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Leader

Real-time whole genome sequencing to control a *Streptococcus pyogenes* outbreak at a national orthopaedic hospital

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SUMMARY

Background: Whole genome sequencing (WGS) of *Streptococcus pyogenes* linked to invasive disease has been used to identify and investigate outbreaks. The clinical application of WGS in real-time for outbreak control is seldom employed.
Aims: A fatal case of bacteraemia at a national orthopaedic hospital prompted an outbreak investigation to identify carriers and halt transmission using real-time WGS.
Methods: Retrospective surveillance was conducted to identify patients with *Streptococcus pyogenes* infections in the last year. Upon contact tracing, four patients and 179 staff were screened for *Streptococcus pyogenes* carriage. All isolates identified were *emm*-typed. WGS was performed in real-time on a subset of isolates.
Findings: Twelve isolates of *Streptococcus pyogenes* from the index case, two patients and eight staff were identified. Six isolates were *emm* 1.0, including the index case and five staff isolates. The remaining isolates belonged to distinct *emm* types. WGS analysis was undertaken on the six *emm* 1.0 isolates. Five were indistinguishable by single nucleotide polymorphism (SNP) analysis, with 0 SNP distance, and one had one SNP difference, supporting the hypothesis of recent local transmission. All screen-positive healthcare workers were offered treatment with penicillin or clindamycin. No further cases were identified.
Conclusion: The increased molecular discrimination of WGS confirmed the clustering of these cases and the outbreak was contained. This demonstrates the clinical utility of WGS in managing outbreaks of invasive *Streptococcus pyogenes* in real-time and we recommend its implementation as a routine clinical service.

Introduction

Streptococcus pyogenes (Lancefield Group A streptococcus; GAS) is a potentially lethal bacterium with many clinical manifestations, ranging from tonsillitis to toxic shock syndrome

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and necrotizing fasciitis. The current global burden of serious GAS disease (including acute and chronic GAS-associated conditions) is estimated at 18.1 million cases with an annual incidence of 1.78 million cases worldwide and 3.33 per 100,000 population in the UK [1,2].

Outbreaks of invasive GAS (iGAS) have been reported in hospital settings, especially in surgical, maternity, and long-term care facilities [3]. Historically, outbreaks have been defined by conventional descriptive epidemiological methods; with two or more cases of GAS related by place or person occurring within a year of one another and sharing the same molecular lineage, determined by sequencing the GAS M protein gene, the *emm* gene. However, the methodology is unable to further differentiate strains of the same *emm* subtype. To support medical interventions, whole genome sequencing (WGS) of GAS isolates linked to invasive or severe disease is being used more frequently to identify and investigate outbreaks, allowing differentiation of strains recovered from cases, household contacts, and healthcare workers in the context of epidemiologically and geographically distinct isolates [4—7]. However, there are few reports of the clinical application of this technique in real-time to control an outbreak.

We describe an outbreak of iGAS infection linked to a fatal case at a UK specialist orthopaedic hospital and the application of WGS in real-time to contain the outbreak.

Case report

A 69-year-old female with neurofibromatosis was admitted to hospital on the day prior to the elective excision of two nerve sheath tumours of the left sciatic nerve. Following an uneventful procedure, she was transferred to a single room on the ward. Fourteen hours later the wound began to ooze with pain surrounding the incision and she developed severe abdominal pain associated with diarrhoea. She deteriorated further with hypotension, hypoxia and lactic acidosis, requiring transfer to the intensive care unit (ICU) 27 h postoperatively with suspected cardiogenic shock, secondary to pulmonary embolism, ischaemic bowel, and refractory sepsis. She responded poorly to broad-spectrum antibiotics, ventilatory and inotropic support, and died from cardiac arrest 41 h postoperatively. Blood cultures were positive with Gram-positive cocci in chains several hours after death and *Streptococcus pyogenes* was identified on culture 24 h later.

Outbreak meeting

On notification of death of the patient from iGAS, an outbreak meeting was convened within 48 h of fatality consisting of the infection control team, director of infection prevention and control, director of nursing, the operating surgeon, local public health representatives, occupational health practitioner, cleaning manager, and communications team. The priorities of the team were to conduct retrospective and enhanced prospective surveillance; initiate environmental control measures; identify potentially exposed patients and staff to arrange screening, and offer prophylaxis or treatment where appropriate.

