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Whole-genome sequencing in the investigation of recurrent invasive group A streptococcus outbreaks in a maternity unit

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SUMMARY

Background: The clinical manifestations of group A streptococcus (GAS) (*Streptococcus pyogenes*) are diverse, ranging from asymptomatic colonization to devastating invasive disease. Maternity-related clusters of invasive GAS (iGAS) infection are complex to investigate and control, especially if recurrent.

Aim: To investigate three episodes of *emm* 75 GAS/iGAS infection in maternity patients at one hospital site over a four-year period (two with monophyletic ancestry).

Methods: The episodes are described, together with whole-genome sequence (WGS) isolate analyses. Single nucleotide polymorphism differences were compared with contemporaneous *emm* 75 genomes.

Findings: Over the four-year study period, seven mothers had *emm* 75 GAS/iGAS and one mother had *emm* 3 iGAS (in year 4) (subsequently discounted as linked). Three (clinical/screening samples) of the seven babies of *emm*-75-positive mothers and three screened healthcare workers were positive for *emm* 75 GAS. WGS similarity suggested a shared ancestral lineage and a common source transmission, but directionality of transmission cannot be inferred. However, the findings indicate that persistence of a particular clone in a given setting may be long term.

Conclusions: Occupational health procedures were enhanced, staff were screened, and antibiotic therapy was provided to GAS-positive staff and patients. The definitive source of infection could not be identified, although staff–patient transmission was the most likely route. The pattern of clonal GAS transmission over the four-year study period suggests that long-term persistence of GAS may have occurred.

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Introduction

Group A streptococcal infections have a spectrum of clinical manifestations (asymptomatic colonization to severe invasive infections) and occur sporadically or in clusters. The incidence of invasive infection ranges from one to three cases per 100,000 population, with a case fatality of approximately 17% [1,2]. Transmission is mostly via respiratory droplets/discharges or infected skin lesions [3]. Most group A streptococcus (GAS) isolates are from the throat, but skin, vaginal, anal and perineal carriage have been noted; asymptomatic throat carriage accounts for 5–12% of isolates [4–6].

Onward transmission can be reduced by robust infection prevention and control, prompt treatment of infection in cases, and decolonization through chemoprophylaxis in prolonged close contacts and colonized mother–neonate contacts. Wider chemoprophylaxis may be appropriate in some outbreaks (e.g. closed communities).

After a hospital-acquired case of invasive GAS (iGAS), the probability of a second case in the same hospital is approximately 40% within 12 months, and 9.3% of hospital-associated GAS infections are linked to others [1].

There is a particular association between iGAS and the peripartum/postpartum period [1,7]. The age-specific attack rate for GAS in the peripartum/puerperal period is 20 times that of non-pregnant females [8]. In 2003–2004, 2–11% of all severe GAS infections were associated with childbirth. Likewise, between 1997 and 1999, puerperal GAS constituted 2% of all iGAS infections in the USA/Canada [5,7]. Pregnancy-associated iGAS occurs mainly in the peripartum, but has been reported during pregnancy and the later postpartum period. Peripartum iGAS carries a substantial maternal morbidity/mortality burden (25%); the risk to the baby is greatest in antenatal iGAS, but still poses a substantial hazard to the newborn [9].

GAS strains can be distinguished by *emm* gene sequence analysis. In England between September 2015 and March 2016, the most common *emm* types associated with iGAS were *emm* 1 (30%), 12 (12%) and 89 (11%) [10]. Puerperal sepsis is fortunately rare. Indeed, few reports of outbreaks in maternity settings have been published in recent years, but they may remain unrecognised. Outbreaks have been associated with infected healthcare workers (HCWs) and shared bathroom facilities [11–13].

Two clusters and an isolated case of infection with *emm* 75 GAS were noted over a four-year period in an acute hospital maternity unit providing in/outpatient and community maternity services. (These are hereafter named as the 'initial cluster', the 'isolated case two years later' and the 'last cluster four years after the initial cluster'). This article reports the investigations into the source, the control measures instigated and the application of whole-genome sequencing (WGS).

