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Genomic interrogation of invasive group A *Streptococcus* (iGAS) epidemiology and COVID-19 impacts in Victoria, Australia: a 6-year retrospective study

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Summary

Background Invasive group A *Streptococcus* (iGAS) cases have increased globally in 2022–2023, raising concerns within the medical and public health communities, including in Australia, while this impact is polyclonal in nature the worldwide spread and dominance of $M1_{UK}$ has been particularly concerning.

Methods To investigate these changes and prepare to implement routine genomic surveillance of iGAS for public health purposes, we performed whole genome sequencing (WGS) on iGAS isolates from Victoria, Australia between 2017 and 2022. Genomic analyses were conducted to determine the epidemiology, genetic diversity, and population dynamics of iGAS.

Findings Analysis of 955 confirmed iGAS cases over a 6-year period revealed a polyclonal population. Fewer iGAS cases were noted between 2020 and 2021 in addition to genetic bottlenecks, likely reflecting the implementation of strict public health measures during the COVID pandemic, followed by a resurgence in cases post-COVID. Low levels of antimicrobial resistance were observed, primarily to macrolides and tetracyclines. Phylogenetic analysis identified a previously undescribed *emm1* sub-lineage, designated $M1_{Aus}$, detected in Australia (Victoria and Queensland), Belgium and the United Kingdom. In Victoria, $M1_{Aus}$ was the dominant *emm1* variant in 2017 and 2018, more recently replaced by the $M1_{UK}$ lineage as the dominant variant, further demonstrating the worldwide impact of $M1_{UK}$.

Interpretation This comprehensive genomic study of iGAS in Victoria, Australia provides valuable insights into the population dynamics, genetic diversity, and impact of pandemic public health measures on iGAS epidemiology. The identification of the $M1_{Aus}$ sub-lineage emphasises the need for continued genomic surveillance and monitoring of iGAS strains, particularly in the context of emerging global sub-lineages and shifts in population structure.

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Keywords: Streptococcus pneumoniae; Invasive group A streptococcus; Genomic epidemiology; Streptococcal typing

Research in context

Evidence before this study

Prior to becoming nationally notifiable, increased cases of invasive group A *Streptococcus* (iGAS) were observed at the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) and throughout the northern hemisphere. PubMed was used to search for "*Streptococcus pyogenes*" "invasive" "genome" "epidemiology" with no limit on language or date of publication. We considered all studies that conducted genomic epidemiology and epidemiology of iGAS.

Added value of this study

Our study is the first to report a six-year genomic epidemiology study in Victoria, Australia. This study has allowed a baseline dataset for genomic epidemiology of *Streptococcus pyogenes* causing invasive disease, allowing continued close analysis of the epidemiological trends in Victoria, Australia, as well as the application to Australian and global settings. With the use of whole genome sequencing, we uncovered a novel *emm1* sub-lineage—$M1_{Aus}$, first detected in Victoria, then Queensland and globally. $M1_{Aus}$ was the dominant *emm1* sublineage in 2017 and 2018 in Victoria, prior to the dominance of $M1_{UK}$ in 2019. A bottleneck impacting *emm1* detections occurred due to public health measures implemented to restrict the spread of SARS-CoV-2 between 2020 and 2022. Subsequently in 2022 we noted an increase $M1_{UK}$.

Implications of all the available evidence

Genomic epidemiology on such a comprehensive genomic dataset has informed local genomic surveillance, enabling outbreak detection, to then inform public health measures tailored to local conditions. This data highlights the ongoing burden of *S. pyogenes* causing invasive disease.

Introduction

Streptococcus pyogenes, or group A *Streptococcus* (GAS), is a human-restricted pathogen, transmitted through close contact of infected individuals.[1] *S. pyogenes* causes a range of superficial infections (such as pharyngitis, scarlet fever and impetigo) to severe invasive infections (such as bacteraemia, cellulitis, necrotising soft tissue infections, and endocarditis) and streptococcal toxic shock syndrome (STSS).[1] Repeated *S. pyogenes* infections can trigger serious immune-mediated disorders, such as acute post-streptococcal glomerulonephritis (APSGN), acute rheumatic fever (ARF), and rheumatic heart disease (RHD).[1]

In a recent global assessment of the burden of disease associated with 33 bacterial pathogens in 2019, *S. pyogenes* infections was ranked 12th by mortality and 14th by number of years of life lost, which was primarily attributed to skin and soft tissue infections (SSTIs) and bloodstream infections.[2] Furthermore, in 2019, of 23 pathogens assessed, *S. pyogenes* was ranked 16th in a systematic review analysing the burden of antimicrobial resistance (AMR).[3] *S. pyogenes* remains susceptible to penicillins, the treatment of choice for iGAS, with lincosamides/macrolides commonly used for penicillin-allergic patients, and tetracyclines and fluoroquinolones being other alternatives. Resistance to lincosamides, macrolides, tetracyclines and low-level resistance to fluoroquinolones have been reported across *S. pyogenes* populations, with *ermB*-carrying *S. pyogenes* isolates regarded as concerning by the US Centres for Disease Control and Prevention (CDC).[4]