Retrospective surveillance

Six patients with GAS infections over the preceding 12 months were identified from the hospital pathology database. Four patients were not linked epidemiologically to the index case and were not examined further. Two patients had been inpatients on the same ward as the index case, patient 1 and patient 2. Patient 1 had suffered from an episode of GAS bacteraemia at another hospital secondary to a peripherally inserted central catheter line infection seven months prior to the index case's admission at this hospital and had been diagnosed with an iGAS infection of the shoulder at this hospital five months prior to the index case's admission. She had received multiple prolonged courses of antimicrobial therapy and GAS had not subsequently been isolated. Patient 1 had been an inpatient for three months in the same single room into which the index case was later admitted following patient 1's discharge. The single room, furniture, and equipment were cleaned with detergent and water followed by hypochlorite 1000 ppm between patient 1's discharge the index case's admission. However, this raised the concern of a transmission event of GAS from patient 1 to the index case. Patient 2 had been diagnosed with an iGAS infection of the hip two months prior to the index case's admission. He had been receiving antimicrobial treatment since diagnosis and GAS had not been subsequently isolated. He was a current inpatient on the same ward as the index case in an open bay.

Patients 1 and 2 were isolated using standard contact precautions according to national guidelines, with the use of a single room with appropriate isolation poster for guidance if they were considered clinically safe, and with the use of personal protective equipment by healthcare workers including disposable gloves and aprons when in contact with the patient, their equipment or immediate surroundings and adherence to strict hand hygiene; washing with soap and water or decontamination with alcohol hand rub before and after contact with the patient or their environment [8].

Screening

Four patients and 45 clinical members of staff, including staff from the ward, ICU, theatre and surgical team members, who had had direct contact with the index case, were screened initially with throat, wound, or skin lesion swabs as indicated for culture.

None of the four patients screened was colonized with GAS. Eight of the 45 (17.8%) staff who were screened were found to have oropharyngeal GAS colonization only; all isolates were susceptible to penicillin and clindamycin (Table I). Four of these eight staff contacts were from the same ward as the index case, three were members of the intensive care team and one was the operating surgeon. Due to the high GAS colonization rate, screening was extended to cover all 134 staff in these areas. No further GAS colonization was identified on extended screening.

Management of staff

Two members of staff who were not confirmed as colonized with GAS but had undertaken intubation were offered prophylaxis with penicillin V or clindamycin in case of penicillin allergy, for three days according to current guidelines [8]. Six

Table I
Outbreak isolates for *emm* typing

Case	Genome accession ERS number	Sample date	Type	Pen	Cli	Ery	Tet	Van	*emm* type
Index patient	ERS2868880	24/06/2017	Blood	S	S	S	S	S	1.0
Patient 1	N/A	26/01/2017	Tissue	S	R	R	R	S	77.0
Patient 1	N/A	25/11/2016	Blood[a]	S	R	—	—	—	89.0
Patient 2	N/A	26/04/2017	Fluid	S	S	S	S	S	12.37
Ward nurse	ERS2868879	28/06/2017	Throat swab	S	S	S	S	S	1.0
Ward nurse	ERS2868869	29/06/2017	Throat swab	S	S	S	S	S	1.0
Ward nurse	N/A	29/06/2017	Throat swab	S	S	S	R	S	5.100
Ward student nurse	N/A	28/06/2017	Throat swab	S	S	S	S	S	12.0
ICU/HDU nurse	ERS2868885	29/06/2017	Throat swab	S	S	S	S	S	1.0
ICU/HDU nurse	ERS2868863	29/06/2017	Throat swab	S	S	S	S	S	1.0
ICU/HDU nurse	N/A	29/06/2017	Throat swab	S	S	S	S	S	252.0
Surgeon	ERS2868875	30/06/2017	Throat swab	S	S	S	S	S	1.0

Pen, penicillin; Cli, clindamycin; Ery, erythromycin; Tet, tetracycline; Van, vancomycin; ICU, intensive care unit; HDU, high dependency unit; S, susceptible; R, resistant.
Genomes were accessioned as detailed in the text; N/A not applicable, the genomes of non-*emm* 1.0 isolates were not sequenced.
[a] Full antimicrobial susceptibilities for blood culture not available.

of the eight (75%) GAS colonized staff reported symptoms of pharyngitis at the time of screening, but none had had any such symptoms at the time of surgery of the index case. All eight GAS-colonized staff were offered treatment with penicillin V or clindamycin for 10 days and were advised to refrain from work until they had completed 24 h of treatment and were asymptomatic [8]. Other staff members were advised to refrain from work if any symptoms developed until their symptoms had resolved.