Methods

Outbreak control teams were established for each episode to investigate the potential source and implement control measures.

Case definitions

- Confirmed – a maternity staff member/associated HCW working at the unit, or a maternity inpatient or baby born at the hospital in the four-year period when cases occurred, with a confirmed isolate of *emm* 75 GAS, with either clinical infection or asymptomatic colonization.
- Probable – a maternity staff member/associated HCW working at the unit, or a maternity inpatient or baby born at the hospital in the four-year period when cases occurred, who had a suspected GAS infection and a GAS isolate identified, or colonized with a GAS isolate but with a non-*emm* 75 strain or no typing data available. The initial probable case definition was kept broad to include any *emm* typing positive isolate to maximize case capture until the situation was clear. However, any non-*emm* 75 clone isolates/cases were subsequently discounted as being part of the outbreak.
- Possible – a HCW from, or inpatient admitted to, another part of the hospital with suspected or confirmed GAS infection of any type/strain in the hospital in the four-year period when cases occurred, with suspected GAS infection but without microbiological confirmation or typing data/matching *emm* type.

Incidents

Over the four-year period, seven mothers, three neonates and three HCWs who were all confirmed, clinical cases were reported with *emm* 75 GAS/iGAS infection. One other mother from the last cluster had *emm* 3 and was subsequently discounted as being part of the outbreak. Six mothers had iGAS and one mother (the index case) had GAS infection. One baby and three staff members had non-invasive disease, and two babies were colonized but not infected. There were no deaths. The mothers (aged 22–39 years) received all their maternity care from the hospital; their babies were 1–44 days old at diagnosis. No neonatal cases were delivered by caesarean section. All mothers were well at admission for delivery, although one had an elevated white blood cell count. Three had recognized risk factors other than pregnancy.

In the initial cluster, the index case re-presented to hospital with postnatal complications; GAS was identified from vaginal sampling. Over the next five days, three maternal iGAS cases occurred. One baby of these mothers developed a GAS skin infection within 10 days of the presentation of the index case, another was found to be colonized, and the baby of the index case was found to have a positive throat swab seven days after presentation of the index case. All cases in this initial cluster had *emm* 75 isolates. A patient with puerperal sepsis also presented 10 days after the index case, but no microbiological isolates were available for that case.

A single, maternal, confirmed case of *emm* 75 iGAS was identified two years after the initial cluster, followed by two confirmed maternal cases of *emm* 75 iGAS four years later in the final cluster (symptomatic within six days of each other), and a single maternal *emm* 3 case who became symptomatic 20 days after the first case in this last cluster. This latter case was subsequently discounted as being part of the outbreak. There were no neonatal cases associated with the isolated case that occurred two years after the initial cluster or in the last cluster.

Investigations and control measures undertaken

Cases and their close contacts were treated as per standard guidelines. Staff screening sampling results were obtained.

GAS-positive staff were excluded from work, treated and rescreened for clearance. Wider staff prophylaxis was not employed.

For the last cluster, risk factors and symptoms, microbiological results and patient–staff interactions (dates/locations) were obtained. Ward movements were examined for staff/case overlaps and mapped against staff sickness absence records. Genome sequencing and *emm* sequence typing were undertaken. Environmental sampling was undertaken on the delivery suite after a deep clean had occurred and a month later (specific sampling site details are not available).

Microbiological investigation

emm gene typing method

The *emm* types were determined using the Centers for Disease Control protocol (Table I). When sequence data obtained were ambiguous, alternative primers (MF1, 5′-ATAAGGAGCATAAAAATGGCT-3′ and MR1, 5′-AGCTTAGTTTTCTTCTTTGCG-3′) were used [14].

