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Investigation of a Cluster of ST22 MRSA Transmission in a Community Setting

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SUMMARY

This study identifies a community cluster of MRSA transmission missed by routine practice and provides genomic evidence for transmission of a typically-nosocomial lineage within community rather than hospital settings. Systematic whole-genome sequencing may increase detection of such outbreaks.

ABSTRACT

Background Whole-genome sequencing (WGS) has typically been used to confirm or refute hospital/ward outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) identified through routine practice. However, appropriately targeted WGS strategies that identify routinely 'undetectable' transmission remains the ultimate aim.

Methods WGS of MRSA isolates sent to a regional microbiological laboratory was performed as part of a 12-month prospective observational study. Phylogenetic analyses identified a genetically-related cluster of E-MRSA15 isolated from patients registered to the same General Practice (GP) surgery. This led to an investigation to identify epidemiological links, find additional cases and determine potential for on-going transmission.

Results We identified 15 MRSA-positive individuals with 27 highly-related MRSA isolates who were linked to the GP surgery, two of whom died with MRSA bacteremia. Of the 13 cases who were further investigated, 11 had attended a leg ulcer/podiatry clinic. Cases lacked epidemiological links to hospitals, suggesting that transmission occurred elsewhere. Environmental and staff screening at the GP surgery did not identify an on-going source of infection.

Conclusions Surveillance in the United Kingdom shows that the proportion of MRSA bacteremias apportioned to hospitals is decreasing, suggesting the need for greater focus on the detection of MRSA outbreaks and transmission in the community. This case study confirms that the typically nosocomial lineage (E-MRSA15) can transmit within community settings. Our study exemplifies the continued importance of WGS in detecting outbreaks

including those which may be missed by routine practice, and suggests that universal WGS of bacteremia isolates may help detect outbreaks in low-surveillance settings.

Keywords:

MRSA, epidemiology, community, general practice, genome sequencing.

BACKGROUND

In the United Kingdom (UK), the emergence of the epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA)-15 lineage (multilocus sequence type (ST) 22) in 1991 was followed by its rapid dissemination throughout UK hospitals and long-term care facilities (LTCFs) [1-4]. This was associated with a dramatic increase in the rate of MRSA bacteremia until rates began to decline in the mid-2000s [5]. Voluntary reporting of MRSA bacteremia in England was replaced by mandatory reporting in 2001 [6], to which mandatory enhanced epidemiological surveillance was added in 2005 [7]. Since 2013, investigation of MRSA bacteremia requires a locally administered Post-Infection Review (PIR), which aims to identify how the case occurred and preventative actions to avoid recurrence. Consequently, responsibility for cases are attributed to the organisation best placed to implement these actions [8].

Currently, only 40% of these reported bacteraemia cases are attributed to a hospital, which suggests that transmission outside of hospitals is a substantial contributor to overall MRSA bacteremia rates [9]. Definitive evidence for community transmission as a driver of MRSA infection in the UK is limited, but is supported by a recent epidemiological and bacterial genomic survey that captured transmission events over a 16,000km² area of the East of England [10]. This argued against conventional wisdom that ST22 is largely healthcare associated in the UK [1,11], and provided evidence for a substantial burden of MRSA transmission outside of hospital settings (i.e. in the community).

Despite this, the focus on MRSA prevention and control remains hospital-centric. Here, we characterise the community-based transmission of EMRSA-15 (typically considered a nosocomial lineage) in a General Practice (GP) surgery. This study argues for a renewed

focus on infection control in community settings and demonstrates the role of bacterial whole-genome sequencing (WGS) in community MRSA surveillance and infection control.

METHODS

Study design

A cluster of 13 MRSA-positive individuals registered to a single GP surgery in Cambridgeshire was first detected during a 12-month prospective study of all MRSA-positive samples processed by the Public Health England (PHE) Clinical Microbiology and Public Health Laboratory, Cambridge University Hospitals NHS Foundation Trust (CUH) in Cambridge, UK. This study has been described in detail elsewhere [10]. In brief, 1,465 individuals were identified with MRSA isolated at least once from either screening swabs and/or clinical specimens, and WGS of 2,282 MRSA isolates from these cases. Combined analysis of WGS data revealed a single large cluster of closely-related MRSA (defined based on a pairwise single nucleotide polymorphism (SNP) distance <50 SNPs) that contained 22 isolates from these 13 individuals. This formed the starting point for a public health investigation and the study described here.