Follow-up screening of GAS-colonized staff contacts was undertaken at weeks 1, 3, 6, and 12 after completing treatment. All staff showed initial clearance of GAS; however, two ward staff were found to be recolonized at 12 weeks, by which time they had left the organization and were lost to follow-up.

Enhanced prospective surveillance

No further cases or colonization were identified during a period of enhanced prospective surveillance lasting 30 days from the first outbreak meeting.

General measures

The index case's room was closed to new admissions. As a further precaution against new cases, the single room, ward, ICU room, and operating theatre underwent cleaning of the floor, fixtures, and all furniture using detergent and water followed by 0.1% sodium hypochlorite (1000 ppm available chlorine) and steam cleaning. Disposable curtains were changed. In addition, the index case's single room as well as all the common areas of the ward were decontaminated using ultraviolet light. Environmental screening was not conducted.

Methods

Cases and contacts

The outbreak investigation was conducted between June 2016 and July 2017. iGAS was defined as isolation of GAS from a sterile site. Contacts were determined by epidemiological linkage to the index case and screened by throat or wound or skin swabs as indicated.

Bacterial culture and antibiotic susceptibility testing

Swabs were plated on to Columbia Blood Agar and cultured overnight at 37°C in 5% CO_2. Isolates were confirmed as GAS by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (Bruker, Bremen, Germany). Antibiotic susceptibilities were determined by disc diffusion and interpreted in accordance with European Committee on Antimicrobial Susceptibility Testing guidelines (http://www.eucast.org).

Emm typing

The Public Health England (PHE) National Streptococcal Reference Laboratory (National Infection Services) performed *emm* gene sequence typing on referred isolates obtained as previously described using a crude DNA extract for polymerase chain reaction (PCR) and Sanger sequencing [9,10]. In brief, the *emm* types were determined according to the protocol and guidelines available on the CDC website (https://www.cdc.gov/streplab/protocol-emm-type.html). When PCR amplicons obtained using the Centers for Disease Control and Prevention-recommended primers generated ambiguous sequence, alternative primers (MF1, 5′-ATAAGGAGCATAAAAATGGCT-3′; and MR1, 5′-AGCTTAGTTTTCTTCTTTGCG-3′) (Sigma–Aldrich, St Louis, MO, USA) were used for the amplification of the *emm* gene.

Genomic sequencing

Sequencing and trimming were undertaken as previously described [11]. Reads were mapped to reference strain NC_018936 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000307535.1/) using bwa (version 0.7.12) [12]. Variants were called using GATK 2.6.5 [13]. Variants were parsed to retain high-quality SNPs (conditions: depth of coverage ≥5, AD ratio

(ratio between variant base and alternative bases) ≥0.8, Mapping Quality ≥30, ratio of reads with MQ0 to total number of reads ≤0.05). Positions that fulfilled the filtering criteria in >0.9 of the samples were joined to produce a multiple fasta format file where the sequence for each strain consists of the concatenated variants. The FASTQ files for the isolates described in this study were submitted to the European Nucleotide Archive (ENA) study number ID PRJEB29459. Areas of recombination were identified and removed using Gubbins (version 2.0.0), and distances were calculated using the reduced fasta produced [14]. The phylogenetic tree was generated using RAxML (version 8.1.17) and visualized using ggtree [15,16].

Results

Emm typing

Eleven GAS isolates from the hospital were referred to the reference laboratory for typing; one from the index case, eight from colonized staff members, and two from epidemiologically linked patients 1 and 2. Additionally, the historical iGAS bacteraemia isolate from patient 1 from a different hospital had also been sent to the reference laboratory, making a total of 12 isolates available for typing (Table I).

Six isolates were *emm* 1.0, including the index case and five healthcare workers, including the operating surgeon. All these healthcare workers reported pharyngitis following surgery on the index patient, including two staff members who were subsequently recolonized. Interestingly, patient 1 had been infected with two different *emm*-type iGAS isolates within the space of two months, one isolated from blood culture on entry to the emergency department at a local hospital and the second from a shoulder wound; both were distinct from the index isolate (Table I).

Whole genome sequencing

Whole genome sequencing was undertaken on the six *emm* 1.0 isolates and compared with WGS data from 18 *emm* 1.0 contemporaneous sporadic iGAS isolates from the same geographical region one week after the *emm* typing results were available and while screening of staff members was still being conducted. Phylogenetic analysis revealed that the six epidemiologically linked *emm* 1.0 isolates co-located in one phylogenetic cluster, separate from the remaining sporadic isolates. Five isolates were indistinguishable by genomic SNP analysis, with 0 SNP distance, and one isolate had one SNP difference. Thus, zero to one SNP were identified for the isolates referred from the cluster under examination and an average distance of 48 SNPs was observed between the sporadic contemporaneous isolates (Figure 1).