Genome sequencing and single nucleotide polymorphism (SNP) calling was performed on 13 GAS isolates from the hospital and contemporaneous *emm* 75 isolates from England as described previously using the *emm* 2 MGAS10270 reference genome, RefSeq accession number NC_008022.1 as the closest available GenBank sequence [15]. This was used in the absence of an *emm* 75 reference genome using bwa-mem 0.7.5a [16]. Gubbins 1.3.3 was used to remove loci with elevated base substitution densities indicative of recombination, producing a multiple sequence alignment file containing non-recombining variant positions alone [17]. These data were used to generate a maximum-likelihood tree using RAxML Version 8.1.17 using the following parameters:

- – f a (rapid Bootstrap analysis and search for best-scoring ML tree in one program run);
- – m (substitution-model) GTRGAMMA;
- – p (random-seed for parsimony inferences) 12,345;
- – x (random-seed for bootstrapping) 12,345; and
- – # (number of runs) 1000 [18].

The FASTQ files for isolates described in this study are available on the European Nucleotide Archive (ENA) study numbers ID PRJEB17773 and PRJEB21833.

Results

Staff sickness absence was only available for the last cluster. However, staff sampling results from incidents in the initial cluster (nose/throat swabs alone), isolated case two years later (sample sites unknown) and last cluster (nose/throat, +/- perineum sampling if first nose/throat screen was positive) were obtained.

In the initial cluster, at least 170 staff were screened with throat swabs/cultured for GAS; the specialities of the staff sampled were not known. One was positive for *emm* 75 GAS and had become symptomatic with throat symptoms only after all cases were diagnosed, apart from the baby of the index case who was detected on screening.

With the isolated case two years later, 54 staff were documented as appropriate to screen by throat/wound swabbing and culture for GAS. These included 32 maternity staff, with the remainder from other teams (e.g. intensive care). Of the 54 eligible staff, 35 were sampled (25 from the maternity department) and all were screen negative.

In the final cluster, staff screening was undertaken over three months. Throat/wound swabs were taken initially, with the subsequent addition of nose and perineum samples for those screened later. In the first round, 371 staff (208 maternity staff) were sampled, and in the second to fourth rounds, 85, 78 and 72 staff (37, 35 and 31 maternity staff) were repeat sampled, respectively; the number eligible for sampling was not known. Although four staff members were GAS positive on screening, only one maternity staff member had the *emm* 75 strain and became unwell four days after the second *emm* 75 case in the last cluster, but before the *emm* 3 case. The other staff members had strains unrelated to the outbreak.

Table I

emm 75 cases by incident and timing

Incident	Patient	Clinical status	Onset of symptoms
Initial cluster	Mother 1	GAS	Index of initial cluster
	Mother 2	iGAS	14 days after index of initial cluster
	Mother 3	iGAS	15 days after index of initial cluster
	Mother 4	iGAS	
	Baby of Mother 2	GAS	
	Baby of Mother 4	GAS	Asymptomatic
	Baby of Mother 1	GAS	Asymptomatic
	Staff member 1	Screening	Detected on screening, symptomatic only after cases presented
Isolated case two years after initial cluster	Mother 5	iGAS	Isolated case two years after initial cluster
Last cluster	Mother 6	iGAS	Index of last cluster
	Mother 7	iGAS	Six days after index of last cluster
	Staff member 2	Screening	Nine days after index of last cluster

GAS, group A streptococcus; iGAS, invasive GAS.

Additionally, a symptomatic staff member from a different department was identified as having an *emm* 75 isolate three months later. This suggests that *emm* 75 may have been circulating more widely in other staff within the hospital, and supports the hypothesis that staff were the unwitting source in these incidents.

Routes of transmission

Four possible routes of transmission were investigated: mother-to-mother, staff–patient–staff, staff–staff and environmental.

Mother-to-mother transmission

No mother was present in more than one cluster (i.e. did not deliver a baby at the maternity unit in each of the affected year clusters); it is therefore unlikely that an individual chronic carrier mother could have been the primary vector responsible for mother-to-mother transmission in all three clusters. Two cases overlapped for one day in the initial cluster on the delivery suite but were in separate rooms; all four cases had been in the maternity unit within a seven-day period. The first and second mothers in the last cluster overlapped in the delivery suite on the day the first was re-admitted with a positive GAS isolate, occupied the same delivery room during delivery, and occupied the same postnatal ward bay but with a three-day separation.

