Journal of Hospital Infection 103 (2019) 454–460

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Circulation of a community healthcare-associated multiply-resistant meticillin-resistant *Staphylococcus aureus* lineage in South Yorkshire identified by whole genome sequencing

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ARTICLE INFO

Article history:
Received 3 May 2019
Accepted 6 August 2019
Available online 10 August 2019

Keywords:
Meticillin-resistant
Staphylococcus aureus
Meticillin resistance
Whole genome sequencing
Epidemiology
Healthcare-associated

SUMMARY

Background: A cluster of seven cases of skin and wound infections caused by a multiply resistant meticillin-resistant *Staphylococcus aureus* (MRSA) were detected in a small-town community in South Yorkshire. Initial microbiological investigations showed that all isolates belonged to a *spa* type observed rarely in England (t1476).
Aim: To describe the epidemiology of t1476 MRSA in South Yorkshire.
Methods: Retrospective and prospective case ascertainment was promoted through communication with local microbiology laboratories. Public health investigation included a detailed review of clinical notes for a subset of nine cases. Genomic and phylogenetic analysis was undertaken on t1476 MRSA.
Findings: Thirty-two cases of t1476 MRSA infection or colonization were identified between December 2014 and February 2018. Cases were older adults (aged 50–98 years). Healthcare exposures for a subset of nine cases indicated frequent contact with a team of district nurses, with all but one case receiving treatment on the same day as another case prior to their own diagnosis. No cases were admitted to hospital at the time of specimen collection. Despite detailed investigations, no carriers were detected among district nursing staff. A long-term carrier/super-shedder was not found. Phylogenetic analysis indicated that t1476 MRSA cases from South Yorkshire were monophyletic and distant from both MRSA of the same lineage from elsewhere in the UK ($N = 15$) and from publicly available sequences from Tanzania.

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https://doi.org/10.1016/j.jhin.2019.08.006

Conclusion: Genomic and epidemiological analyses indicate community-based transmission of a multiply resistant MRSA clone within South Yorkshire introduced around 2012–2013, prior to the detection of a spatial–temporal cluster associated with a distinct risk group. Surveillance data indicate continued circulation.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) continues to be responsible for a considerable public health burden globally. Although this burden is largely dominated by healthcare-associated (HA) MRSA, the 1990s and 2000s saw the emergence of genotypically and epidemiologically distinct lineages in the community-associated (CA) and livestock-associated (LA) MRSA, leading to increases in rates of MRSA infection across the entire health landscape [1–3].

At a global level, most MRSA infections in humans are associated with healthcare exposure. However, following a raft of initiatives to drive down MRSA rates in England, there have been marked changes in the epidemiology and rates of MRSA such that the majority of cases are now community-onset [4]. These infections may be diagnosed within the community following acquisition of the organism during an exposure related to provision of healthcare, be that in a hospital, general practice, or care received in the home. Just as established transmission of CA-MRSA is observed, through the existence of complex networks of healthcare-related contacts and care provision, HA-MRSA clones such as the EMRSA-15 clone (ST22-IVh) dominant in the UK may also become established in community environments [2,5].

In England, the rise in MRSA rates led to the implementation of mandatory reporting of cases and a raft of infection control measures which collectively proved successful in reducing MRSA rates from >40% to ~6.6% [4]. This reduction in incidence has had a major impact on clonal distribution, with attendant decreases in the healthcare-associated lineages (EMRSA-15 and -16; CC22-IVh and ST36-II, respectively) which have dominated in England since their emergence in the 1980s [6]. Although shifts in clonal distribution are well documented, drivers for epidemic waves are not clearly understood [7]. Mandatory MRSA bacteraemia surveillance has remained in place in England to monitor changes in circulating strains and to detect the emergence of new lineages, including those that may be due to importation from other countries [4,6].

In November 2015, a cluster of seven cases of MRSA skin and wound infections was detected in a small town in South Yorkshire (population ~9000). MRSA isolates from all cases had an identical *spa* type (t1476) and antibiogram, exhibiting non-susceptibility to oxacillin/cefoxitin, ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, and trimethoprim. All cases were older adults (≥70 years), registered at the same general practice, and had received recent care from a pool of district nurses. An incident control team was convened and, due to uncertainty around the size and extent of the cluster, it was decided to undertake further retrospective and prospective case finding. Here we report the investigations undertaken to describe the epidemiology and dissemination of this MRSA clone in South Yorkshire.