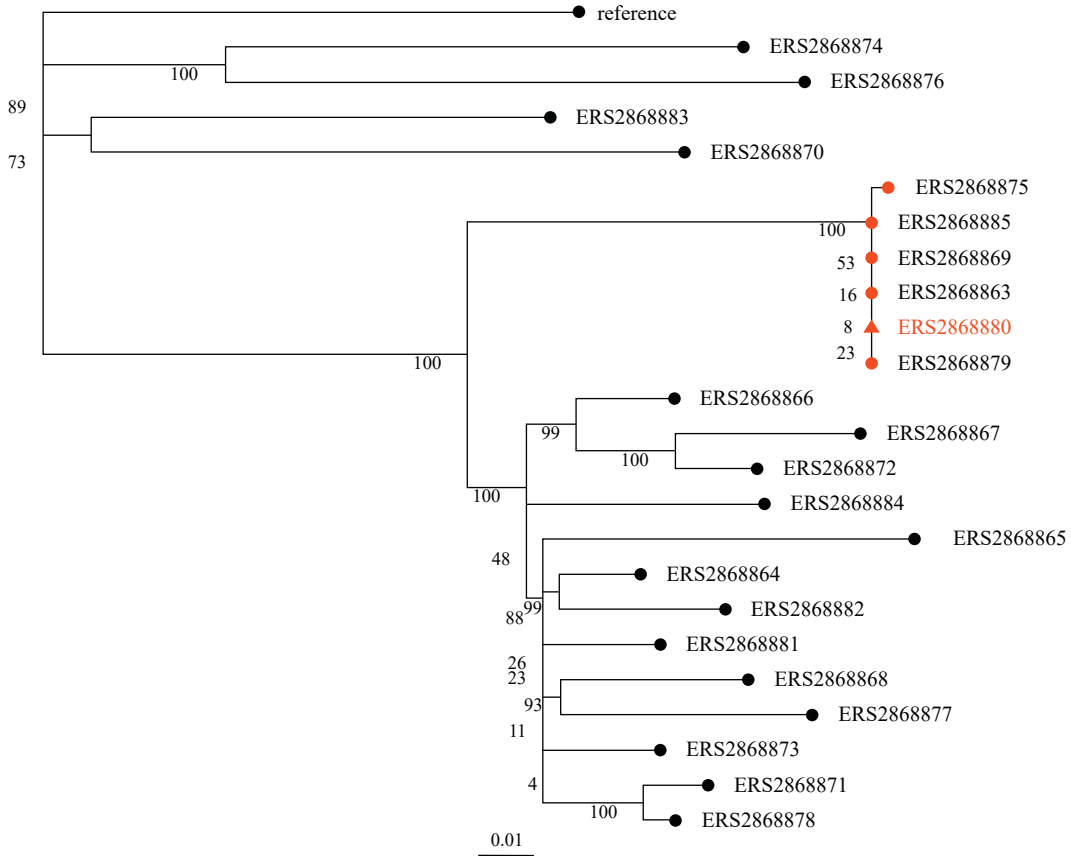

Figure 1. Phylogenetic tree of incident and contemporaneous isolates. Red indicates isolates associated with the index case and healthcare workers; index isolate is indicated with a red triangle. Black circles indicate other *emm* 1.0 contemporaneous sporadic iGAS isolates from the same geographical region.

Antimicrobial susceptibility

All *emm* 1.0 isolates from the investigation site referred to PHE had the same susceptibility profile and were susceptibility to penicillin, clindamycin, erythromycin, tetracycline, and vancomycin. One isolate was resistant to erythromycin and tetracycline and two isolates were resistant to clindamycin (Table I).

Discussion

We describe a rapidly fatal case of iGAS *emm* 1.0 in a patient that occurred postoperatively. GAS isolates of the same *emm* type were isolated from five healthcare workers including one surgeon. WGS data available during the outbreak investigation demonstrated the close clustering and low SNP variation among the outbreak isolates and supported the hypothesis of recent local transmission, and a common exposure could not be excluded. However, the directionality of infection could not be inferred, limited by low potential transmission and exposure period.

Healthcare-associated GAS infection is defined as a GAS infection that is neither present nor incubating at the time of admission, but is considered to have been acquired following admission to hospital, or as a result of healthcare interventions [8]. In 2017/18 *Streptococcus* spp. caused just under 5% of all types of surgical site infections reported to PHE [17].

