Evaluating the role of asymptomatic throat carriage of *Streptococcus pyogenes* in impetigo transmission in remote Aboriginal communities in Northern Territory, Australia: a retrospective genomic analysis

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Summary

Background ***Streptococcus pyogenes*, or group A *Streptococcus* (GAS), infections contribute to a high burden of disease in Aboriginal Australians, causing skin infections and immune sequelae such as rheumatic heart disease. Controlling skin infections in these populations has proven difficult, with transmission dynamics being poorly understood. We aimed to identify the relative contributions of impetigo and asymptomatic throat carriage to GAS transmission.**

Methods **In this genomic analysis, we retrospectively applied whole genome sequencing to GAS isolates that were collected as part of an impetigo surveillance longitudinal household survey conducted in three remote Aboriginal communities in the Northern Territory of Australia between Aug 6, 2003, and June 22, 2005. We included GAS isolates from all throats and impetigo lesions of people living in two of the previously studied communities. We classified isolates into genomic lineages based on pairwise shared core genomes of more than 99% with five or fewer single nucleotide polymorphisms. We used a household network analysis of epidemiologically and genomically linked lineages to quantify the transmission of GAS within and between households.**

Findings **We included 320 GAS isolates in our analysis: 203 (63%) from asymptomatic throat swabs and 117 (37%) from impetigo lesions. Among 64 genomic lineages (encompassing 39 emm types) we identified 264 transmission links (involving 93% of isolates), for which the probable source was asymptomatic throat carriage in 166 (63%) and impetigo lesions in 98 (37%). Links originating from impetigo cases were more frequent between households than within households. Households were infected with GAS for a mean of 57 days (SD 39 days), and once cleared, reinfected 62 days (SD 40 days) later. Increased household size and community presence of GAS and scabies were associated with slower clearance of GAS.**

Interpretation **In communities with high prevalence of endemic GAS-associated skin infection, asymptomatic throat carriage is a GAS reservoir. Public health interventions such as vaccination or community infection control programmes aimed at interrupting transmission of GAS might need to include consideration of asymptomatic throat carriage.**

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Introduction

The global burden from *Streptococcus pyogenes*, or group A *Streptococcus* (GAS), is estimated to be 33 million cases of severe disease and more than 600 000 deaths per year.[1] This burden is particularly borne by people in low-income and middle-income countries, and some minority populations in high-income countries, such as Aboriginal communities in Australia and New Zealand.[2] GAS is a human-restricted pathogen, with the throat and skin providing niches for asymptomatic colonisation and infection.[3] GAS causes direct infections (eg, impetigo, pharyngitis) that can have toxin-induced sequelae (eg, scarlet fever, streptococcal toxic shock syndrome) and post-infectious immunological sequelae (eg, acute rheumatic fever, rheumatic heart disease, acute post-streptococcal glomerulonephritis).[3]

The estimated global population of children with impetigo is 162 million at any one time.[2] The highest rates have been reported from Oceania (median prevalence of 40% among 19 studies), with a median prevalence of 45% in Aboriginal communities in northern Australia.[2] By contrast, symptomatic pharyngitis in these communities is uncommon.[4] Acute rheumatic fever and rheumatic heart disease collectively affect 33 million people, and caused in excess of 300 000 deaths worldwide in 2015.[5] Rheumatic heart disease is the

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Research in context

Evidence before this study

In regions with endemic *Streptococcus pyogenes* (or group A *Streptococcus* [GAS]), high prevalence of infection, and a heavy burden of post-infectious sequelae, interventions targeted at skin infections alone have not been sustainably effective. While many factors contribute to this situation, interventions that only target symptomatic skin infections can potentially leave a reservoir of GAS in the asymptomatic members of the population, which can reseed transmission. In this study, we aimed to investigate the role of asymptomatic throat carriage and impetigo in the transmission of GAS in a remote endemic setting. We searched PubMed for clinical and laboratory studies published from database inception to June 1, 2022, using the search terms "asymptomatic", "carriage", "impetigo", "transmission", "*Streptococcus*", and "*pyogenes*". The search was restricted to English language articles. The literature suggested that direct skin-to-skin contact is the primary driver of impetigo transmission, while respiratory droplet spread is the primary mechanism of spread for pharyngitis. While there have been documented cases of throat infections linked to subsequent skin infections, asymptomatic throat carriers are classed as low risk for the transmission of GAS.

Added value of this study

Most of the 64 genomic lineages (defined as sharing more than 99% of genome and separated by 5 or fewer single nucleotide polymorphisms) were recovered from both asymptomatic throats and impetigo lesions. Genomic lineages were commonly transmitted within and between households, with isolation from throats frequently preceding isolation from impetigo lesions. Asymptomatic throat carriers are likely to be a source of GAS isolates that cause skin infections.

