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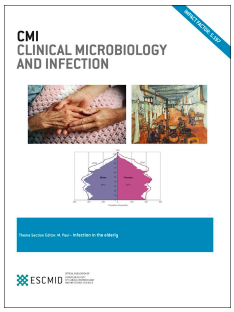

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The ongoing *Streptococcus pyogenes* (Group A *Streptococcus*) outbreak in London, United Kingdom in December 2022: a molecular epidemiology study.

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Abstract

Objectives: Epidemiological and whole genome sequencing (WGS) analysis of the ongoing outbreak of *Streptoccous pyogenes* (Group A *Streptococcus)* in London (United Kingdom).

Methods: Prospective identification of Group A *Streptococcus* cases from a diagnostic laboratory serving central and south London between November 27th and December 10th 2022. Case notes were reviewed and isolates retrieved. Case numbers were compared with the previous 5 years. Whole genome sequencing was performed with long-read, nanopore technology for *emm* typing and identification of superantigen genes. Associations of pathogen-related factors with invasive disease were assessed by single variable and multivariable logistic regression.

Results: Case numbers began increasing in October 2022 from a baseline of 2.0 cases per day, and in December 2022 the average daily case numbers reached 10.8 cases per day, 4-fold the number usually seen in winter. 113 cases were identified during the prospective study period. Three quarters (86/113, 76%) were paediatric cases, including two deaths. 11/113 (10%) cases were invasive. 56 isolates were successfully sequenced including 10/11 (91%) invasive isolates. The *emm12* (33/56, 59%) and *emm1* (9/56, 16%) types were predominant, with 7/9 (78%) *emm1* isolates being from the M1uk clone. The majority of invasive isolates had superantigens *spea* (7/10, 70%) and *spej* (8/10, 80%), whereas in non-invasive isolates these superantigens were found less frequently (*spea:* 5/46, 11% and *spej:* 7/46, 15%). *spea* (OR 8.9, CI 1.4-57, p=0.020) and *spej* (OR 12, CI 1.8-78, p=0.011) were associated with invasive disease by multivariable analysis.

Conclusions: *emm12* and *emm1* types predominate in the ongoing outbreak, which mainly affects children. In this outbreak, the *spea* and *spej* superantigen genes are associated with the severity of presentation.

Introduction

On 2nd December 2022 the UK Health Security Agency (HSA) alerted clinicians to an increase in scarlet fever and invasive disease caused by *Streptococcus pyogenes* (Group A *Streptococcus* [GAS]) [1]. Invasive disease, defined as isolation of GAS from a normally sterile site [2], has resulted in the death of around 30 children [1]. It is unclear whether this increase is associated with circulation of particularly invasive types or clones. We therefore undertook a review of the epidemiology and performed rapid whole genome sequencing (WGS) of GAS from community and hospital cases in London (United Kingdom).

Methods

Study period, population and case details.

Cases of GAS were identified and prospectively collected over 2 weeks (27th November until 10th December 2022). Cases were captured from our laboratory reports, which serves two adult hospitals, one paediatric hospital, and community services for approximately 500,000 people in central and south London. Linked clinical data were retrieved from electronic patient records. Clinical information retrieved included sex, age, area of residence, sampling date, sample site, geographical location of sampling (categorised as general practice, outpatients, emergency department [not admitted], or hospital inpatients). Where area of residence was not available, location of swab was used (n=7). Invasive cases were defined as per Centre for Disease Control definitions [3]. Outcomes included GAS-related hospitalisation, ICU admission and death, censored after 23rd December 2022. Retrospective reports of GAS isolation for the past 5 years were also retrieved from the laboratory reports. Incidence graphs were made in StataMP (StataCorp LLC, TX). Geospatial data was mapped using Geopandas v0.12.2 (https://geopandas.org/), with cases inside of Greater London (n=101) mapped onto middle layer super output areas (Office of National Statistics). Cases outside Greater London are not shown (n=12).

Whole genome sequencing of isolates.

