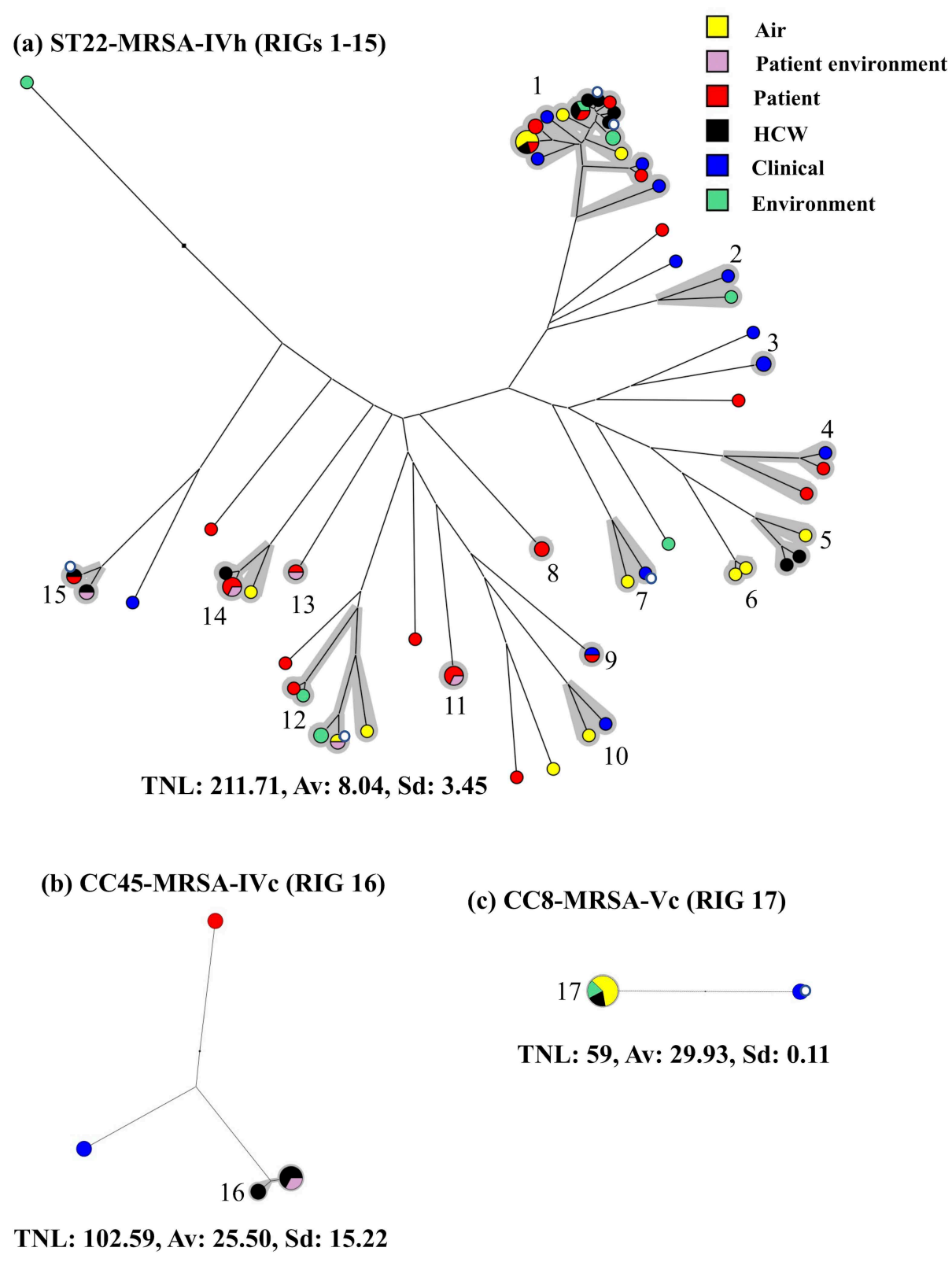
Figure 1. Clonal complexes represented by best-fit UPGMA trees generated by investigating the similarity between core-genome MLST profiles of 110 CC22-MRSA-IVh, CC45-MRSA-IVc and CC8-MRSA-Vc/IVh isolates sequenced. Trees were generated using the categorical differences algorithm and the UPGMA method using the BioNumerics suite of bioinformatics software. Isolates exhibiting ≤24 allelic variations clustered in related isolate groups (RIGs) consisting of isolates from patients (patient volunteer participants and clinical isolates), HCWs and environmental sources. RIGs are indicated by grey shading and annotated by numerals 1-17. Panel (a) shows all 76 ST22-MRSA-IVh isolates recovered of which 65 segregated into RIGs 1-15. Panel (b) shows all six CC45-MRSA-IVc isolates recovered of which four segregated into RIG-16. Panel (c) shows all 11 CC8-MRSA isolates recovered of which 10 oxacillin-susceptible CC8-MRSA-Vc isolates segregated into RIG-17. The node colours represent isolates from different sources as indicated by the legend. The total network length (TNL) comprising the number of allelic differences within each CC is shown beneath each CC, with the average (Av) number of allelic differences and standard deviation (Sd). The associated *n* x *n* matrices generated for each CC group was calculated using BioNumerics and are provided in Table SII. RIGs 1-17 isolates are detailed in Table SI along with study isolates unrelated to all other isolates, which for convenience were assigned to RIG-0 (Table SI). The 110 *mecA*-positive isolates sequenced consisted of 92 MRSA and 18 oxacillin-susceptible MRSA (indicated by small-white circles) (Table SI).