Implications of all the available evidence

The effectiveness of strategies to reduce the burden of impetigo and associated sequelae depends on interrupting GAS transmission, the drivers of which can differ according to the social and environmental contexts. The focus of control programmes on skin-to-skin transmission, where direct contact between individuals is required, might not account for the possible persistence and transmission of GAS via asymptomatic throat carriage. We show that asymptomatic throat carriers could act as reservoirs for strains that cause impetigo, probably introducing new strains into households and the broader community.

principal cause of acquired heart disease in young people aged under 25 years, and disproportionally affects Aboriginal Australians, who are more than 60 times more likely to develop the disease than non-Aboriginal Australians.[6]

Key strategies to reduce the burden of GAS-related disease include primordial prevention (ie, addressing the underlying social determinants of disease, including targeting social and environmental factors) and primary prevention, which is largely based on antibiotic treatment of direct infections with the aim of preventing subsequent acute rheumatic fever. Vaccines have been in development for more than five decades, and are hoped to contribute to primary prevention of infections and sequelae.[7] The effectiveness of these strategies will crucially depend on the transmission dynamics of GAS, which can differ according to the social and environmental context.[8] For example, where symptomatic pharyngitis is more common, the major route of transmission involves droplet spread.[9] However, if pyogenic skin infections outnumber symptomatic pharyngitis, as is seen in remote Aboriginal Australian communities, skin-to-skin contact could be more important.

In settings with endemic skin infections, GAS clones cultured from impetigo lesions have been shown to be shared within and between households.[10] The contribution of the carrier state to GAS transmission is unclear, and it has been suggested that asymptomatic throat carriage does not play a role in transmission of impetigo-related GAS.[11,12] A study of children living at the Red Lake Indian Reservation (MN, USA) in the 1960s[13] revealed that the typical acquisition cascade of GAS infection sequentially progressed from normal skin to impetigo lesions to asymptomatic nose or throat carriage. Within individuals, there were no instances of recovery of GAS from the respiratory tract preceding recovery from normal skin. However, this Red Lake study has not been replicated (in part due to the intensive nature of sampling), and the study only involved children, had a limited time range, and a single GAS M-type (M57) was the predominant circulating strain. A more recent study suggests that strains present in asymptomatic throat carriage can be recovered from the surrounding environment.[14]

If transmission is predominantly skin-to-skin, then interventions targeting this mode of transmission will probably have a large impact in reducing skin infections. However, if asymptomatic throat carriage is an important reservoir of infection, strategies focusing entirely on interrupting skin-to-skin transmission might not be as effective.[15] Similarly, vaccines that target GAS disease, but are without efficacy against asymptomatic throat carriage, might only be able to have limited impact at the population level.

We aimed to elucidate the relative contributions of impetigo and asymptomatic throat carriage to the transmission of GAS by conducting a whole-genome sequence (WGS)-based network analysis of GAS isolates recovered during a 2-year longitudinal surveillance study[4] in remote Aboriginal communities in the Northern Territory of Australia.

Methods

Sample collection and parent study

Full methodological details are in the appendix (pp 2–6). Briefly, *S pyogenes* isolates were collected as part of a prospective surveillance project conducted between Aug 6, 2003, and June 22, 2005 in three remote Aboriginal communities (recorded as community 1–3) in northern Australia with high prevalence of acute rheumatic fever and rheumatic heart disease.[4,16] Upon monthly visits by research staff, individuals were asked about sore throats and impetigo. Swabs were taken from active skin lesions and all throats, regardless of symptoms. Swabs were inoculated on to culture plates, which were transported by air to a laboratory in Darwin, NT for incubation and identification of beta haemolytic streptococci. Group A *Streptococcus* isolates were then stored at –70 degrees Celcius at the Menzies School of Health Research, Darwin, NT, Australia.

Across the three communities, researchers aimed to visit 49 households monthly for a total of 531 household visits. 1173 individuals were enrolled (4842 total consultations) and asked about sore throat symptoms and examined for pharyngitis and impetigo. An estimated 26% of the total population of the communities were enrolled. Sore throat symptoms were reported on nine occasions (0·2% of assessments), with clinical signs of purulent pharyngitis observed twice. Of 1173 individuals, 919 (78·3%) never had GAS recovered, 183 (15·6%) had GAS recovered on one occasion, 50 (4·3%) had GAS recovered on two occasions, and 21 (1·8%) had GAS recovered on three or more occasions. Throat swabs were taken from all participants regardless of symptoms and signs, and swabs were taken from skin lesions. 780 people (67%) were seen on fewer than five occasions, 294 (25%) on five to nine occasions, 77 (7%) on ten to 14 occasions, and 22 (2%) on more than 15 occasions. Time between household visits ranged from 2 days to 313 days (median 81 days). GAS was recovered from at least one individual sampled from 48 out of 49 households over the study duration. The median number of visits for each household during which at least one individual had GAS recovered was six (range one to 22). The consistency of sampling visits to communities was affected by severe weather events and funeral ceremonies (appendix pp 7–8).