Methods

Case definition and case ascertainment

A case was defined as an individual from whom MRSA *spa* type t1476 was identified from a clinical or screening specimen taken between December 1st, 2014 and March 31st, 2018 and processed at a National Health Service (NHS) laboratory in South Yorkshire. In May 2016 retrospective and prospective case finding was undertaken through communication with local microbiology laboratories in South Yorkshire, requesting that all new and existing MRSA cultures with specimen dates after December 1st, 2014 and a matching antibiogram (non-susceptibility to oxacillin/cefoxitin, ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, trimethoprim; susceptible to chloramphenicol, fusidic acid, mupirocin, rifampicin) be sent to the Healthcare Associated Infections and Antimicrobial Resistance Division at Public Health England (PHE) for polymerase chain reaction (PCR) characterization and *spa* typing.

Epidemiological investigation

A spatial–temporal cluster of nine cases resident in a small town with specimen dates between December 2014 and December 2015 was investigated to identify common exposures between cases. Information on community social group attendance, private care providers and meal provision services was collected through a questionnaire completed by cases. In May 2016 a detailed review of hospital admission notes and electronic primary care records was undertaken for these nine cases. Details of prior history of MRSA carriage, indwelling devices, tissue integrity, antibiotic prescriptions, and treatment received from community and trust healthcare workers between December 1st, 2013 and December 10th, 2015 were collected using a study-specific data collection tool. Dates of treatment received, including inpatient admissions, were examined for common exposure periods between cases. Data on treatment by individual healthcare professionals were used to investigate healthcare networks and to inform screening.

Screening of district nursing staff

Organizations providing district nursing care were identified from patient notes. All district nursing staff employed at these

organizations (including bank and night staff) in June 2016 were contacted by occupational health and asked to consent to screening. Swabs were taken from the nose, axilla, hairline, and throat of each staff member on two separate occasions approximately one week apart.

Microbiological characterization

MRSA isolates were characterized by PCR to ascertain their *mecA*, *mecC*, and *luk-PV* status [8]. *Spa* typing was performed as described elsewhere [9]. Whole genome sequencing (WGS) was performed as described previously [8,10]. In brief, genomic DNA was extracted using the Qiagen DNA mini kit (Qiagen, Hilden, Germany) and QIAsymphony instrument (Qiagen). DNA libraries were prepared with the Nextera XT kit (Illumina, Cambridge, UK) and sequenced on the Illumina HiSeq 2500 instrument (Illumina), generating 100 bp paired end sequence fragments. Sequence types (ST) were derived from WGS sequences using MOST [11]. By mapping (bowtie2) WGS reads against reference sequences, a broad range of loci were screened for virulence factors, the immune evasion cluster, arginine catabolic mobile element, heavy metal and biocide resistance genes in addition to genes and chromosomal mutations associated with antimicrobial resistance [12,13]. Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types were deduced based on the detection of the *mec* complex and *ccr* genes by BLAST on assembled genomes generated using Spades.

Phylogenetic analysis

The phylogenetic relationship between isolates was determined at the core genome level by single nucleotide polymorphism (SNP) analysis using an in-house pipeline (phenix; https://github.com/phe-bioinformatics/PHEnix). Sequence reads were mapped to an ST8 MRSA reference sequence (NC_007795) using BWA software and SNPs were called and filtered using GATK2 (ad_ratio: 0.9; min_depth: 10; qual_score: 40; mq_score: 30; mq0_ratio: 0.1). Filtered SNPs were concatenated; only core genome SNPs were considered. Sites due to probable recombination were excluded using Gubbins. The resulting alignment was used to estimate the evolutionary rates and time to the most recent ancestor (tMRCA) using GTR substitution models (BEAST v1.8.4) with uniform rate variation among sites and under relaxed clock rate using the sample timeframe 2013–2018. The best tree was drawn using ITOL application [14]. Previously sequenced lineage-matched (ST and *spa* type) MRSA from (i) the PHE database ($N = 15$) and (ii) the human sources in Tanzania ($N = 6$) available in the public domain were included as comparators [15]. Sequencing data have been deposited in the European Nucleotide Archive under project number PRJEB31479.