Public health investigation

The detection of the MRSA cluster resulted in an investigation conducted in May 2015 by the local PHE health protection team. The GP surgery had more than 10,000 registered patients and provided specialist services including diabetic and podiatry clinics. The 13 people involved in the MRSA cluster (defined as cases) were sent an information sheet and details of opt-out consent prior to individual GP record review. If consent was not withheld and records were available, data were collected on demographics, co-morbidities and date of first MRSA detection. In the six-months prior to each patient's first recorded positive

MRSA result, healthcare attendance (primary care, hospital outpatient, or in-patient) and microbiological samples that were MRSA-negative were recorded. Incidence rates of MRSA-positive individuals were calculated per 10,000 registered patients at the study surgery. The CUH laboratory information system was used to determine incidence of MRSA positivity based on samples submitted to CUH from four comparable practices within the same region (defined as practices with >10,000 registered patients in the same GP Classification Group) [12]. All data were collected and analysed within the context of the public health investigation.

Staff at the GP surgery were invited to undergo MRSA screening (nose/throat/groin swabs) following attendance at an information session and written consent. Environmental MRSA screening was performed at 40 sampling points in the building. Samples were taken from high-contact equipment and surfaces in the following areas: two randomly selected medical clinic consultation rooms, two nursing clinic rooms (where the ulcer clinic, which was the strongest epidemiological link between patients, was held), and shared patient waiting areas. At each sampling point, an area of approximately 10cm-by-10cm (or entire surface of handles) was swabbed and cultured for MRSA using direct plating onto chromogenic agar [13].

Extended case-finding was performed to identify further cases that might be linked to the cluster over a longer time period, and for whom MRSA isolates had been stored and could be retrieved for sequencing. This involved three different approaches. (i) A retrospective search was performed of the CUH information system for MRSA-positive samples submitted by the GP surgery between January 2006 until June 2015. These data were then cross-referenced with the bacterial archive database to determine if isolates had been stored at -

80°C. (ii) Laboratory surveillance was conducted in the laboratory between November 2015 and February 2016 to detect MRSA-positive individuals from the GP surgery. (iii) Recent PIRs at the GP surgery were reviewed. Isolates were requested from the receiving hospital for WGS and patient records reviewed as described above.

WGS, typing and data analysis

DNA was extracted, libraries prepared, and 150-bp paired end sequences determined on an Illumina HiSeq2000 (original study isolates) or MiSeq (isolates identified through additional case finding). Methods were as previously described [14]. Details of reads, depth of coverage/N50 are provided in Table S1. Sequence data were submitted to the European Nucleotide Archive (www.ebi.ac.uk/ena), accession numbers are also listed in Table S1. STs were assigned using sequence data, an in-house script and the MLST database (<http://saureus.mlst.net/>), and STs were assigned to clonal complexes (CC). Isolates were mapped using SMALT (<http://www.sanger.ac.uk/science/tools/smalt-0>) to the E-MRSA15 reference genome (strain HO 5096 0412, accession HE681097). Mobile genetic elements, indels and regions of high-density SNPs were excluded to identify the phylogenetically informative core genome for each isolate, and SNPs used to create a mid-point rooted, maximum-likelihood phylogeny using RAxML with 100 bootstraps [15]. Trees were visualised using Figtree (<http://tree.bio.ed.ac.uk/software/figtree/>) and ITOL (<http://itol.embl.de/>). *In-silico* PCR of the variable X-region of the *spa* gene was undertaken using the genome data and published primers [16]. Spa-type was then determined using SpaTyper (<http://spatyper.fortinbras.us>).