Invasive GAS disease (iGAS) is defined as the isolation of *S. pyogenes* from a usually sterile body site and is associated with high case fatality rates of up to 20–50%.[5] iGAS infections most frequently occur in older people and young children and, in Australia, the incidence of iGAS is 2.1-fold higher in Aboriginal and Torres Strait Islander children.[1] Outbreaks of iGAS disease have been described most frequently in vulnerable populations including young children, residents of long-term care facilities, people experiencing homelessness and people who inject drugs.[1,6]

Increasing incidence of iGAS have been reported in multiple countries in recent years.[7–10] In Australia, iGAS was added to the National Notifiable Diseases list from 1st July 2021, and became officially notifiable in Victoria in February 2022.[11] Many distinct lineages have been detected in iGAS isolates, which typically mirrors isolates detected in non-invasive infections.[8,12,13] The rise of new highly virulent sub-lineages, such as $M1_{UK}$, may result in outbreaks and provide an explanation for the increased incidence.[14,15] Understanding the genomic epidemiology of iGAS in specific jurisdictions is crucial for evaluating population dynamics and the impact of the disease.[14–19] Genomic analysis of iGAS strains can potentially give insights into the transmission patterns, genetic diversity, and potential

factors contributing to the spread and severity of disease. This knowledge is valuable in informing public health strategies, surveillance efforts, and the development of effective interventions to control and manage iGAS infections.

In this study, we conducted retrospective genomics analyses of iGAS cases in Victoria, Australia from 1st January 2017 to 31st December 2022. Using a combination of epidemiological and bioinformatic approaches, we describe the genomic epidemiology of confirmed iGAS isolates in Victoria, explore the impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) public health restrictions, and establish a baseline and recommendations for ongoing genomic surveillance of iGAS in Australia.

Methods

Study setting and inclusion criteria

From 1st January 2017 to 31st December 2022, 21 laboratories across Victoria referred 1030 iGAS cultures to Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) for identification and further characterisation. From the 22nd February 2022, iGAS cultures were received at MDU PHL under the auspices of the National Notification Surveillance System and Public Health and Wellbeing Regulations (2019). An iGAS case was defined as *S. pyogenes* isolated from a normally sterile site of an individual.[20] A single isolate per case was included in the dataset; where multiple samples for a single case were available, the earliest isolate was selected unless multiple *emm* types or MLST were present (if present, they were included in the dataset as separate cases due to separate infections). Samples are received with patient demographic details, sample type, and date of collection, without additional epidemiologic data.

Whole genome sequencing, assembly and typing

Samples were processed according to accredited laboratory workflows and all isolates underwent whole genome sequencing on the Illumina NextSeq platform (Supplementary Material). Sequences underwent quality control, with draft genome length between 1.6 and 2.0 Mb and containing less than 120 contigs included in downstream analyses.

De novo draft assemblies were constructed using Shovill v1.1.0 (https://github.com/tseemann/shovill) based on the SPAdes (v3.13.0) assembler, species taxonomic classifications were determined using kraken2 (default parameters) and read/assembly statistics were summarised through the bohra pipeline v2.0.0 (https://github.com/kristyhoran/bohra) (Supplementary Material). For each isolate, the *emm* type, multi-locus sequence type (ST) and genome-wide variable-length k-mer cluster was assigned (Supplementary Material).

Mapping, single nucleotide polymorphism (SNP) calling and species phylogeny

All read mapping and variant calling was performed using snippy v4.4.5 (https://github.com/tseemann/snippy), applying a minfrac value of 0.9 and mincov value of 10. The MGAS5005 reference was used (GCF_000011765.3) and core genome alignments were produced using snippy-core. Maximum likelihood phylogenies were produced with IQ-Tree v1.6.12,[21] using the GTR + F + G4 model with rapid bootstrapping -bb 1000 and -alrt 1000.

Antimicrobial resistance (AMR) gene and virulence factor gene profiles

All genomes were screened for AMR genes using abritAMR v1.0.11 incorporating the AMRFinderPlus database v2021-09-30.1[22] using default parameters. To date, no known resistance due to mutations in the *S. pyogenes*-specific penicillin binding proteins have been validated and included in the AMRFinderPlus database. Additional screening of all isolates was conducted against *S. pyogenes* serotype M3 strain ATCC BAA-595/MGAS315 protein reference (WP_011106648.1) using screen_assembly3.py v1.0.0[12] to search for a proposed mutation, PBP2x T553K substitution.[23] All genomes were screened against an amino acid database of 31 known *S. pyogenes* known virulence factors (including superantigens, streptodornases, streptokinase, toxins, hyaluronic acid, regulators and a *sof* operon gene) using screen_assembly3.py v1.0.0 with the flags -p 85 and -l 90[12] (Supplementary Material).