Results

Descriptive epidemiology

A total of 32 cases of *spa* type t1476 MRSA infection or colonization were identified from specimens taken during a four-year period between December 2014 and February 2018. Cases resided in four neighbouring local authority areas (Figure 1) and were older adults (median age: 86 years; range: 50–98), predominantly female (63%, 20/32). There were 21 infections (66%): most (90%, 19/21) were skin or wound infections, two were invasive (bacteraemia). Nine cases were identified as part of routine MRSA pre-admission screening for MRSA; one case was diagnosed from an unspecified swab and another from a sputum sample.

Epidemiological links

The nine cases for which clinical notes were reviewed were diagnosed from wound swab specimens taken between December 2014 and December 2015. All nine were resident in the same local authority (YH1) and none were admitted to hospital at the time of specimen collection. Eight cases were elderly patients with chronic foot or leg ulcers of between eight months and 10 years duration. Five of these cases were diabetic. The final case had a surgical site infection diagnosed two months after wide local excision. All nine cases had been prescribed antibiotics in the 180 days prior to MRSA isolation. Six cases (67%) had been prescribed tetracyclines (67%), six β-lactams (67%), three macrolides (33%) and one glycopeptide (11%). No links were identified between the cases through community social groups, private care providers or meal provision services.

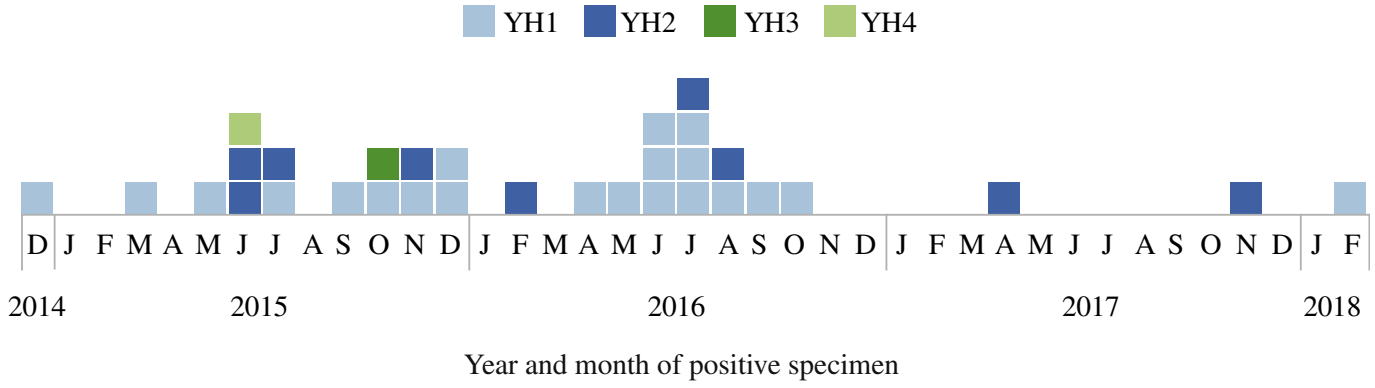

Figure 1. Cases of *Staphylococcus aureus spa* type t1476 infection or colonization, Yorkshire and Humber, December 2014 to February 2018. YH1–4, South Yorkshire local authority areas.

District nursing

All nine cases had received regular wound care from the same group of district nurses prior to identification as part of the t1476 MRSA cluster. Cases received treatment from district nurses on a median of 20 (range: 6–30) different days in the 30 days prior to isolation of t1476 MRSA. Patients received at least one visit per week from district nurses for a median of 15 (range: 3–55) continuous weeks prior to isolation of MRSA. Furthermore, apart from the first recognized of these nine cases, all had received treatment from a district nurse on the same day as another case within the cluster prior to their own diagnosis. There were 68 individual district nurses named in patient notes; 39 of whom had treated more than one case within the initial cluster. Interactions with district nurses were highly complex within the network of nine cases; 18 individual nurses were recognized as having had contact with five or more cases within the cluster and three district nurses had treated all nine of these cases on at least one occasion between December 1st, 2013 and December 10th, 2015. Additional investigation into nursing practices, equipment storage, and usage identified no specific concerns. Three cases had contact with staff from housing and care services for elderly people.