RESULTS

During the year-long prospective MRSA study of carriage and clinical MRSA isolates in the East of England between April 2012 and April 2013, we identified a number of potential outbreaks based on genomic relatedness and epidemiological links [10]. One potential outbreak consisted of thirteen MRSA-positive individuals (22 isolates) registered with the same general practice (GP) surgery in Cambridgeshire and therefore was of particular interest. All thirteen isolates were ST22 and part of the EMRSA-15 clade (Figure 1). We initiated an investigation to rule out on-going transmission, and to elucidate if this represented community-based transmission or ‘spill-over’ from a hospital/LTCF. Extended case finding identified additional MRSA-positive individuals attending the same GP with samples available for sequencing (Figure 2). First, retrospective review of electronic laboratory records identified four individuals with a total of seven isolates retrievable for sequencing, one of whom (Patient(P)04) had already been identified in the initial 13 cases. Second, prospective surveillance of MRSA-positive samples sent from the GP surgery over three months between November 2015-February 2016 and surveillance of new positive MRSA samples by the infection control team identified three retrievable isolates from three individuals. Third, two PIRs had been undertaken in 2014/2015 (P12/P13). Both patients had died with MRSA bacteremia in another regional hospital. A single isolate from each blood culture was obtained for each patient from the admitting hospital. A summary of the 22 patients (34 isolates) from the original study and additional case-finding is provided in Table 1. The median number of MRSA isolates per patient was 1 (range 1-4). Four patients had only screening samples submitted. Of those clinical samples submitted, 61% were reported as superficial swabs of lower limbs/foot, while three were from blood cultures and one from pus (all from different patients.)

Genetic analysis

Spa-genotyping showed that the cluster was formed of two main *spa* types (t032, t294) with three additional variants (Figure S1). STs were derived from WGS data for the 12 MRSA isolates identified through additional case finding. The predominant ST was ST22 (7 isolates, 3 individuals), the remainder being ST45 (3 isolates, 3 individuals), ST6 (1 isolate) and ST1539 (a single-locus variant of ST221, 1 isolate). The non-ST22 cases were excluded from further analysis. After combining the 22 ST22 isolates from the original study and 7 from additional case-finding to a total of 29 ST22 isolates, a maximum-likelihood tree was constructed based on SNPs in the core genome compared to the EMRSA-15 reference genome. This demonstrated clustering of 27 of the 29 ST22 isolates from 15 individuals (Figure 3), now referred to as cases. Of these, 13 had been identified in the original cluster and the additional two isolates were from two cases (P12 and P13) identified during PIRs of fatal bloodstream infections. The median pairwise SNP distance between the 27 cluster isolates from these 15 cases was 21 (range 0 to 58, interquartile range 10 to 37). The median pairwise SNP distance for cluster isolates from the same person (in 8 cases with more than one isolate) was five (range 0 to 60, interquartile range 1.5 to 15.5). One patient (P04) had cluster isolates that extended over a period of 34 months (a basal isolate in 2012, and three isolates in 2014/15 with pairwise distances of 60, 59 and 57 SNPs from the 2012 isolate).

Public health investigation

To further understand the cluster, a public health investigation was performed to investigate the 15 genomically linked cases, together with screening of staff and the environment for the presence of MRSA. Two of the 15 cases (P14/P15) were excluded from further epidemiological analysis due to missing patient records or refusal of consent. Of the remaining 13 cases, the median age was 80 years (range 12-91, IQ range: 61.5-81.5) at the

time of the investigation, and six cases (46%) were women. Geographical mapping of first MRSA isolation date and place of residence for each case demonstrated that cases lived within 5.6km of each other and two individuals (P10/P11) lived on the same street. No cases lived in the same household or LTCF.

Review of sample requesting information showed a predominance of lower limb swabs (cases with samples including lower limb, 9; screen alone, 1; bacteremia alone, 2). GP medical records revealed that the date of first recorded MRSA positive sample for cases ranged from 2006 to 2015 (Figure 4A). Healthcare contact by each case in the six months prior to first MRSA detection was extensive for all but two patients (Figure 4B). Six of the 13 cases had attended hospital in this period, of whom three cases (P08/P11/P12) had attended only one hospital, two cases (P05/P13) had attended two different hospitals, and one (P10) had attended three different hospitals. Crucially, no overall link could be made between cases and attendance at a hospital (Figure 4A). Six individuals had one or more samples that were negative for MRSA in the six-months prior to their MRSA first detection date, and had no record of hospital attendance in the intervening time. Eleven of the 13 cases had attended the GP leg ulcer clinic. P5 and P11 had not, but P11 lived in the same road as P10.

A total of 57 GP surgery staff (approximately 90% of current clinical/non-clinical employees) received multi-site MRSA screens, all of which were negative. This included four nurses who had worked at the ulcer clinic since the first positive MRSA samples in 2008. Forty environmental samples were taken from communal waiting areas and clinic rooms, all of which were also MRSA-negative.