Characterisation of *emm1* sub-lineages

The *emm*1 sub-lineages were further investigated with an *emm*1-specific phylogeny (as described above). In addition, for global context 233 representative publicly available *emm*1 genomes were compared to all Victorian iGAS *emm*1 genomes, and the alignment was masked from known mobile genetic elements[24] and regions of high variation (Supplementary Material). The *emm*1 pan-genome analyses were conducted using panaroo v1.2.8 using the –strict mode, –refind_prop_match 0.5 –search_radius 1000.[25] Bayesian Analysis of Population Structure (BAPS) clustering from the *emm1* core genome alignment was performed in R studio using RheirBAPS v1.1.3.[26] The subsequent clusters were used to interrogate the core genome alignment and pan-genome analyses to examine the presence of unique and shared mutations/indels and genes to each lineage using Scoary v1.6.7.[27]

Epidemiological and statistical analyses

Descriptive analyses were performed on metadata collected for all isolates, summarising the specimen collection date and *emm* type per month and year. Public health measures implemented due to the COVID-19

global pandemic (due to SARS-CoV-2) were overlaid to identify any potential impacts on iGAS case numbers.

Data visualisation

Epidemiological analyses, statistical analyses, phylogenetic tree visualisations with accompanying metadata were performed using R version 4.1.1 and RStudio version 1.4.1717[28] (Supplementary Material).

Ethical approval

All data received under the National Notification Surveillance System were collected in accordance with the Public Health and Wellbeing Regulations 2019. All data received prior to 22nd February 2022 was submitted voluntarily to MDU PHL by diagnostic laboratories. Ethical approval was received from the University of Melbourne Human Research Ethics Committee (1954615).

Role of the funding source

The funders played no role in the writing of the manuscript or decision to submit for publication.

Results

Characteristics of the Victorian iGAS cases

1030 suspected iGAS isolates were submitted during the study period. Of this, 19 isolates did not meet the inclusion criteria, sequence data was not available for 14 isolates, and 34 duplicate isolates were excluded and 8 isolates were excluded due a combination of the above reasons, yielding a total of 955 isolates included in the final dataset.

Isolates originating from blood cultures were the most common specimen type submitted (882 isolates (92.4%)), followed by pleural cavity (25 isolates (2.6%)) and other sterile sites (including tissue, fluid, aspirate, abscess and lymph node; 48 isolates (5%)) (Table 1 and Supplementary Material). An increased number of non-blood culture isolates from most age groups (except for 75 years and over) were submitted in 2022 after mandatory notification commenced and made up almost a half of the isolates received from the 0–4 age group (Table 1 and Supplementary Figure S1). A higher number of iGAS isolates were submitted from males compared to females, except for the ages 5–39 years where iGAS isolates were more frequent among females (Table 2).

The highest annual number of isolates were submitted in 2017 (241 isolates), followed by 2019 (201 isolates) (Table 1 and Fig. 1). December 2022 had the highest number of isolates per month (39 isolates), followed by September 2019 (30 isolates) (Fig. 1). In 2020 and 2021, 70 and 74 isolates were received, respectively, aligning with the implementation of public health measures to control the spread of SARS-CoV-2 (Fig. 1). Following easing of public health restrictions, case numbers increased in 2022 to 174 isolates for the year (Fig. 1).

Genomic surveillance of iGAS reveals a dynamic, polyclonal landscape impacted by the COVID-19 pandemic

In silico typing and clustering identified a diverse, polyclonal populations of 78 *emm* types (consisting of 110 *emm* sub types), 123 STs and 104 genomic clusters (Supplementary Table S1 and Supplementary Figure S2). Approximately two-thirds (65.4%) of *emm* type groups consisted of a single ST and a single genomic cluster, leaving another one-third of *emm* types with more than one ST (Supplementary Material). As such, we decided to routinely use the combination of *emm* type and ST for epidemiological surveillance reporting.

Over the 6-year period, the most prevalent *emm*-types were *emm*1 (263 isolates, 27.5%), *emm*12 (172 isolates, 18%, covering ST36, ST807 or ST1128), *emm*89 (81 isolates, 8.5%), *emm*3 (56 isolates, 5.9%, covering ST15 and ST315) and *emm*4 (49 isolates, 5.1%) (Fig. 1, Supplementary Table S1 and Supplementary Figure S2B). Sixty-one *emm*-types were uncommon with <10 occurrences each (172 isolates, 18%) (Supplementary Table S1 and Supplementary Figure S2B).