During the prospective study period, new growths of GAS were identified by scientists in the diagnostic lab and saved for WGS. GAS isolates were then grown on purity plates before being subjected to bead beating for nucleic acid extraction as previously described [4]. Library preparation and indexing were performed using SQB-RBK110.96 and sequenced on R.9.4.1 flow cells (Oxford Nanopore Technologies). Multilocus sequence typing was performed using krocus v1 (https://github.com/andrewjpage/krocus). Genome assembly was performed using flye v.2.8.3 (https://github.com/fenderglass/Flye) and polished with medaka v1.7.2

(https://github.com/nanoporetech/medaka). *emm*-typing was performed on completed assembly using emmtyper (https://github.com/MDU-PHL/emmtyper) and in parallel by mapping reads with BLAST against the CDC reference dataset (https://www2.cdc.gov/vaccines/biotech/strepblast.asp). Reads were interrogated for presence of virulence factors using BLAST against Virulence Factor DataBase (http://www.mgc.ac.cn/VFs/), specifically detections of superantigens smez, *spej*, speg, *spea*, spec, ssa, spem, spei, speh, spek and spel genes based on previous reports [5]. Superantigen genes were considered detected if reads mapped with 90% coverage and 90% identity, as previously described for antimicrobial resistance genes [4]. Basecalled and demultiplexed read data is available at the Sequence Read Archive (BioProject: PRJNA914624). Use of samples and linked clinical data was approved by the Research Ethics Committee (18/NW/0584).

Statistical analysis of the association of pathogen-related factors with invasive disease

Single variable analysis assessing the association of pathogen-related factors with invasive disease utilised the likelihood ratio test. In this analysis age was treated as categorical data: either paediatric (<18 years of age) or adult. *emm* type was categorised into *emm1* or other, due to previous association of emm1 with invasive disease. Variables with a significance value of $p \leq 0.2$ were taken forward to multivariable analysis, conducted using backwards stepwise binary logistic regression. Significance threshold was set at $p<0.05$. Analysis was performed using SPSS v28 (IBM, NY).

Results

5 year epidemiological surveillance of laboratory-identified GAS infections

Cases of GAS between 2017 and 2023 are shown in Figure 1 (see also Supplemental Table 1 & 2). GAS infections usually peak in late spring and early summer, as previously described [1]. During the two pandemic years (March 2020 to March 2022), GAS cases fell below the monthly nadir seen in previous years. In 2022, an increase in case numbers in December 2022 reached 10.8 cases per day over 4-fold higher average daily case numbers than in December of the pre-pandemic years, amounting to 238 cases in December 2022 in total. Cases still remain higher than other years as of February 2023.

Figure 1: Daily cases (14-day moving average) of laboratory reported GAS isolation from clinical samples between January 1st 2017 and January 31st 2023.

Description of GAS cases captured within the study period

113 cases were identified (Table 1, Supplementary Table 3) during the prospective study period of 27th November until 10th December 2022. 59/113 (52%) were female and median age was 7 (IQR 4-

13), with 86/113 (76%) being paediatric cases under 18 years old. 11/113 (10%) were considered invasive. A similar proportion of paediatric cases (7/86, 8%) and adult cases (4/27, 5%) were invasive. No demographic or case details were missing. Geospatial data on incidence is shown in Supplementary Figure 1 (see also Supplementary Table 4)

17/113 (15%) cases were admitted to hospital, with 2 deaths in children. Most (7/11, 64%) invasive cases were lower respiratory tract infections.Of the 7 cases of invasive respiratory disease, 4/7 had co-infection with respiratory viruses (human metapneumovirus=2; influenza=1, respiratory syncytial virus=1), 2 did not, and 1 was not tested. Most of the non-invasive cases (74/113) (73%) were pharyngitis (Table 1).

Whole genome sequencing analysis and molecular typing.

Isolates were retrieved from 65/113 (58%) cases. WGS was successful for 56/65 (86%) of isolates retrieved (Supplementary Figure 2), comprising 10/11 (91%) invasive cases and 46/102 (45%) non-invasive cases. Similarly to the whole cohort, the majority of successfully sequenced isolates were from paediatric cases (41/56, 73%), with a median age of 6 (IQR 4-29), and most (39/56, 70%) were cases of pharyngitis.

Table 1: Description of cases and summary of typing results.