General practice

At the time of identification, all nine cases where notes were reviewed were registered with the same general practice, had received home visits by general practitioners, and had attended the surgery to see practice nurses. There were 31 dates on which ≥2 cases received home visits or visited the general practice; on 29 of these occasions the cases saw different healthcare professionals. No single named healthcare professional from the general practice had contact with all nine cases, with the highest level of contact observed for one general practitioner who treated five cases. However, all nine identified cases can be connected indirectly through contact with healthcare workers over the duration of the cluster investigation.

Screening of district nursing staff

In June 2016, 153 nursing staff working across the local authority (including night teams, rapid response teams, student placements, and bank shift workers) were approached for MRSA screening. Overall, 95% of these ($N = 145$) consented to multisite screening for MRSA. Three members of nursing staff, identified from the review of patient notes, who had treated all nine cases were fully screened. One staff member, with no known contact with the cases, tested positive for an unrelated MRSA strain.

Microbiology and molecular epidemiology

Enhanced case ascertainment led to the identification of a total of 32 t1476 MRSA cases, including the seven associated with the initial cluster. Most were from the YH1 local authority area and the immediately surrounding local authorities YH2, YH3, and YH4 ($N = 21$, 9, 1, and 1, respectively). The majority of cases were identified in 2015 ($N = 12$) and 2016 ($N = 16$) with fewer cases recognized in 2014, 2017, and 2018 ($N = 1$, 2, and 1, respectively).

Bioinformatic analyses showed that all t1476 MRSA belonged to ST8 and encoded SCC*mec* type V. All but one of the 32 MRSA encoded the toxic shock syndrome toxin (*tst*). Enterotoxins J and R were also frequently identified ($N = 26$; 81.3%).

Resistome data correlated with the observed phenotype of non-susceptibility to oxacillin/cefoxitin, erythromycin, clindamycin, gentamicin, trimethoprim doxycycline, and ciprofloxacin. Genes encoding *mecA*, *erm(C)*, *aacA-aphD*, and *dfr(G)* were detected universally; *tet(K)* was frequently encoded ($N = 30$; 94%) likely indicating loss of this mobile genetic element in two isolates on storage. Chromosomal mutational resistance to quinolones (*grlA*/*gyrA*) was universally observed (Figure 2). Genes associated with non-susceptibility to chlorhexidine (*qacC*) and mercury (*merA*/*B*) were also highly conserved in this clade. Loss of *tet(K)* together with *merA*/*B* was observed in two isolates likely indicating plasmid loss.

Phylogenetic analysis indicated that all isolates of MRSA *spa* type t1476 from South Yorkshire belonged to a monophyletic clade (SY clade) with a time to the most recent ancestor estimated to be 5.6 years (95% highest posterior density: 4.4–6.9) (Figure 2). The SNP distance range within the SY clade was 1–49 core genome SNP. By comparison, the cases from other regions of England were more distantly related (55–190 SNPs). Similarly, publicly available genomes from cases in Tanzania where this lineage is frequently reported among MRSA were more clearly phylogenetically distinct (>850 SNPs).

Discussion

This study has provided valuable insights into the established but previously unrecognized circulation of an unusual MDR MRSA clone in South Yorkshire. Detection of this South Yorkshire clade was associated with a cluster within a clear demographic: older adults with frequent contact with healthcare facilities, both in primary care settings and their homes, with clear risk factors for community MRSA colonization including older age, longstanding wounds/ulcers and recent antibiotic usage [16]. Moreover, where clinical notes were reviewed in detail for a spatial–temporal cluster of cases, district nursing contacts provided a plausible mechanism for direct or indirect exposure to the organism and as potential facilitators of onwards transmission. No long-term carriers of t1476 MRSA were detected from district nursing staff screened.

Although the origin of this strain of MRSA in South Yorkshire is uncertain, phylogenetic analysis supports a clonal expansion within the region. The ST8-t1476-SCC*mec*V clone is among the most frequently identified MRSA clones in the Democratic Republic of Congo and Tanzania [15,17,18]. We found no evidence of links to these regions in this study, and phylogenetic analysis of publicly available sequences from this area showed that they were quite distant from the current cluster. During the period of active public health management of the initial cluster, t1476 MRSA was a minority *spa* type in South Yorkshire and continues to be so at the current time. The establishment of national surveillance of MRSA bacteraemia isolates by WGS since April 2017 will afford greater opportunity to monitor for clones and changes in the epidemiology of MRSA nationally.