GAS is transmitted by inhalation or contact with large droplet secretions. In hospitals, healthcare workers, other patients, contaminated equipment, or the environment can be sources of the infection. Thus, in any healthcare-associated GAS infection it is vital to screen, treat, and offer prophylaxis to patients and healthcare workers to prevent the spread of infection.

A number of postoperative outbreaks of iGAS, most commonly associated with wound infections, have been reported and linked epidemiologically to the operating surgical team with documented colonization of their hands, oropharynx, or rectum [18—20]. In this outbreak, a wound swab from the operative incision following surgery from the index case was not sent for microbiological culture. The patient in the room before the index case (patient 1) was infected five and seven months before the index case with two iGAS strains of different *emm* types (89.0 and 77.0) to the index case. Currently, there are no recommendations that patients with a previously diagnosed iGAS infection should be screened and isolated for GAS carriage [8].

There has been an increased incidence of GAS over the past three decades and PHE now conducts active surveillance of scarlet fever and iGAS infection. In 2017/18 the number of laboratory notifications of iGAS was almost 73% higher than average for the previous five years and remains elevated in the current 2018/19 season [21,22]. Upsurges in iGAS are usually associated with the emergence of new virulent strains [23,24]. GAS *emm* type 1.0 is the most common strain of iGAS currently circulating in the UK and accounted for 24% of all iGAS typed between January and December 2017 and 22% between January and February 2019 [21,22]. GAS *emm* 1.0 strains are associated with severe infections, necrotizing fasciitis, streptococcal toxic shock syndrome and a high mortality rate due to the carriage of virulence genes such as *sdaD2*, *speA2*, *nga*, and *slo* that encode streptodornase, the superantigen speA, NAD^+-glycohydrolase, and streptolysin O [25—27]. The finding of GAS *emm* type 1.0 bacteraemia in this patient was in keeping with the rapid fatality of her illness. Indeed, the five other healthcare workers that were colonized with the same strain as the index case all suffered with pharyngitis, illustrating the pathogenicity and virulence of *emm* type 1.0 isolates. It is important to note that staff reported pharyngitis after the death of the patient and were off duty while symptomatic. This highlights that detection and action regarding outbreaks with this *emm* type is vital to prevent secondary cases.

In this outbreak the timely intervention of the outbreak management team may have halted the development of secondary cases. By following published guidelines for the control of GAS in healthcare settings on notification of the positive culture, a retrospective investigation of all iGAS infections over the past year was conducted and patients and staff who had contact with the index case were screened [8]. This led to the identification of colonized and exposed healthcare workers who were offered timely treatment or prophylaxis and retested to ensure eradication of carriage and prevent further spread. No further cases were detected on enhanced prospective surveillance.

There are only a few reports of the use of WGS in the management of GAS outbreaks and the majority of these clusters were small, although some indicated multiple healthcare worker or environmental involvement [3—5,7,28]. Thus, comparable WGS data on GAS that may indicate transmission events, transmissibility, or virulence in outbreak settings are lacking, as is WGS data on community carriage isolates. Such information may give early indications in the evolution of both community and healthcare outbreaks for early intervention measures. Indeed, a recent retrospective analysis of 93 referred clinical GAS isolates by WGS from a single region in the UK revealed both highly diverse and closely related isolates, with clusters of *emm* types 1.0 and 3.1 with no obvious epidemiological linkage on clinical analysis, suggesting cryptic community transmission [26]. Conversely, the genomic discrimination provided by WGS can sanction the inclusion or exclusion of epidemiologically linked isolates [3].

The increased discrimination provided by WGS in this outbreak helped to delineate the cluster involved and to establish the timing of the transmission event and differentiate this cluster from other concurrent isolates. The limitation of WGS in this setting, however, is causality, due to the short time-frame of this outbreak and the small numbers involved. In addition, screening for GAS is rarely undertaken outside of an outbreak setting, making pathways of transmission often difficult to determine. Nevertheless, the versatility of WGS and the abundance of data provided in such settings are far superior to that provided by conventional *emm*-typing alone, especially for commonly identified types by providing finer typing resolution. WGS-based *emm*-typing for GAS has shown good correlation with conventional typing methods, so we recommend implementation as a routine service [6].

In conclusion, this case provides a demonstration of the successful containment of an outbreak of iGAS using WGS to aid epidemiological investigations, emphasizing the robust clinical relevance and applicability of this technology that should be implemented as a routine service. It also reminds us of the severity of this infection and the continuous need for

maintaining high standards of infection prevention and control with ongoing surveillance.

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None declared.

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