Typing data for the 56 sequenced isolates is presented in Table 1 (and Supplemental Table 5 & 6). The majority were of two *emm* types: *emm12* (33/56, 59%) and *emm1* (9/56, 16%). A higher proportion of invasive disease was caused by emm1 (5/10, 50%), compared to non-invasive disease (4/46, 9%). In addition, *emm1* type comprised a higher proportion of paediatric (8/41, 20%) compared to adults (1/15, 7%) cases.

The *emm1* types were further characterised to identify whether they were from the M1uk clone, which has previously been associated with increases in scarlet fever and increased superantigen production [6] . 7/9 (78%) *emm1* types were highly related to the M1uk clone, sharing 26 of 27 characteristic SNPs, including 4/5 (80%) of the invasive *emm1* isolates. Single SNP differences have also been documented in another study describing the M1uk clone [7]. The remaining 2/9 (22%) *emm1* isolates were unrelated to the M1uk clone, lacking any of the M1uk defining SNPs. GAS isolates from the two paediatric deaths were caused by an *emm12* and a non-M1uk *emm1* type.

Detection of superantigen genes by whole genome sequencing.

Each successfully sequenced isolate was analysed for the presence of 11 superantigen genes (Supplementary Table 5 & 7). The superantigen *spea* (streptococcal pyrogenic exotoxin) was found in a majority of invasive isolates (7/10, 70%) but a minority of non-invasive isolates (5/46, 11%) Similarly, the superantigen *spej* gene was more common in invasive isolates (8/10, 80%), compared to non-invasive isolates (7/46, 15%). Most (7/9, 78%) of *emm1* had *spea* and *spej*, including all (5/5, 100%) of the invasive *emm1* isolates. Most M1uk isolates had *spea* (6/7, 86%) and *spej* (5/7, 71%) detected. Both paediatric deaths carried *spea* and one also carried *spej*.

Single variable and multivariable analysis of pathogen-factors associated with invasive disease

From the successfully sequenced isolates, *emm* type, M1uk clone, and presence of the 11 superantigen genes were investigated for association with invasive disease by single variable analysis (Table 2). The superantigens speK, speL, speG and speM were omitted from analysis due to collinearity. Of pathogen-related factors, *emm1* type (OR 10.5; 95% CI 2.1 - 52), M1Uk clone (OR 9.6l 95% CI 1.7 - 52), superantigens *spej* (OR 22; 95% CI 3.9-27) and *spea* (OR 19; 95% CI 3.7-99) were significantly associated with invasive disease ($p<0.05$).

For the binary logistic regression model, pathogen-related factors from the univariable analysis with a p value≤0.2 were taken forward for backwards step selection. Variables in the multivariable model therefore included *emm* type, M1Uk clone, and genes for superantigens *spej*, *spea*, speC and ssa. Table 2 shows the final model with two variables retaining significance for association with invasive disease: *spea* (OR 8.9; 95% CI 1.4-57; p=0.020) and *spej* (OR 12; 95% CI 1.8 – 78; p=0.011). The final model can predict invasiveness 89% correctly (96% of non invasive and 60% of invasive cases).

Table 2: Single variable and multivariable analysis of the association of pathogen-related factors with invasive disease.

Discussion

The epidemiology presented here from London reflects the pattern seen nationally with a dramatic increase in GAS infections in late 2022, after two years of below average incidence during the COVID-19 pandemic. The reasons for this epidemiological pattern are unclear, but postulated to be caused by decreased GAS exposure particularly amongst children due to non-pharmaceutical interventions (social distancing and/or masking) during the COVID-19 pandemic, leading to lower levels of immunity [1]. This may also be compounded by high circulating rates of respiratory viruses this winter, including influenza and COVID-19, which may pre-dispose to subsequent GAS infections [9,10]. Although first recognised in the UK, an increase in GAS infections is now being reported in other countries in the European region (Ireland, France, the Netherlands and Finland) [10] and in the United States [11].

There is an association between certain *emm* types, high superantigen production, and invasive disease [12]. The majority (75%) of isolates sequenced were *emm1* and *emm12* types, similar to what has been reported nationally. [1]. *Emm1* types were overrepresented in the invasive cases, and the majority of the *emm1* isolates were of the M1uk clone. Prior to the pandemic, M1uk was recognised to comprise the majority of UK emm1 isolates, with high superantigen production [6]. This clone has also been reported in North America [7].