For communities 1 and 3, 1089 people were enrolled (548 in community 1 and 541 in community 3). 330 GAS isolates were obtained, 210 from the throat (of whom no participants reported having a sore throat or had purulent pharyngitis) and 120 from skin.[16]

Study design

For this retrospective subanalysis and genomic study, we recovered GAS isolates from two of the three communities (community 1 and 3). Isolates from the other community (community 2) were excluded due to the short sampling period and sparseness of sampling. Ten isolates from communities 1 and 3 could not be revived from storage or were contaminated. We conducted WGS on included isolates using the Illumina MiSeq platform. We conducted in silico analysis using standard pipelines for quality control, genome assembly, phylogenetic analysis, and annotation (see appendix pp 2–3) and removed any genome from downstream analysis that showed signs of contamination of species other than *S pyogenes*, or was of low quality based on read depth and genome assembly metrics (appendix p 2). Using this in silico analysis, we were able to assign typing schemes, phylogeny, single nucleotide polymorphism (SNP)-clusters, and pan-genome analysis.

See Online for appendix

This study received ethics approval from the Human Research Ethics Committee of Northern Territory Health and Menzies School of Health Research (approval number 2015-2516).

Inferring genomic lineages for transmission analysis

Isolates with highly related chromosomal backbones (same *emm* type, multilocus sequence type [MLST] and >99% shared total gene content) were called whole genome clusters (WG-clusters). Within WG-clusters, isolates separated by five or fewer pairwise SNPs were assigned to SNP-clusters, which can be regarded as the same clone and herein are referred to as genomic lineages.

Transmission windows and community transmission

A timeseries analysis of the sampling intervals to determine the most appropriate windows over which to investigate potential transmissions was conducted. To investigate potential transmission, we used two date-of-isolation thresholds: a within-visit range of 0–5 days to calculate the links within each visit, and a range of 14–43 days to calculate the links occurring between each visit. We constructed network models in which nodes represented households and edges represented established links or transmissions between pairs of individuals. Edges were assigned based on two isolates being of the same genomic lineage and being isolated within or between visits. The number of links and source-recipient relationship values were calculated from transmission matrices.

Statistical analysis

We conducted univariable and multivariable survival analyses to determine the factors that influence the time to clearance of GAS, using individuals and households from all three communities as epidemiological units in separate analyses. A Cox proportional hazards survival (time-to-event) analysis was conducted to establish the impact of each variation on the time to clearance of GAS within individuals and households.

Role of the funding source

The funders of the study had no role in study design, data collection, analysis, interpretation or writing of the report.

	Community 1 (n=210)	Community 3 (n=110)	Total (n=320)
Asymptomatic throat swabs	127 (60%)	76 (69%)	203 (63%)
Impetigo lesion swabs	83 (40%)	34 (31%)	117 (37%)
Body or face	5 (2%)	1 (1%)	6 (2%)
Upper limbs	8 (4%)	4 (4%)	12 (4%)
Lower limbs	65 (31%)	27 (24%)	92 (29%)
Other	5 (2%)	2 (2%)	7 (2%)

Data are n (%).

Table 1: **Body sampling sites and frequency of isolation of *Streptococcus pyogenes*, cultured from asymptomatic throat and impetigo lesion swabs**

Results

We performed WGS on 320 GAS isolates from community 1 (127 [40%] asymptomatic throat, 83 [26%] impetigo) and community 3 (76 [24%] asymptomatic throat, 34 [11%] impetigo; table 1). Long-term carriage or infection of a single *emm* type was rare, with only six individuals carrying the same *emm* type at consecutive visits (mean days apart 31 [SD 7]), and two individuals with the same *emm* type recovered at visits more than 120 days apart and with intervening negative samples. 14 individuals had different *emm* types recovered at separate timepoints, and 13 individuals had multiple GAS isolates cultured from different body sites during a single consultation (appendix pp 9–11).