The dynamics of *emm* types detected fluctuated over time (Fig. 1 and Supplementary Figure S1). Prior to the implementation of the first public health measures to

Year collected	Blood (%)	Pleural cavity (%)	Other (%)	Total
2017	233 (96.7)	4 (1.7)	4 (1.7)	241
2018	186 (95.4)	1 (0.5)	8 (4.1)	195
2019	193 (96.0)	5 (2.5)	3 (1.5)	201
2020	62 (88.6)	0 (0.0)	8 (11.4)	70
2021	68 (91.9)	0 (0.0)	6 (8.1)	74
2022[a]	140 (80.5)	15 (8.6)	19 (10.9)	174
Total	882 (92.4)	25 (2.6)	48 (5.0)	955

[a]iGAS became notifiable in Victoria in February 2022.

Table 1: **Sample types of Victorian iGAS cases between 2017 and 2022.**

Age group, years	Female (%)	Male (%)	Total
0–4	48 (41.0)	68 (58.1)	116
5–19	42 (50.0)	41 (48.8)	83
20–39	93 (55.4)	74 (44.0)	167
40–59	82 (36.3)	144 (63.7)	196
60–74	68 (40.0)	102 (60.0)	170
75+	92 (48.4)	98 (51.6)	190
Total	425 (44.5)	527 (55.2)	952[a]

[a]Sex data unavailable for 3 cases.

Table 2: **Demographic characteristics of confirmed Victorian iGAS cases between 2017 and 2022.**

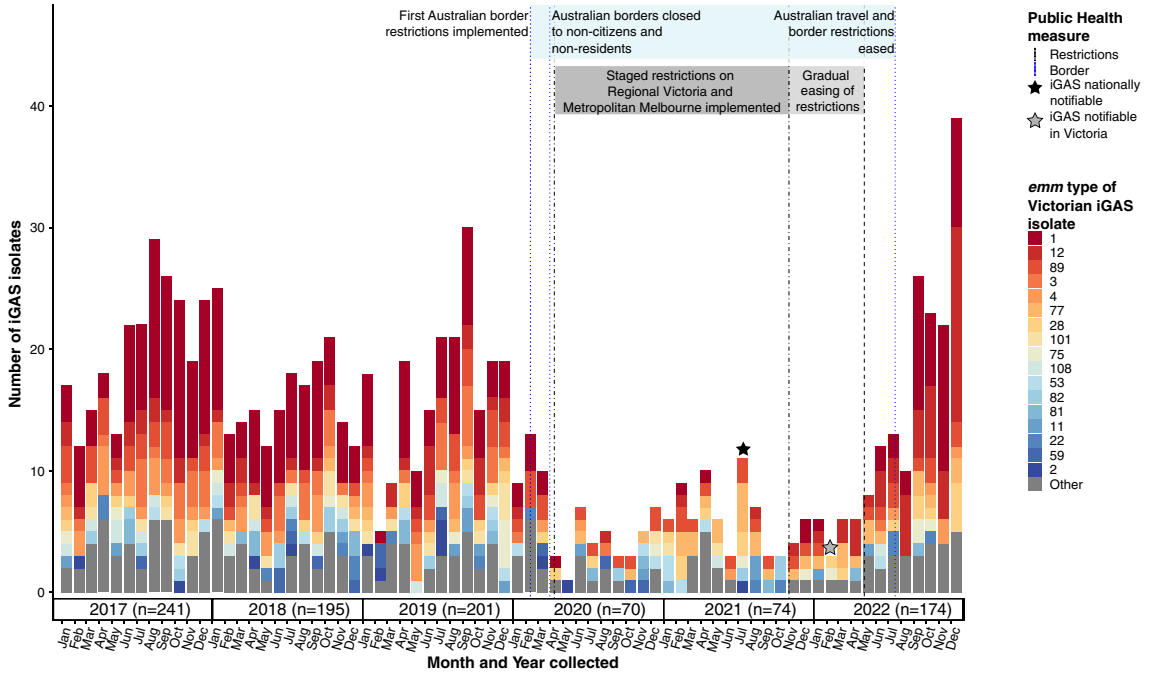

Fig. 1: **The detection of Victorian iGAS isolates and impacts of public health measures implemented due to COVID-19**. Victorian iGAS isolates are plotted each month from 1st of January 2017 to the 31st of December 2022. The seventeen most common *emm* types (each with ≥10 occurrences) are coloured from most common (red) to less common (dark blue). Sixty-one less common *emm* types (with <10 occurrences), are grouped together as 'Other' (grey). Major public health measures implemented for both SARS-CoV-2 and iGAS are highlighted including Victorian staged restrictions (black lines), Australian border restrictions (blue lines), when iGAS became nationally notifiable (black star symbol) and when iGAS notifiable actions were implemented in Victoria (grey star symbol).

reduce the spread of SARS-CoV-2 in Australia (March 2020), *emm*1 isolates were most frequently detected (Fig. 1 and Supplementary Figure S2B). The *emm*1 type disappeared between February 2020 and May 2022, with 21 months without a single *emm*1 isolate detected (Fig. 1). As gradual easing of restrictions in Victoria occurred, from June 2022, detections of *emm*1 increased (Fig. 1).