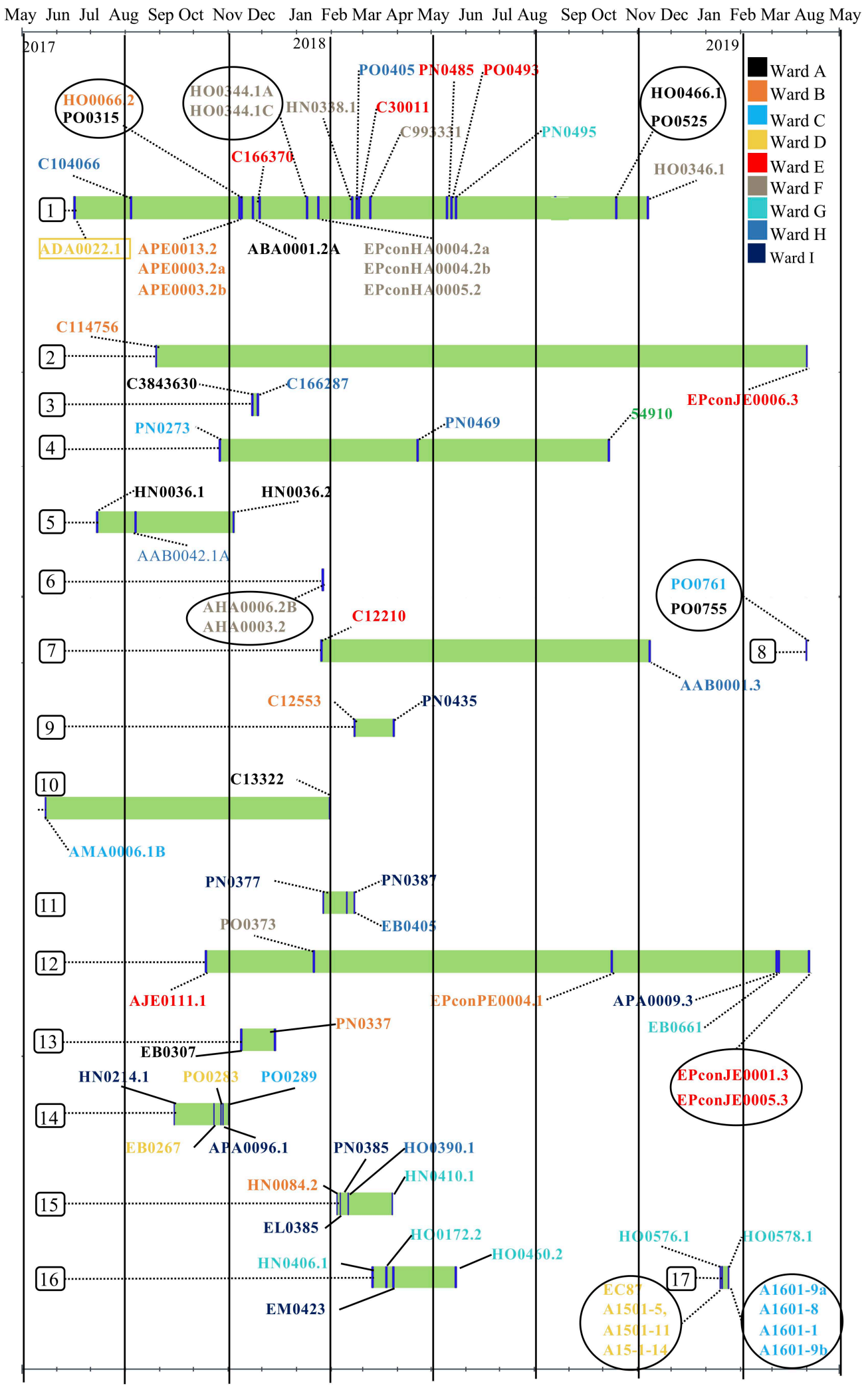
Figure 2. Recovery timeline for 79 MRSA isolates segregated into related isolate groups (RIGs) by cgMLST analysis. Isolates within each RIG exhibited ≤24 allelic variations and were deemed very closely relayed based on previously suggested relatedness thresholds [23,24]. RIGs are numbered 1-17 on the left-hand side of the figure. A green bar indicates the timeline for recovery of the related isolates that comprise each RIG with blue lines indicating the time point an isolate(s) was recovered. Isolates recovered at individual time points are labelled using coloured font indicating different wards as indicated by the legend in the top right corner of the figure. Multiple isolates recovered from the same ward or different wards on the same date are enclosed within a circle. Isolates recovered from HCWs, patients, clinical samples, air and other environmental sites are indicated beginning with a capital H, P, A, C and E, respectively.