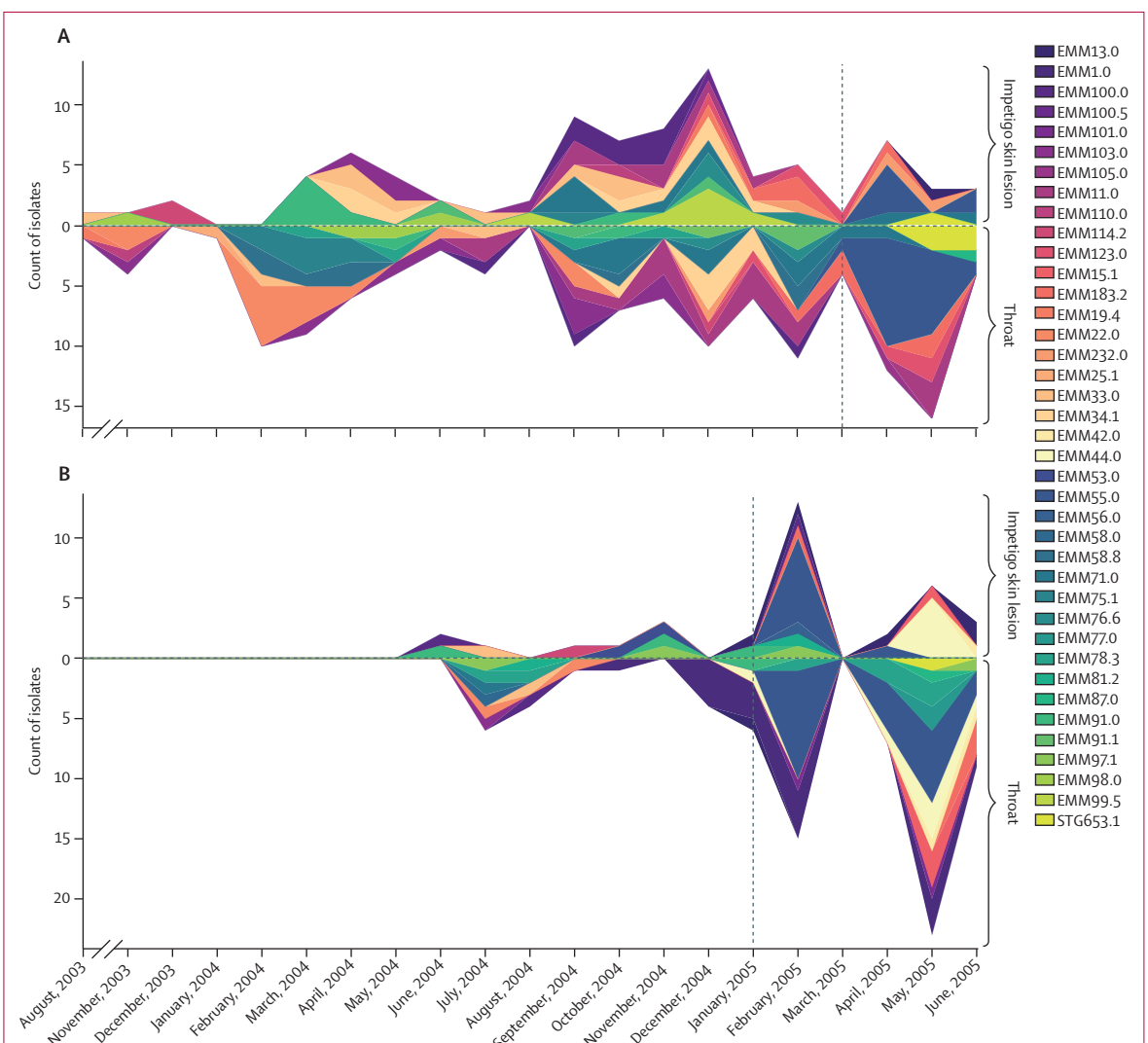

Figure 1: **Distribution of *Streptococcus pyogenes* in the two study communities**
Counts of isolates by GAS *emm*-subtype over the study period are shown for community 1 (A) and community 3 (B), facetted according to recovery from impetigo (top) and asymptomatic throat carriage (bottom). The dotted line represents the month before the first observation of the reported GAS *emm*55 outbreak in each community. GAS=group A *Streptococcus*.

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There was a diverse population of 39 *emm*-subtypes that fluctuated in prevalence over time, with replacement of *emm* types occurring in less than 4 months on average (figure 1; appendix p 12). Some *emm* types were seen at a single visit, others persisted for 10 months. There were no long-term carriage or infections observed in any individuals. Frequently isolated *emm* types were common in both impetigo and asymptomatic throat swabs. However, *emm*1 (n=16), *emm*22 (n=15) and *emm*78·3 (n=11) were exclusively isolated from throat swabs. During the study period an outbreak of *emm*55 isolates arrived suddenly in both communities (as marked by the dotted line in figure 1), disseminated widely, and peaked as the most prevalent *emm* type.

We used a WGS approach to assign genomic lineages based on a whole genome identity of greater than 99% and pairwise core threshold of five or fewer SNPs. 64 genomic lineages were identified from 38 MLSTs and 39 *emm*