Prior to 2020, *emm*12 (ST36, ST807 or ST1128), *emm*89, *emm*3 and *emm*4 were the most common *emm* types after *emm*1. From February 2020, *emm*89 isolates were among the most frequent *emm* types detected (between 10 and 12 isolates each year), with 12 *emm*89 isolates (17.1%) the most frequently detected in 2020. The highest number of isolates detected during 2021 were *emm*77 (16 isolates, 21.6%). In 2022, *emm*12 (ST36 or ST1128) isolates were most prevalent, re-emerging with 47 isolates detected (27%, Fig. 1 and Supplementary Figure S2). No clear gain or loss of known virulence factors was noted in the 2022 increase of *emm*12 isolates (Supplementary Material).

Acquired antibiotic resistance

Among the Victorian iGAS isolates, 140 (14.7%) were found to carry genes associated with resistance to tetracyclines, macrolides, aminoglycosides, streptomycin, trimethoprim or chloramphenicol (Table 3). Specifically, 133 isolates (13.9%) contained resistance genes for tetracycline (mostly *tet(M)*, 120 isolates (12.6%)) or macrolides (mostly *erm(B)*, 36 isolates (3.8%)); 46 isolates (4.8%) had resistance genes for both classes (Table 3 and Supplementary Table S1). Seventeen isolates carried three or more resistance genes, while four isolates (0.4%) had six resistance genes, the highest number observed. Trimethoprim, macrolide, or chloramphenicol resistance genes were less prevalent, occurring in eight isolates (0.8%) or fewer, and not associated with any emm types (Table 3 and Supplementary Material). None of the recorded *pbp2x* mutations associated with increased tolerance to penicillin were observed (Supplementary Table S1).

Genomic surveillance revealed a new *emm*1 sub-lineage, $M1_{Aus}$, and *emm*1 population shifts in Victoria

Further analyses of the 263 Victorian *emm*1 lineage identified that 107 isolates belonged to the $M1_{UK}$ sub-lineage (40%), while 23 isolates belonged to the $M1_{Global}$ sub-lineage (8.7%) (Fig. 2A and B). The remaining 133 Victorian *emm*1 isolates (50.5%) formed a discrete, monophyletic lineage within the *emm*1 phylogeny (Fig. 2A). SNP-based analyses supported by

Class	Gene	Prevalence (%)	No. of unique *emm* types where gene present
Tetracycline	*tet(M)*	120 (12.6)	37
Tetracycline	*tet(L)*	1 (<1%)	1
Tetracycline	*tet(O)*	4 (<1%)	1
Tetracycline	*tet(T)*	1 (<1%)	1
Macrolide	*erm(A)*	15 (2%)	7
Macrolide	*erm(B)*	36 (4%)	16
Macrolide	*erm(T)*	2 (<1%)	1
Macrolide	*mefA*	8 (1%)	6
Macrolide	*msrD*	8 (1%)	6
Amikacin/Kanamycin	*Aph(3)′-IIIa*	16 (2%)	8
Streptomycin	*ant6′-1a*	10 (1%)	7
Chloramphenicol	*catA*	7 (1%)	5
Trimethoprim	*dfrF*	8 (1%)	5

Table 3: **Summary of acquired AMR genes by class in Victorian iGAS.**

BAPS groupings (Supplementary Tables S4 and S5), confirming the presence of a novel *emm*1 sub-lineage, which we have designated $M1_{Aus}$, that could be differentiated from the global *emm*1 population by 16 core SNPs and 6 deletions in intergenic, regulatory, and metabolic genes (Fig. 2 and Supplementary Table S4). Recombination and pangenome analyses provided no evidence for a strict gene gain, loss or homologous recombination event being a feature of $M1_{Global}$, $M1_{Aus}$ or $M1_{UK}$, but rather that bacteriophage and virulence prevalence varied within each sub-lineage (Fig. 2A and Supplementary Table S4).

In Victoria, the highest number of $M1_{Aus}$ isolates were in 2017 and 2018 (64 and 45 isolates respectively, Fig. 2 and Supplementary Figure S4A). In 2019, the dominant *emm*1 sub-lineage shifted to $M1_{UK}$ with 28 isolates (Fig. 2B). In 2020 and 2021, fewer than 5 isolates were detected for all sub-lineages. From 2022, $M1_{UK}$ detections increased, and are now the dominant sub-lineage (Fig. 2B and Supplementary Figure S4).