Staff–patient–staff transmission

emm 75 was found on staff screening in the initial cluster (one maternity staff member found to be colonized) and in the last cluster (one maternity staff member and a non-maternity staff member found to be infected).

No direct link between a single staff member and all cases was identified; however, two staff members had contact with one or more cases in each of the episodes.

The staff member who was GAS positive in the initial cluster cared for five of the cases, but only became symptomatic after all the cases had presented, except the baby of the index case whose isolate was detected on screening. This staff member was also involved in care of the single case two years later, but was not present for the last cluster. The GAS-positive maternity staff member in the last cluster had contact with the index case of the last cluster more than two weeks before onset, but did not develop symptoms until after the last cluster index case had presented, but did have contact with three of the cases (two mothers, one baby) in the initial cluster within three days of their infection being identified. One staff member was common to two cases in the last cluster, and another was common to all three cases in that cluster, but no positive isolates were obtained from them.

Staff–staff transmission

In the last cluster, some staff members had worked while symptomatic with potential GAS infections (e.g. sore throat). However, this was only after the index and second case in the last cluster had already presented, making it less likely that they were the primary vectors (more likely to have been downstream recipients). In the first round of screening in the last cluster, 371 staff were sampled. Follow-up sampling was attempted for a further three rounds to try to increase detection, but fewer staff were resampled on each occasion – 85, 78 and 72, respectively – meaning that other asymptomatic staff who were not sampled could have been the primary vectors but remained undetected, or that – where negative results were obtained – the organisms present were below the limit of detection.

Environmental transmission

During the investigations of the last cluster, the unit was examined for compliance with infection prevention and control. Some deficiencies were noted, such as blood contaminating a privacy curtain, and clinical equipment placed on top of waste bins. Recommendations for improvements were made. GAS was not isolated from environmental sampling; however, the sites sampled were not available to the outbreak team, so the appropriateness of sampled sites or the sampling methods used is not known.

Genomic sequencing analysis

WGS analysis showed that the initial and last cluster isolates were located within a clade with a shared ancestral lineage suggesting common source transmission; however, transmission directionality could not be inferred. The isolates were separated from all others by at least nine SNPs. No other contemporaneous isolates from national *emm* 75 GAS strains were located within this clade (Figure 1). The single case isolate that arose two years after the initial cluster did not have an immediate common ancestor to the initial or last cluster isolates.

Discussion

No mother was present in more than one cluster; it is therefore unlikely that an individual chronic carrier mother could have been the primary vector responsible for mother-to-mother transmission in all the clusters. However, mother-to-mother transmission may have occurred within the individual cluster (e.g. two mothers overlapped in the initial cluster on the delivery suite for one day but had no direct contact, making this less likely, and in the last cluster, two mothers occupied the same bay/bathroom and delivery suite room but on different days). It is also unlikely that the mothers were separately importing GAS leading to multiple re-introductions into the hospital environment, as there was little evidence of *emm* 75 circulating in the community (only 3.2% nationally and 3% regionally during the study period), and all of the regional *emm* 75 isolates resulted from this incident, suggesting that *emm* 75 is relatively uncommon in invasive disease.

The recurrence of clonal GAS clusters over this four-year period is unusual. The initial and final cluster isolates were closely related with a common ancestor, and were distinct from other *emm* 75 isolates. The variation observed between the initial and final cluster isolates is indicative of acquired variation consistent with genetic drift over time. The number of single point mutations (SNPs) estimated to occur in the GAS genome (based on analysis of *emm* 1/12 type strains) has been estimated at 1.37–1.8 SNPs per core genome per year [19,20]. The genetic distance between the initial and last cluster maternity unit clade of isolates is consistent with these estimates. The nine SNPs that separate the maternity unit clade from all other isolates in this study represent an approximate time of 6.5 years.