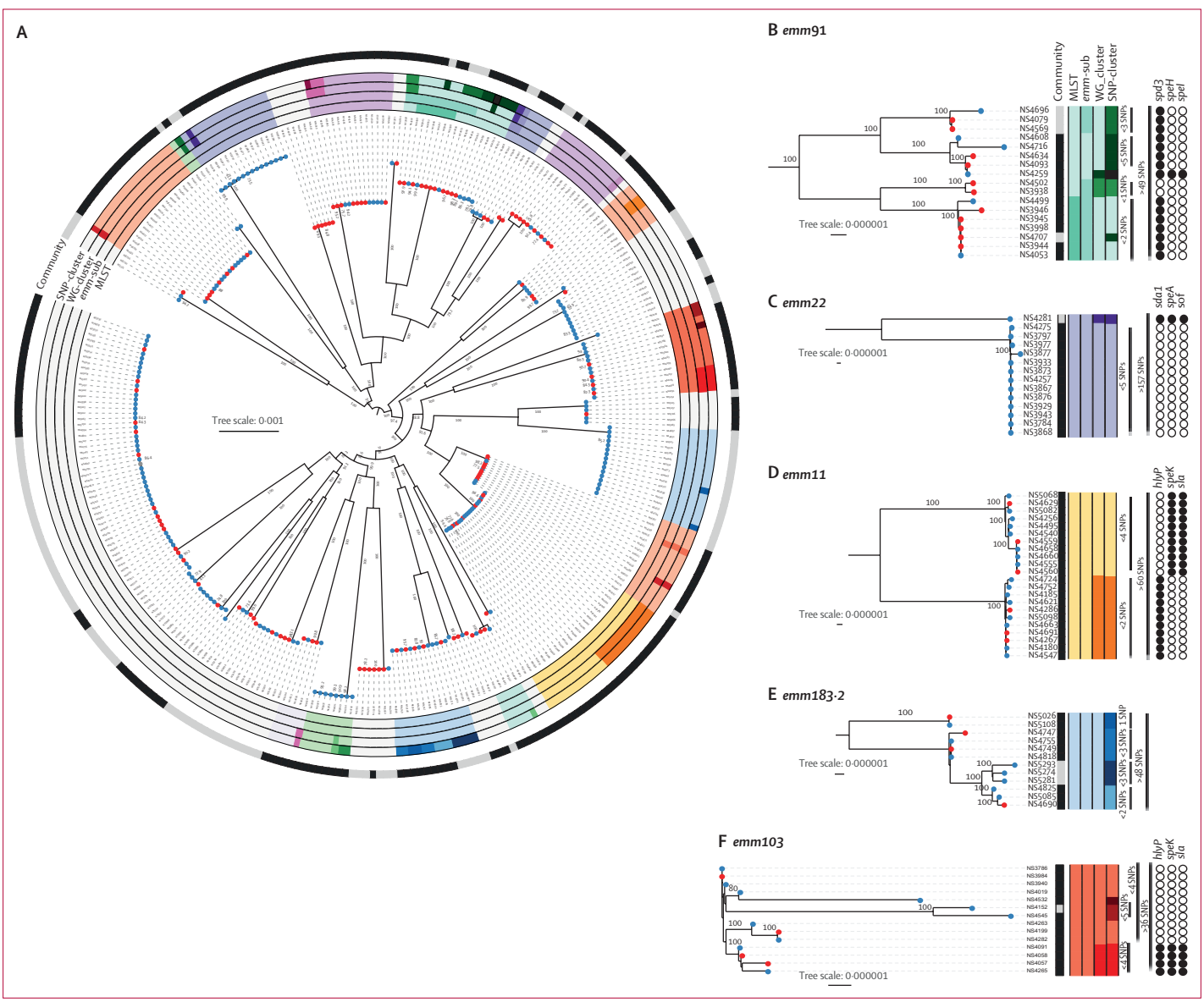

Figure 2: **Maximum-likelihood phylogeny of 320 whole-genome sequences of *Streptococcus pyogenes***

(A) Maximum-likelihood phylogeny of 320 whole genome sequences of *Streptococcus pyogenes*, or Group A *Streptococcus*. Tips are coloured by site of isolation (asymptomatic throat swab in blue, impetigo in red). Concentric rings in grey or coloured from innermost to outermost represent the genomic clustering schemes; MLST, *emm*-subtype, WG-cluster, and SNP-cluster (or genomic lineage). Where each section in the rings is light grey, there is no resolution change between the genomic clustering schemes. Sections that are coloured represent clusters with changes to clustering based on clustering scheme. The outermost ring represents the community where the sample was isolated (community 1 in black, community 3 in grey). (B–F) Maximum-likelihood phylogeny of five different *emm* types used to represent the different diversity within GAS lineages, cluster designation, major accessory variation, and pairwise SNP comparison. Tips are coloured by site of isolation (asymptomatic throat swab in blue, impetigo in red). The grey and black column represents the community where the sample was isolated (community 1 in black, community 3 in grey). Coloured columns from left to right represent the genomic clustering schemes; MLST, *emm*-subtype, WG-cluster, and SNP-cluster (genomic lineage): *emm*91 (green; B), *emm*22 (purple; C), *emm*11 (yellow and orange cluster; D), *emm*183·2 (blue; E), and *emm*103 (red; F). GAS=group A *Streptococcus*. MLST= multi-locus sequence type. WG= whole-genome.

subtypes, and were highly supported through concordance of core genome phylogeny, virulence, and pan-genome analyses (figure 2B–F, appendix p 13). The diversity within *emm* types varied (figure 2). 14 *emm* types, including the *emm*55 outbreak clone, comprised a single genomic lineage. 16 *emm* types were diverse, with variations in core and accessory genome content (figure 2B–F). Key examples include *emm*91, which comprised four genomic lineages with more than 49 SNPs between lineages, and variation in prophage and accessory gene content (figure 2B).