In comparison to the M1 global database (3989 genomes), 211 isolates (5.2%) were identified as $M1_{Aus}$ lineage. In this dataset, in addition to the Victorian $M1_{Aus}$ isolates, there were 51 $M1_{Aus}$ isolates from iGAS cases in Queensland, Australia, 24 isolates from the United Kingdom and 2 isolates from Belgium (Fig. 2A and Supplementary Table S5). Seventeen intermediate isolates ($M1_{Aus\text{-}inter}$) were identified, which contained a subset of the lineage-defining mutations in a similar manner to $M1_{UK\text{-}inter}$ reported previously.[15,29] Among these isolates, 15 were from the UK, and one isolate each was from Belgium and the USA. No intermediate isolates were found among the isolates from Australia. The earliest isolate of $M1_{Aus}$ in Victoria, confirmed by whole genome sequencing, was in December 2012 and $M1_{UK}$ in September 2014.

Discussion

This study represents one of the most comprehensive genomic epidemiology studies of iGAS in Australia to date, including retrospective sequencing analyses of isolates dating back to 2017, before mandatory notification status (February 2022). This study presents an important step in implementation of prospective genomic surveillance, defining optimal typing methods (*emm* type and MLST), and establishing baseline data (including AMR and virulence factors), allowing us to better understand and respond to increased iGAS case numbers after easing of the COVID-19 pandemic restrictions.

Increases in iGAS cases were reported globally during the latter half of 2022 and early 2023, deviating from the usual seasonal variation of some jurisdictions,[7,8] raising concerns within the medical and public health communities including the World Health Organisation and Australian Department of Health. The decline observed in iGAS cases in Victoria during times of intense public health measures during the COVID-19 pandemic, and significant changes in genomic population structures, echoes experiences across Australia, Europe, Scandinavia, and the USA.[8] This reduction, particularly notable for age groups below 20 years and above 60 years, is likely due to reductions in *S. pyogenes* transmission reflecting extended school and childcare closures and visitor restrictions in aged care settings and hospitals.[17,30]

Previous studies have demonstrated significant genomic diversity, confirmed by whole genome sequencing, with many genotypes that are equally likely to cause invasive and non-invasive disease.[18,19,31,32] Our data also demonstrates a dynamic polyclonal genomic population, with multiple genotypes contributing to the recent increase in Victorian iGAS cases, rather than a single outbreak of an *emm* type or acquired pathogenicity element. Understanding the dynamics of this pathogen in each community is critical to inform public health interventions, including vaccine development.

Understanding local genomic epidemiology within a wider global context is also critical, particularly addressing concerns about the spread of highly virulent pandemic lineages or sub-linages, such as $M1_{UK}$. In this study, we identified a previously undescribed *emm*1 sub-lineage we designated $M1_{Aus}$, which was the dominant *emm*1 sub-lineage in Victoria during 2017 and 2018, and has subsequently declined in the post COVID-19 pandemic years alongside the increase is $M1_{UK}$. The origins of $M1_{Aus}$ are not clear at this stage, but we note that it was detected in Victoria as early as 2012 and has also been detected from public data from other regions of the globe. Further research is needed to explore the pathogenesis and molecular mechanisms of this *emm*1 sub-lineage, as well as to investigate its evolutionary