Following recognition that this MRSA clone was circulating in a relatively small area, a review of exposures for nine cases identified that spread of this clone was likely facilitated through a network of district nurses. As carried out in similar

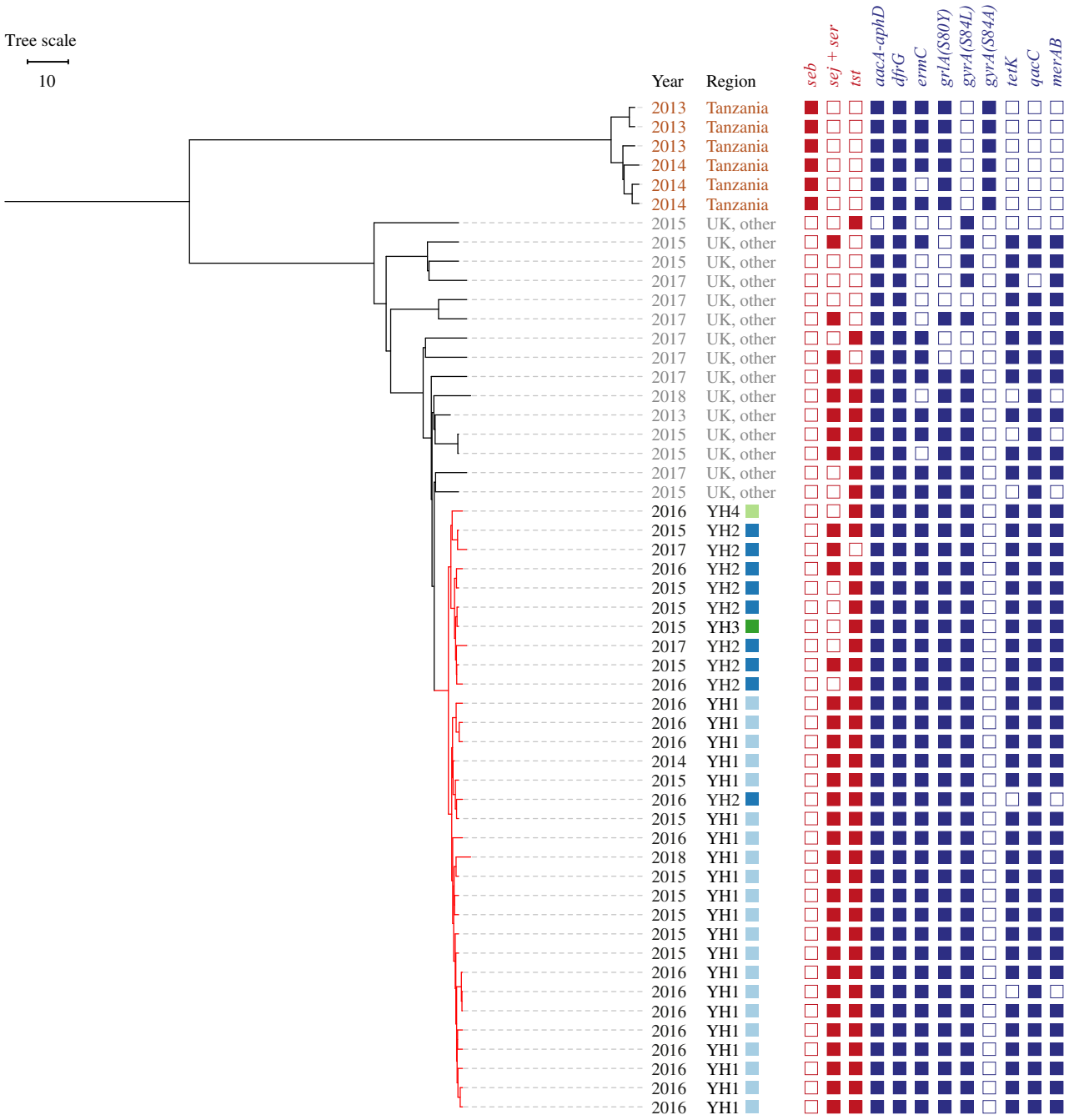

Figure 2. Phylogenetic tree, and profiles of virulence factors and antimicrobial resistance genes of isolates of *Staphylococcus aureus spa* type t1476 infection or colonization, Yorkshire and Humber, December 2014 to February 2018. YH1–4, South Yorkshire local authority areas.