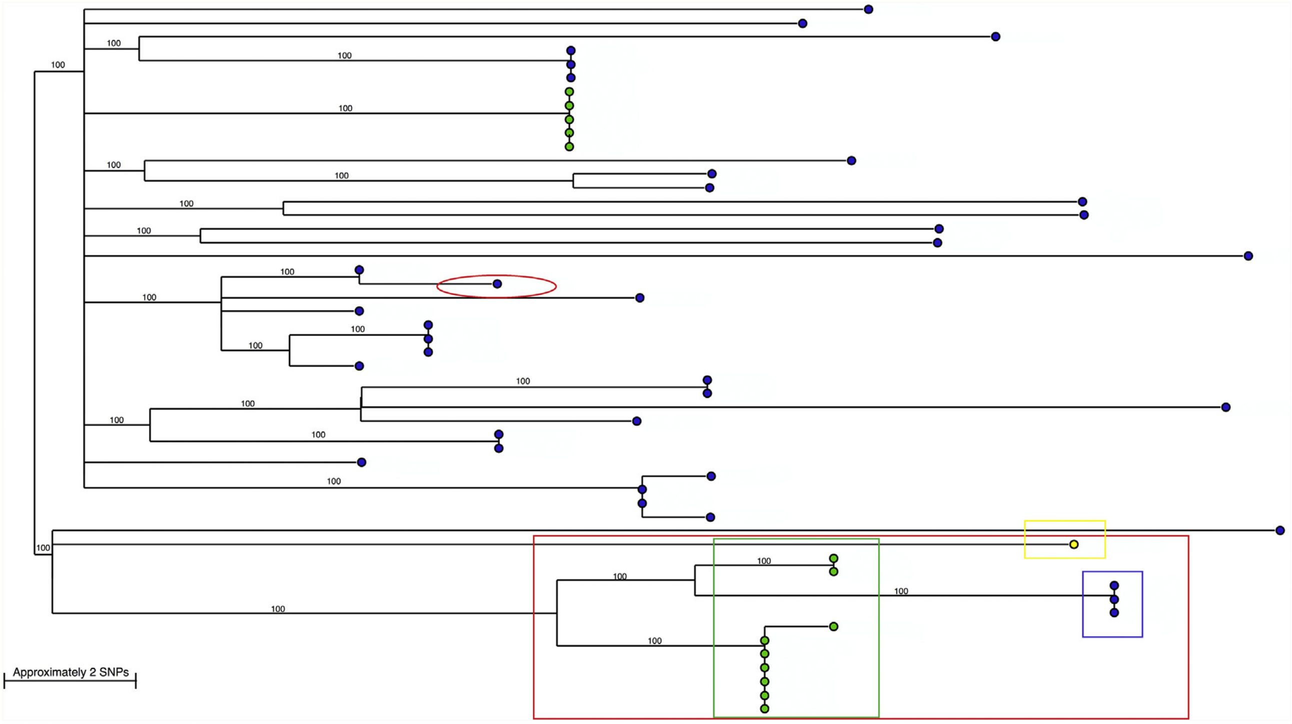

Figure 1. Phylogenetic tree of incident and contemporaneous isolates. Red rectangle, incident isolates over the whole four-year period; green square, isolates from initial cluster; yellow rectangle, isolates from isolated case two years after initial cluster; blue square, isolates from final cluster; red oval, contemporaneous isolate from same geographical area. SNP, single nucleotide polymorphism.

It is possible that the initial cluster strain could have persisted in the hospital environment and/or in one or more colonized staff members until the last cluster; however, such a long duration (greater than two years) of clonal persistence has not been described previously.

There are three possible explanations for the emergence of a second clonally linked cluster four years after the first infection episode:

- Treatment/screening failure in the initial cluster with one or more staff members retaining asymptomatic *emm* 75 GAS colonization/shedding, leading to ongoing staff–staff, staff–patient or staff–environment transmission. It has been demonstrated previously that asymptomatic carriage is cleared less effectively by treatment than symptomatic carriage [21].
- A persistent external source/s re-introducing GAS to the hospital (e.g. staff members, relatives).
- Persistence of environmental contamination. GAS has been documented to persist in fomites for up to 6.5 months [22].