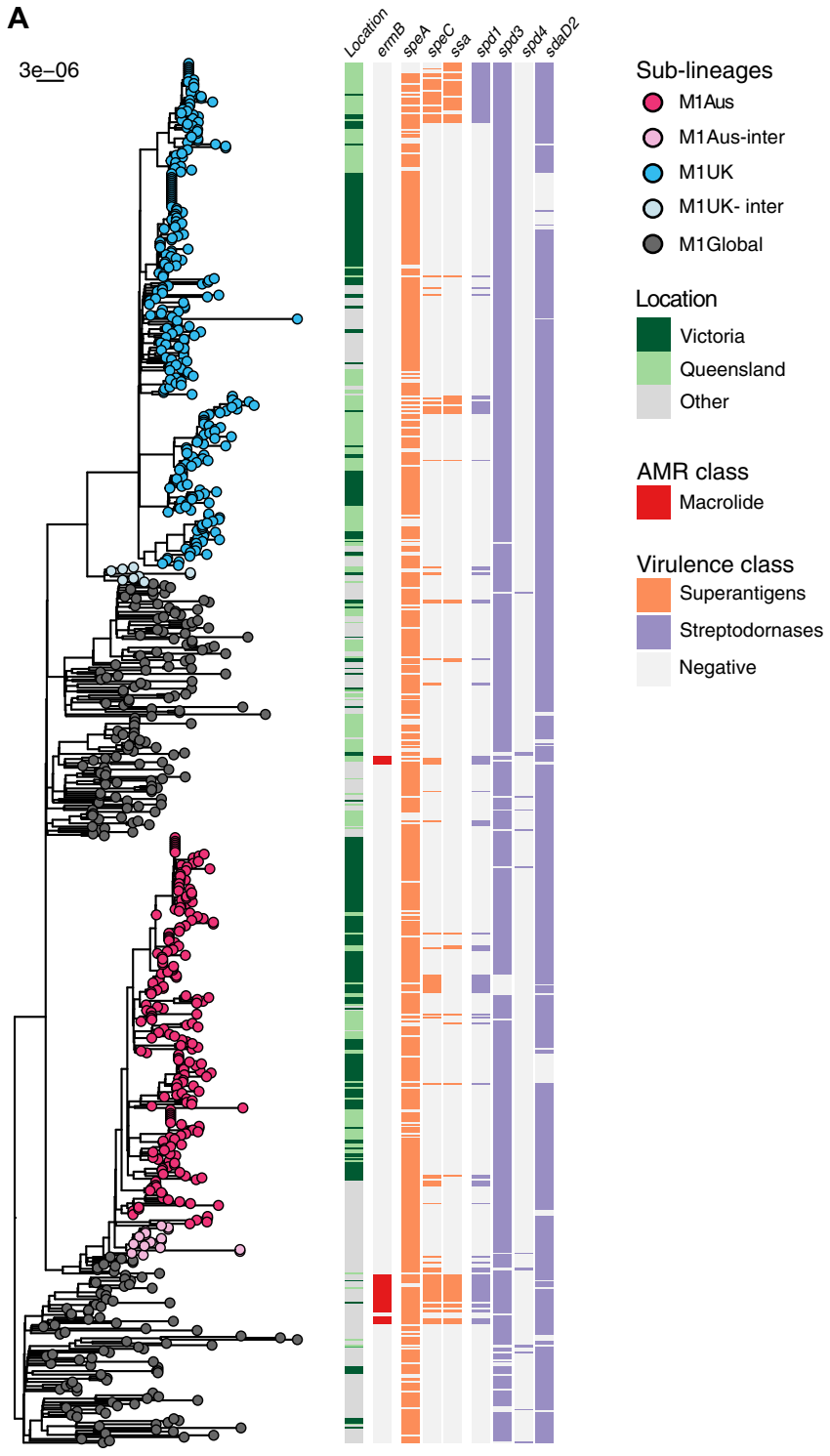

Fig. 2: **Victorian iGAS *emm1* diversity reveals a novel lineage $M1_{Aus}$.** A) The maximum likelihood phylogenetic relationship of the 263 Victorian iGAS isolates are compared with an additional 233 global representative isolates. Tree tips are coloured by sub-lineages; $M1_{UK}$ (blue), $M1_{UK\text{-}inter}$ (light blue) $M1_{Global}$ (grey), $M1_{Aus}$ (pink) and $M1_{interAus}$ (light pink). The heat map shows the location within Australia (shades of green) vs global genomes (grey), followed by the presence of erythromycin resistance gene (*ermB*, red), superantigens *speA*, *speC* and *ssa* (orange), and streptodornase (DNAse) *spd1, spd3, spd4* and *sdaD* (purple). B) The distribution of each Victorian iGAS *emm*1 isolates is plotted, grouped by sub-lineage per month and year.