580 In relation to HCW isolates, isolates beginning with a HO or HN designation indicate oral
581 or nasal isolates, respectively.

582

583


(a) ST22-MRSA-IVh (RIGs 1-15)
Air
Patient environment
Patient
HCW
Clinical
Environment
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
TNL: 211.71, Av: 8.04, Sd: 3.45
(b) CC45-MRSA-IVc (RIG 16)
16
TNL: 102.59, Av: 25.50, Sd: 15.22
(c) CC8-MRSA-Vc (RIG 17)
17
TNL: 59, Av: 29.93, Sd: 0.11


May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Aug May
2017
2018
2019
Ward A
Ward B
Ward C
Ward D
Ward E
Ward F
Ward G
Ward H
Ward I
HO0066.2
PO0315
HO0344.1A
HO0344.1C
HN0338.1
PO0405
PN0485
PO0493
C30011
C993331
PN0495
HO0466.1
PO0525
HO0346.1
C104066
C166370
1
ADA0022.1
APE0013.2
APE0003.2a
APE0003.2b
ABA0001.2A
EPconHA0004.2a
EPconHA0004.2b
EPconHA0005.2
C114756
2
C3843630
C166287
3
EPconJE0006.3
PN0273
PN0469
S4910
4
HN0036.1
HN0036.2
5
AAB0042.1A
6
AHA0006.2B
AHA0003.2
C12210
PO0761
PO0755
7
8
AAB0001.3
C12553
PN0435
9
C13322
10
AMA0006.1B
PN0377
PN0387
11
EB0405
PO0373
12
AJE0111.1
EPconPE0004.1
APA0009.3
EB0661
PN0337
13
EB0307
EPconJE0001.3
EPconJE0005.3
HN0214.1
PO0283
PO0289
14
EB0267
APA0096.1
PN0385
HO0390.1
HN0410.1
HN0084.2
15
EL0385
HO0172.2
HO0576.1
HO0578.1
HO0460.2
HN0406.1
16
17
EM0423
EC87
A1501-5,
A1501-11
A15-1-14
A1601-9a
A1601-8
A1601-1
A1601-9b