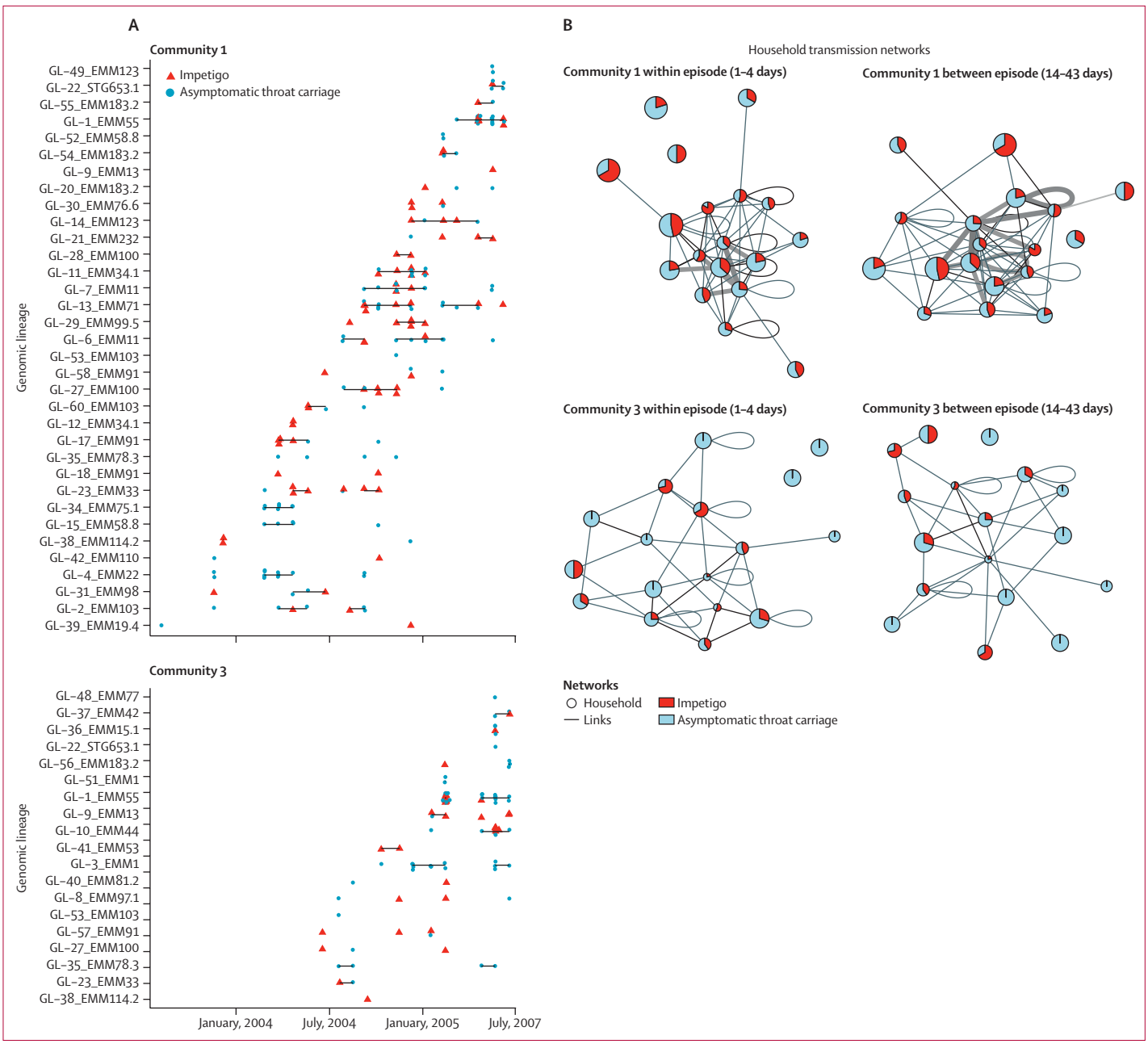

Figure 3: **Lineage timeline plots of *Streptococcus pyogenes***

(A) Lineage timeline plots of *Streptococcus pyogenes*, or GAS, isolates across the study period of both communities clustered by genomic lineage (where number of isolates in lineage is >2). Each point represents an isolate cultured from asymptomatic carriage (blue circles) and impetigo (red triangles). Isolates cultured from a single visit (a single visit, 1–4 days) are represented by overlapping points, and lines connect isolates of the same lineage recovered at consecutive visits (range of 14–43 days between visits). Disconnected points represent genomically linked but epidemiologically unlinked isolates. (B) Household transmission networks of within-visit and between visits for each community. Nodes represent households, size of the node corresponds to the total number of unique individuals within the household and colour shows the proportion of GAS isolates cultured from asymptomatic throat carriage (blue) and impetigo (red). Connected lines show potential links between households when isolates of the same genomic lineage were found in different individuals within the same household (loops) or different households (lines), thickness and darkness of lines corresponds to number of links between nodes. GAS=Group A *Streptococcus*.

	Within-visit links (0–5 days)			Between-visit links (15–43 days)		
	Household	Community	Total	Household	Community	Total
Community 1 (N=185 total links)	10 (5%)	69 (37%)	79 (43%)	14 (8%)	92 (50%)	106 (57%)
Community 3 (N=79 total links)	7 (9%)	37 (47%)	44 (56%)	4 (5%)	31 (39%)	35 (44%)
Total (N=264 total links)	17 (6%)	106 (40%)	123 (47%)	18 (7%)	123 (47%)	141 (53%)

Table 2: **Transmission links within and between households**

13 *emm*22 strains isolated from community 1 formed a single lineage, while the single *emm*22 isolate from community 3 differed to those from community 1 by more than 157 SNPs, and had an additional lysogenic phage encoding the DNase *sda1* and exotoxin *speA* (figure 2C). *emm*11 isolates were separated into two lineages by more than 60 SNPs and the presence of a lysogenic phage encoding superantigen *speK* and phospholipase A2 *sla* (figure 2D), and an approximately 140 kb rearrangement in the chromosome. These two *emm*11 lineages were concurrently circulating in the same community. *emm*183.2 comprised four lineages (figure 2E). *emm*103 also comprised four lineages, one of which had an additional prophage carrying *speK* and *sla* (figure 2F). By comparison with *emm* type classification, genomic lineages persisted for a shorter time (appendix p 14).