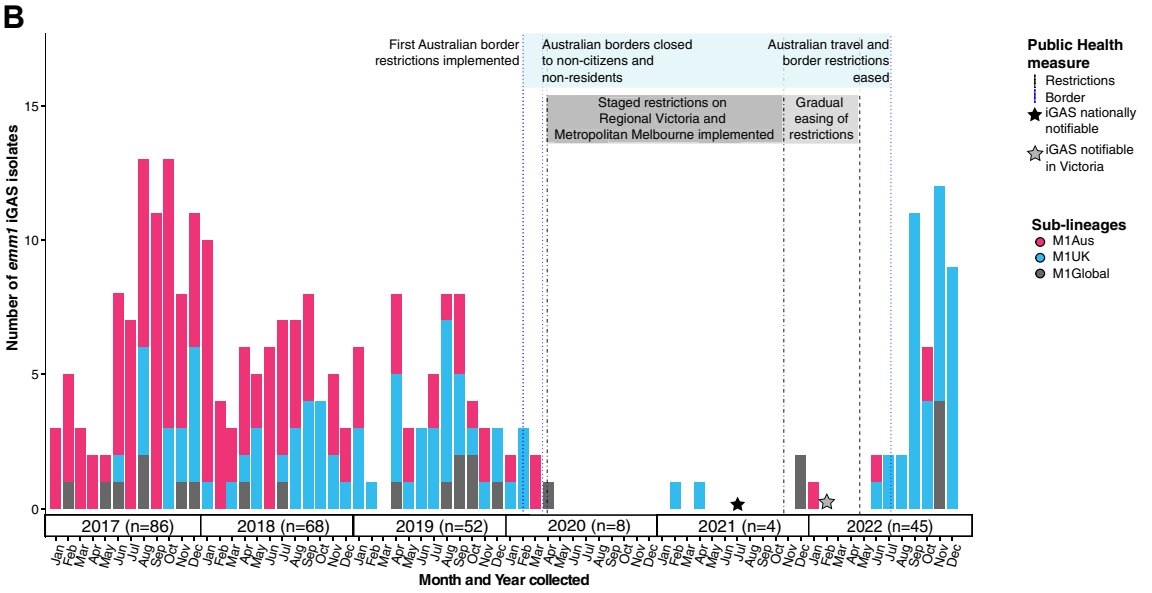

Fig. 2: Continued.

dynamics within global populations and make comparisons across Australia.[15]

From 2019, the dominant sub-lineage shifted to $M1_{UK}$, aligning with global observations.[8,14,15] Population shifts and sub-lineages within the *emm*1 population have been well-characterised, with *atoR* mutations associated with hypervirulence and increased SpeA superantigen production potentially associated with a fitness advantage over the previously described *emm*1 sub-lineages.[14,15] In Victoria, the impact of $M1_{UK}$ as a potential driver of increased iGAS cases is unclear but plausible, and further integrated analyses of clinical and genomic data is planned to extend the utility of genomic surveillance, now possible due to its mandatory notification status.

Antimicrobial resistance is typically low across *S. pyogenes* isolates globally[4,13] and this was also the case in our dataset, with only 14.7% of isolates having an AMR gene detected. Consistent with global trends, resistance to tetracycline and macrolide classes are the most prevalent in the Victorian isolates, with no penicillin resistance mutations detected.[33] Macrolide resistance is of concern internationally, with increasing rates of *erm(B)* acquisition conferring macrolide resistance reported in the United States of America, occurring in 23% of *S. pyogenes* isolates in 2017.[4] Prevalence of *erm(B)*-carrying isolates were somewhat lower in our dataset (3.8%), but it is important to continue monitoring to inform antibiotic recommendations for treatment and prophylaxis. In the absence of comprehensive AMR surveillance of *S. pyogenes*, genomic AMR surveillance (particularly if already being performed for other reasons) provides a reasonable alternative to monitor for emerging AMR threats.

A limitation of this study is that this study period includes both voluntary (2017–February 2022) and mandatory notification and isolate submissions. Therefore, the number of iGAS cases prior to mandatory notification may be underestimated (especially non-bacteraemic cases). However, data from our long-standing Victorian Hospital Pathogen Surveillance System shows the number of blood culture notifications in 2022 are comparable to the number of annual notifications in the 10 years prior, despite the change in notification status.[18] This suggests that the number of missed notifications is likely to be small (Supplementary Material). Further potential confounding factors include the differences in healthcare-seeking behaviour during and after the pandemic, and varying sample submission practices across laboratories.

Future implementation of genomic epidemiologic surveillance for public health should strive to incorporate clinical, epidemiologic, and genomic data, including surveillance of co-infections where possible, to enhance our understanding of the epidemiology, clinical characteristics, and outcomes associated with different strains of iGAS. This study contributes to establishing a genomic framework to provide a foundation for ongoing surveillance and monitoring of iGAS infections in Victoria and Australia. These data can help identify potential outbreaks, track the spread of specific strains, and inform public health interventions to control and prevent further transmission of iGAS.

Contributors

Conceptualisation: JMM, JAL, BPH, NLS; data curation: JMM, JAL, KS, LSK; formal analysis: JMM, JAL; funding acquisition: BPH, NLS; investigation: JMM, JAL, BPH, NLS; methodology: JMM, JAL; project administration: JMM, JAL, KS, NLS; supervision: BPH, NLS; validation: JMM, JAL; visualisation: JMM, JAL; writing—original draft: JMM, JAL, NLS; and writing—review & editing: JMM, JAL, KS, LSK, MW, JS, ME, DH, TMK, AJD, KBG, AWJJ, SYCT, BPH, NLS.

We declare that more than one author has directly accessed and verified the underlying data reported in this manuscript.

Data sharing statement

Whole genome sequence reads from *Streptococcus pyogenes* isolates analysed in this study are deposited in the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the BioProject PRJNA857543 and accession numbers listed in Supplementary Table S1.

Declaration of interests

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2025.101467.

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