Given that this cluster was only identified fortuitously by genome sequencing, we sought to determine if the incidence rate in the practice had been higher than that expected. This was achieved by comparing the incidence rate of MRSA-positive samples submitted to the CUH diagnostic microbiology laboratory between 2006-2013 between the study GP surgery and four other practices of a similar size and patient demographic within Cambridgeshire. This showed a fluctuating rate over time for all four practices, with no identifiable outbreak signal for the general practice under investigation (Figure S2).

DISCUSSION

In this study, we found that routine infection control failed to detect or prevent a community cluster involving fifteen people who carried or were infected with ST22 MRSA. This was despite two fatal cases of bacteremia that were investigated using standard public health procedures [17], but were not linked to each other or the cluster until WGS was undertaken. Overall, epidemiological evidence was consistent with onwards MRSA transmission in the community, although the precise circumstances under which this occurred could not be defined. Most patients were high users of primary care services including a GP leg ulcer clinic, although transmission through other unidentified contacts cannot be ruled out.

One case (P04) had a history of testing MRSA positive since December 2008, and WGS on available isolates confirmed carriage of the same MRSA lineage over a period of 34 months. The diversity within the isolates from P04 encompasses that of isolates from all other cases, potentially suggesting that persistent carriage in this case had contributed to spread of this lineage. Due to limited sampling, it is not possible to rule out re-infection but the most recent common ancestor of these isolates would have dated to around 2006 (based on a

SNP rate of ~ 3.5 SNPs/genome/year in ST22 [24]), consistent with carriage since that date. The important contribution of long-term MRSA carriers to transmission in hospitals has been shown previously [18], and is likely to be relevant in other settings. Decolonisation of persistent carriers with chronic wounds such as leg ulcers is notoriously difficult, and rigorous infection control is required during treatment such as dressing changes when bacterial shedding can occur. MRSA was not isolated from staff or the environment at the GP practice during a point-prevalence survey, but this was performed a considerable period of time after the cluster had become established and was undertaken largely to identify modifiable factors.

MRSA ST22 is the most common MRSA lineage associated with healthcare-associated infection in the UK, and based on the higher overall prevalence of MRSA in hospitals versus the community in the last few decades it has been assumed that the predominant directionality of spread is from hospitals into the community. Previous studies conducted in the UK have isolated ST22 from the community [19,20,21], but bacterial typing lacked sufficient resolution to infer transmission. To support this, *spa* genotyping of the cluster isolates in this study was undertaken, and based on the presence of a number of *spa*-types it is unlikely that such typing would have identified this cluster. WGS has been used to confirm that transmission of a PVL-positive, single locus variant of ST22 occurred from a special care baby unit into the community where it subsequently persisted [22,23]. By contrast, the findings of our study suggest that most cases (9 of 13) associated with the MRSA cluster had either not attended a hospital or had at least one intervening sample that was MRSA-negative in the six months prior to first MRSA detection. The majority of cases had links to clinic attendance in the community (in particular for ulcer care), providing

genomic evidence for transmission of this typically-nosocomial lineage within community rather than hospital settings.

A greater focus is needed to detect MRSA transmission in the community if overall MRSA bacteremia rates are to be further reduced. The role of infection prevention and control in the community will become increasingly relevant as initiatives are rolled out that increase delivery of care outside of hospitals [25], and will require a review of the current predominantly hospital-centric structure of infection services [19]. Several methodological approaches could be considered. A low-cost passive surveillance option would count cases from submitting locations over time, associated with a defined threshold above which an investigation is triggered. However, the protracted period over which transmission occurred in the cluster described here meant rates of MRSA over time for the GP surgery were comparable with other similar practices. Consequently, the real-time analysis of epidemiological data from this practice is unlikely to have triggered an outbreak investigation. However, the addition of WGS allowed robust assessment of the relatedness of MRSA isolates and cases, and the implementation of surveillance WGS to control procedures may be a necessary tool if MRSA transmission is to be targeted by rapid interventions.