To disentangle the association of pathogen factors with invasive disease, multivariable analysis was conducted, showing the association is attributable to *spea* and *spej* superantigen rather than the other analysed pathogen-related factors. *spea* and *spej* superantigen production may therefore explain the pathological mechanisms behind invasive disease in this outbreak. Alternatively, other unassessed pathogen factors that cosegregate with these genes may play a mechanistic role.

As sequence typing data was only obtained for half the isolates our results may not be representative of the outbreak nationally. In addition, viral respiratory infections were not systematically assessed leaving us unable to comment on any association with GAS. Larger studies are required to discriminate the overlapping associations between invasive disease, types, clones and superantigen carriage. Replicating these findings in other regions will be important to confirm whether increased incidence is related to these pathogen specific factors such as superantigens and hypervirulent clones.

This study illustrates the benefit of embedding pathogen sequencing in diagnostic laboratories, allowing rapid outbreak investigation to inform clinical and public health teams. Meanwhile, clinicians in affected areas are recommended to consider Group A *Streptococcus* for presentations usually due to respiratory viral infections [1].

Conflicts of interest

The work was funded by the Medical Research Council (LBS: MR/W025140/1, GN: MR/T005416/1, JDE, AAM, RB, GN, LBS, TC: MC_PC_19041). JDE has received funding from the Guy's and St. Thomas' Charity (TR130505). AAM and members of the Synnovis Microbiology Labroatory Group are full time employees of Synnovis. JDE holds part-time employment contract with Oxford Nanopore Technologies that commenced in October 2022. Guy's & St Thomas' NHS Foundation Trust signed a commercial collaboration agreement with Oxford Nanopore Technology in September 2022. WN, CA, GH, MT, NAY, SDJN, SDG, TGSW have no conflicts of interest to declare.

Authors contributions

AAM, LBS, CA, TC & TGSW were involved in conceptualisation, data acquisition, methodology and formal analysis. AAM & LBS were involved in writing – original draft. The Synnovis Microbiology Laboratory Group, MT, GH & NAY were involved in investigation. WN, SDJN, SDG, GN & JDE were involved in supervision and writing – review and editing. RB was involved in project administration and resources. AAM, LBS, TC, SDJN, GN, RB & JDE were involved in funding acquisition.

Appendix

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Table 1: Description of cases and summary of typing results.

	Paediatric		Adult	
n (%)	86 (76%)		27 (24%)	
Mean age, yrs (IQR)	5 (4-7)		41 (34-57)	
Clinical manifestation				
Non-invasive:				
Upper respiratory tract	65 (76%)		9 (33%)	
Skin and soft tissue	12 (14%)		14 (52%)	
Scarlet fever	2 (2%)		0 (0%)	
Invasive:				
Lower respiratory tract	5 (6%)		2 (7%)	
Septic arthritis	1 (1%)		1 (4%)	
Necrotizing fascitis	0 (0%)		1 (4%)	
Bacteraemia	1 (1%)		0 (0%)	
GAS-related outcomes				
Hospitalisation	10 (12%)		7 (15%)	
ICU admission	3 (3%)		2 (7%)	
Death	2 (2%)		0 (0%)	
Typing (n=56)	**Invasive**	**Non-invasive**	**Invasive**	**Non-invasive**
emm12	2	24	2	5
emm1	5	3	0	1
other	0	7	1	6

Table 2: Single variable and multivariable logistic regression analysis of the association of pathogen-related factors with invasive disease.

Variable	**OR (95% CI)**	**p value**
Single variable analysis		
emm1 type	11 (2.1 - 52)	0.004
M1Uk clone	9.6 (1.7 - 54)	0.010
Superantigen		
smez	0.20 (0.011 - 3.5)	0.27
spej	22 (3.9 - 127)	<0.001
spea	19 (3.7 - 99)	<0.001
spec	0.28 (0.40 - 1.9)	0.20
ssa	0.26 (0.064 - 1.1)	0.065
spei	0.92 (0.21 - 4.1)	0.91
speh	1.9 (0.37 - 10)	0.44
Multivariable analysis: final model		
spea	8.9 (1.4 - 57)	0.020
spej	12 (1.8 - 78)	0.011