To examine GAS strain linkage within and between communities, a household transmission network was constructed by combining epidemiological data (date of isolation, person identifier, and household identifier) with isolate genomic lineages (figure 3). 43 genomic lineages were widespread within the communities covering multiple households (median three; range one to 22), five lineages were found in a single household (represented by two isolates), and eight lineages were found in both communities. 14 lineages were found in more than five households, suggesting widespread community transmission.

We investigated the movement of each genomic lineage within and between community visits. We used dates of isolation and the sampling intervals of 1–5 days (within-visit) and 15–43 days (between community visits) to determine whether isolates assigned to a genomic lineage were epidemiologically linked. From the 296 isolates assigned to a genomic lineage, we determined 264 potential epidemiological linkages (figure 3); 123 links within visits and 141 links between visits; with 35 occurring within and 229 between households (table 2). In comparison to whole genomic lineages, if using *emm* typing as the sole marker for genome similarity, we would observe 12 additional within-visit links and six between-visit links. 47 isolates assigned to genomic lineages were not able to be temporally linked (>43 days apart).

Of the 48 genomic lineages with more than two isolates, 34 contained isolates cultured from both active impetigo and asymptomatic throat carriage, six contained isolates from impetigo only, and eight contained isolates from asymptomatic throat swabs only. To investigate the role of impetigo and asymptomatic carriage in spread of GAS, we inferred the directionality of transmission, and quantified the number of events by asymptomatic carriage and impetigo as the source (appendix p 15). Of the 264 links, the inferred source was asymptomatic throat carriage for 166 (63%) and impetigo for 98 (37%) (table 3).

	Impetigo lesion source	Throat source	Total
Within-visit links			
Community 1	36 (46%)	43 (54%)	79
Community 3	18 (41%)	26 (59%)	44
Between-visit links			
Community 1	35 (33%)	72 (67%)	107
Community 3	9 (26%)	25 (74%)	34
Total	98 (37%)	166 (63%)	264

Data are n (%) or n.

Table 3: **Inferred transmission links by source of transmission link and community**

To examine potential avenues for intervention to reduce the burden of disease, we used this dataset to more broadly investigate factors associated with household GAS clearance. As an individual's time to clearance of GAS is almost certainly shorter than the sampling interval between household visits (range 14–81 days; and appendix pp 16–19 for individual-level analysis), we aggregated individual-level data to form household-level variables (appendix pp 20–21). There were 41 households that had 99 eligible GAS clearance events (with known date of onset and known date of offset of GAS recovery from household members). Households became infected with GAS for a mean of 57 days (SD 39 days) and once cleared, re-infected a mean of 62 days (SD 40 days) later. In the multivariable model, three variables were associated with slower household clearance of GAS: increased household size (adjusted hazard ratio [aHR] per additional individual 0·97, 95% CI 0·96–0·99; p=0·0023), the presence of GAS in the community at the time of household clearance (0·41, 0·22–0·77; p=0·0053), and the presence of scabies in the community at the time of GAS detection (0·60, 0·35–1·00; p=0·054). The model did not violate the proportional hazards assumption (p=0·91).

Discussion

Understanding the transmission dynamics of pathogens in local settings is an important aspect for disease surveillance and control and mitigation programmes.[17] This study has demonstrated that throat carriage may be a reservoir of GAS isolates capable of causing impetigo in endemic disease settings. Within our dataset, most GAS lineages were recovered from both impetigo lesions and asymptomatic throats. Therefore, most of the lineages in our dataset are not restricted to either exclusively a throat or skin niche. Applying a stringent criterion for indicating genomic identity via WGS produced a greater specificity than the use of *emm* typing alone for determining clear evidence of transmission events between throat and skin. We observed highly connected community-wide networks of transmission between individuals presenting with impetigo, and individuals with asymptomatic throat carriage. We observed transmission chains of diverse genomic lineages to pass through multiple individuals, households, and both body sites. In our assessment of the directionality of transmission, the throat appears to be at least as likely as the skin to be a source, and not just a recipient, of transmission.

The Red Lake Indian reservations studies in the 1960s found that among intensively sampled individual household members the temporal sequence involves the presence of GAS on normal skin followed by impetigo lesions, and subsequently the upper respiratory tract.[18] We did not sample intact skin in this study and therefore cannot exclude the possibility of there being no throat-to-skin transmission (ie, that all transmission occurs from skin-to-skin or from skin-to-throat). However, our findings that near-identical clones of multiple genomic lineages were shared across throat and skin, together with existing evidence that strains recovered from asymptomatic throat carriage can be shed and then be cultured from the surrounding environment (including on agar plates placed in classrooms[14]), supports the possibility that transmission from throat to skin may occur. This does not preclude the involvement of intact skin carriage of GAS in the pathogenic pathway of impetigo.[18] Notably, the original source of the GAS on normal skin could not be determined in the Red Lake Indian reservations studies, and the proposed conceptual model by Ferrieri and colleagues[13] indicates an uncertain source. Although not definitive, our findings raise the possibility that the uncertain source is not restricted to skin lesions, and the broader asymptomatic throat carriage within a community could be an important reservoir.