This study has a number of limitations. We cannot exclude that the outbreak may have been detected through other typing methods not undertaken here, such as pulsed-field gel electrophoresis. We did not undertake sampling for asymptomatic MRSA carriers in the wider community, which is likely to have under-represented the extent of the cluster. Only a small proportion of the MRSA isolated from samples submitted by the GP surgery were available for sequencing, reducing the number of cases that could be included from the

retrospective look-back. The study was not sufficiently powered to conduct a case-case design (cases with MRSA assigned to the cluster versus unrelated MRSA cases) to determine specific risk factors for MRSA acquisition, as comparison between practices was limited due to the variation in services provided. Finally, not all staff who may have been involved in the cluster were screened for MRSA due to staff turnover.

In conclusion, the detection of transmission and outbreaks associated with MRSA ST22 carriage and infection in the community is incomplete. In particular, this case-study demonstrates the need to consider GP surgeries as transmission hotspots. Whilst WGS of all MRSA isolates from GP surgeries may not be cost-effective, this case-study demonstrates how universal WGS of bacteremia isolates can detect relatedness and potential transmission events in settings which are not typically regarded as foci of transmission. Systematic WGS strategies could provide more accurate attribution of source, provide a mechanism for more efficient targeting of infection control, and lead to further reductions in the number of people who become colonised by, and go on to develop MRSA bacteremia.

Ethics approval/consent to participate: Study protocol approval for the prospective study was granted by the National Research Ethics Service (ref: 11/EE/0499), the National Information Governance Board Ethics and Confidentiality Committee (ref: ECC 8-05(h)/2011), and the Cambridge University Hospitals NHS Foundation Trust Research and Development Department (ref: A092428). The 13 people involved in the MRSA cluster were sent an information sheet and details of opt-out consent prior to individual GP record review. All data were collected and analysed within the context of the public health investigation.

Competing interests: NB is on the advisory board for Discuva Ltd. All other authors declare that they have no competing interests.

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Authors' contributions: M.S.T. and E.W. undertook the epidemiological and bioinformatic analyses with contributions from T.W., E.M.H., F.C. Laboratory work was conducted by B. B. The public health investigation was managed by B.N. and N.B.; the investigation team also consisted of M.S.T., E.W., B.S., T.W. and S.J.P. Additional screening was undertaken on-site by B.S. and M.S.T. S.J.P. and J.P. supervised and managed the study. All authors had access to the data and read, contributed, and approved the final manuscript.

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Table

Table 1.

Patient and sample information.

Figure legends

Figure 1.

Maximum likelihood tree generated from SNP sites in the core genome for 1,715 CC22 isolates from the 2012-2013 study. The clade highlighted in grey is the largest cluster (with a maximum SNP cut-off of 50) within the collection, and represents patients registered to the study GP surgery.

Figure 2.

Flow diagram summarising patient identification. One patient was captured by both the community cluster and extended retrospective laboratory record review.

Figure 3.

Phylogenetic analyses of 29 MRSA ST22 isolates from 15 cases linked to a GP surgery. Midpoint rooted maximum likelihood tree based on SNPs in the core genome. Each isolate is labelled as patient (P) study number_isolate number_year of isolation. Circles indicate multiple isolates from the same patient, with each color being unique to a patient. Cases without circles signify those with a single isolate.

Figure 4.

A: Date of first known positive MRSA sample (denoted by black star) for 13 individuals investigated in public health investigation, and the preceding 6-month window (grey boxes)

during which contacts with healthcare for each case were established. Red open circles denote date of genomically confirmed cluster lineage MRSA samples for each individual.

B: Timeline summarising healthcare contact for 13 cases in the 6 months prior to first MRSA positive sample. The timeline for each case does not necessarily overlap and ranges between 2006-2015. Recorded contact with healthcare represented by: Open circle, hospital; Red square, ulcer clinic; Open square, any other GP visit. Black crosses indicate date of negative MRSA sample.

Supplementary files

Table S1.

Sequence accession numbers and sequencing information.

Figure S1.

Phylogenetic analysis of 29 MRSA ST22 isolates from 15 cases linked to a GP surgery. Midpoint rooted maximum likelihood tree based on SNPs in the core genome. Colored bars indicate *spa* genotype: red, t294; blue, t032; yellow, t379; orange, t1302; purple t492; grey, not typable/not done.

Figure S2.

Comparative incidence rate of MRSA for the study GP surgery and four comparable general practices. Bold line represents practice studied. Practices not labelled to maintain organisational anonymity.