It is not possible to confirm any of these hypotheses definitively. However, the first hypothesis is the most plausible as staff colonization was found, no inanimate reservoir was detected, and *emm* 75 GAS/iGAS disease is rare. Supporting this, asymptomatic, colonized staff members and symptomatic staff members have been previously demonstrated to account for 34% and 8.2% of all GAS outbreaks, respectively, and carriage with the same strain in close contacts of a GAS-infected case can approach 30% [5,23]. Staff may have unwittingly transmitted GAS to each other, leading to persistence in multiple staff members. Control measures were therefore targeted at interrupting transmission from staff, including the screening of staff to detect carriers, attention to handwashing and infection control procedures.

WGS was useful for supporting the linkage of cases and determining that the clusters were genetically distinct from other *emm* 75 isolates. However, more contemporaneous isolates would have been helpful to indicate more clearly the incidence of infections and prevalence of carriage of this particular clade.

Although staff screening was undertaken, the logistics of sampling, particularly repeat sampling, of a large group of individuals was challenging. Screening effectiveness is also limited by sample quality and false-negative results. Data quality was also an issue; data on the location of mothers was incomplete, not all staff–patient interactions were documented and staff signatures were frequently unclear. Increased duration and proximity of staff–patient interactions may increase the chances of transmission; however, it is unusual for these details to be noted in clinical records.

While environmental surfaces have been recognized as the source of maternity GAS outbreaks (e.g. GAS remain viable on fomites for up to 6.5 months; and shower heads, curtains, baths, bidets and toilet seats have been implicated previously), and some infection control deficiencies were identified, sampling found no evidence of environmental GAS contamination [5,22,24]. Environmental sampling was performed with dry swabs after deep cleaning, which may have reduced detection. The sites sampled were not available to the outbreak team so their relevance was not known. Moistened/specific environmental swabs or directly applied plates may have produced more meaningful results [24]. Although speculative in the absence of clearer environmental sampling data, it is unlikely that a single persistent environmental source was responsible due to the time between the two episodes. It is more likely that the environment acted as a temporary, intermediate vector. Even with systematic, environmental sampling using moistened swabs, areas of contamination may be missed, so negative swabs are not necessarily indicative of a non-contaminated environment. The focus therefore should be on assuming that there may be temporary contamination of the care environment, and robust cleaning to reduce such contamination to a minimum. GAS prevention and control guidelines recommend that 'communal facilities, including bathrooms/toilets, are cleaned and decontaminated after each use especially on delivery suites, postnatal wards and other high-risk areas' [5]. At the time of the last cluster, the cleaning regime was consistent with other National Health Service practice (i.e. twice-daily cleaning); however, cleaning was subsequently increased to decontaminate after each patient use. Further research on the optimal cleaning frequency for such high-risk areas is warranted.

In conclusion, recurrent clonal outbreaks of GAS/iGAS spanning comparable durations to this incident and *emm* 75 outbreaks related to childbirth appear rare. It is postulated that recurrent infections seen with isolates within the *emm* 75 monophyletic group occurred over a four-year period due to asymptomatic carriage in maternity staff members, with transmission to patients. However, other modes of transmission cannot be excluded. Increased monitoring and vigilance is essential, particularly if more cases with the *emm* 75 type are identified. Staff and patient education will be a valuable tool in preventing any future cases. No further cases have been noted at the time of writing (March 2018). Authorities investigating maternity-related outbreaks should be aware that prolonged, clonal isolate outbreaks are possible over a period of years, indicating that robust efforts to identify, control and eradicate the common source are needed, particularly given the seriousness of puerperal sepsis. Fortunately, all invasive cases recovered in these incidents, but some were very ill, so the outcome could have been very different.

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Conflicts of interest

HD is currently employed by GlaxoSmithKline and holds GSK shares. At the time this work was done, she was employed by Public Health England. Meera Chand and Victoria Chalker are affiliated with the National Institute for Health Research Health Protection Research Unit in Respiratory Infections at Imperial College London in partnership with Public Health England. The views expressed are those of the author(s) and not necessarily those of the National Health Service, the National Institute for Health Research, the Department of Health or Public Health England.

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