community outbreaks of MRSA in the UK, intensive screening of community healthcare staff was undertaken, including district nursing staff known to have provided care for several cases [19]. Despite this intensive screening of most of the likely facilitators of transmission, no t1476 MRSA carriers were detected. Although, due to the time elapsed before screening was undertaken and staff turnover over this period, we are not able to conclusively rule out the possibility that an unidentified t1476 MRSA carrier(s) existed within the district nursing staff, the phylogenetic evidence suggests the absence of a long-term asymptomatic carrier responsible for multiple transmission events, at least within the context of spread associated with the investigated cluster of cases [20]. As no additional epidemiological information was collected for the 23 cases outside of this cluster, the mechanism for transmission across the wider community remains unknown. As these cases had a broader geographical and temporal dispersion than the initially identified cluster, we consider it unlikely that a single healthcare network could have facilitated transmission across multiple local authorities between 2014 and 2017. A more plausible hypothesis, supported by the phylogenetic analysis, is that t1476 MRSA has been circulating across the area for quite some

time and was only recognized through the detection of the spatial–temporal cluster associated with a defined risk group. Certainly the structure of the phylogenetic tree supports a single introduction sometime in the past, although it is likely that the nature of any putative founding event will never be known.

Undoubtedly, not all t1476 MRSA cases in South Yorkshire will have been detected as part of this investigation. Voluntarily submitted laboratory data over the course of this study from South Yorkshire indicate that an additional 53 MRSA isolates were identified with the same antibiogram as the SY clade, most of which occurred after July 2016 (43, 81%), suggesting that the apparent decline in cases observed in this study likely represents an under-ascertainment bias rather than a true reduction in prevalence. However, the true prevalence of t1476 carriage in the population is unknown. No patient population-level screening was undertaken in this investigation, and case ascertainment is restricted to isolates taken from clinically significant infections and routine MRSA admission screens. Additionally, there is variation in laboratory testing practices across South Yorkshire, and not all laboratories routinely test MRSA isolates against all of the antibiotics included in the antibiogram we used to identify potential t1476 cases. Isolates sent to these laboratories will not be identifiable through a matching antibiogram and therefore will not have been sent for *spa* typing and PCR characterization.

Despite increased use of antibiotics in the community, it is generally considered that MDR MRSA emerged in, and has been largely restricted to, healthcare settings. CA-MRSA have traditionally been regarded as more susceptible than their HA-MRSA counterparts. However, MDR CA-MRSA clones are increasingly recognized [21,22]. MRSA with larger SCC*mec* elements tend to be less successful compared with community lineages which frequently encode smaller SCC*mec* elements such as IV and V [7,23]. The emergence and dissemination of MDR MRSA is a public health concern, particularly were such lineages to become successful in the community and across the healthcare sector in England, limiting the choice of therapeutic agents. In this study, MRSA were predominantly identified within a vulnerable population/age group at known risk of acquiring MRSA. Fortunately, this strain does not appear to demonstrate increased virulence; only one infection was associated with death, and that individual was elderly with underlying severe comorbidities.

Control measures as part of the public health response to this incident included face-to-face enhanced infection prevention control and MRSA awareness training for district nursing staff. Such measures may have contributed to the decline in the number of confirmed cases, although it is impossible to untangle this effect from stochastic fluctuations in transmission/acquisition rates and case ascertainment biases prompted by increased awareness in response to the active public health investigation. Increased awareness of the risks of MRSA and other MDR organism transmission in community healthcare settings is required to maintain high levels of standard infection prevention control practices. Nonetheless, it appears that this MRSA clade continues to circulate in South Yorkshire; certainly, MRSA with the same antibiogram continue to be isolated sporadically. Due to variation in the range of antimicrobials against which local NHS laboratories screen MRSA isolates, routinely collected surveillance data for antimicrobial resistance does not allow for the prevalence of t1476-ST8-V relative to other MRSA strains to be accurately determined within the region or nationally.

Acknowledgements

We would like to thank all members of the Outbreak Control Team for their invaluable contributions to this investigation. We would also like to thank colleagues from NHS microbiology laboratories for submission of isolates for analysis and members of the Public Health England Field Service Yorkshire and Humber for advice and guidance during this investigation.

Conflict of interest statement
None declared.

Funding sources
None.

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