The main conclusions from this study have several implications in public health and disease intervention. Strategies that identify and treat impetigo lesions are important for the individual.[19] Nonetheless, where there is widespread household and community prevalence of asymptomatic throat carriage, treatment of symptomatic impetigo alone might not significantly interrupt transmission. Several skin infection treatment and control programmes have been trialled in remote communities both in Australia and internationally,[20,21] but have proven challenging, and the initial benefits of these interventions have not been sustained long term. However, widespread detection and eradication of asymptomatic throat carriage is also unlikely to be feasible as continued surveillance is burdensome and household turnover and community migration rates are high.[22] Broader public health interventions such as improvements in quality and quantity of housing to reduce overcrowding, and interventions to reduce minor skin trauma such as addressing the burden of scabies remain crucial.[23,24] The SToP (See, Treat, Prevent) skin sores and scabies trial is an example of a skin control programme that includes environmental health and health promotion activities in addition to surveillance and treatment of impetigo lesions.[20]

Currently there is no clear indication as to the protective effect of vaccines against different disease manifestations and the impact on asymptomatic carriage.[7] If throat-to-skin transmission is an important pathway leading to skin infections, then a vaccine reducing throat carriage will likely be more effective than a vaccine protecting only against symptomatic pharyngitis and invasive disease. We observed a diverse GAS population in which genomic lineages fluctuated in prevalence and appeared equally probable to be associated with disease. An effective vaccine would need to cover a large diversity of lineages.[25]

While our findings have a clear application to settings among disadvantaged communities and developing countries, there might be important parallels with the emerging patterns of invasive GAS disease in people who inject drugs and people who are homeless in the USA and Europe.[26] In these populations, there appears to be an association of invasive GAS with cellulitis and skin or generalist *emm* types (*emm* patterns D and E) rather than the traditional throat types such as *emm* types 1, 3, and 12. Notably, these skin or generalist types have been demonstrated to be more likely to be part of local transmission clusters.[27] The actual dynamics of these transmission clusters have yet to be elucidated. Our findings in Aboriginal communities, where skin infections related to *emm* patterns D and E are predominant, suggest that it could be important to expand future epidemiological sampling investigations beyond the skin to the throat in populations of those who inject drugs or experience homelessness.[28] Similarly, a public health focus on skin hygiene and safe injection practices to reduce invasive GAS might not be sufficient if broader issues of disadvantage, such as household crowding, are not addressed.

There are limitations to our study.[4,16] This study only recruited approximately 26% of the total population within each community, and the households selected

were those with a known risk of acute rheumatic fever. As such, the dynamics and infection rates within the recruited households could differ from the rest of the community. GAS was only isolated from around 30% of the impetigo cases, a rate lower than that reported from other studies,[19] and possibly explained by swabs being obtained from both moist skin lesions and dry skin lesions, the latter being less likely to be culture positive. Some GAS may have been missed among mixed cultures with staphylococci and other streptococci. Future studies should consider the use of culture-independent methods for GAS detection. As the interval of sampling appears to be longer than duration of infection, intervening cases of carriage and impetigo, that are important to the transmission chain, will have been missed. We did not sample intact skin or perform environmental screening.

This study raises important aspects to consider for further surveillance and research. Studies that sample more frequently, including sampling the intact skin and with repeated sampling from individuals' skin and throat, together with WGS of recovered isolates, would better quantify the respective contributions of intact skin and asymptomatic throat carriage in transmission pathways.[29] Such studies should be considered in different epidemiological contexts, as the respective contributions to transmission may differ. Similarly, better understanding the role of minor skin trauma and the contributions to such trauma may allow targeted interventions (eg, contribution of scabies).

Asymptomatic throat carriage probably plays an important role in GAS transmission, and is linked to impetigo disease in remote tropical communities. We could not assess the potential role of intact skin carriage. However, our findings could help to explain the absence of durability of treatment programmes targeted at impetigo, as asymptomatic individuals probably remain as an important reservoir of GAS.

Contributors

JAL, AJM, RHC, PTC, CZ, NG, JMV, and SYCT contributed to the conception of this project. JAL, PTC, MIM, PMG, DCH, BJC, JRC, RMA, MRD, and SYCT were responsible for collection of epidemiological laboratory and genomic data. JAL undertook bioinformatic analysis of whole genome sequence data. JAL, AJM, RHC, PTC, CZ, DJP, TBJ, MRD, NG, JMV, and SYCT interpreted or analysed data. JAL, AJM, and SYCT accessed and verified the data in this study. JAL, AJM, RHC, PTC, CZ, DJP, TBJ, JMM, CLG, ACB, JRC, MRD, NG, JMV, and SYCT prepared and edited the manuscript. All authors contributed to the interpretation of results and critical review of the manuscript. All authors approved of the manuscript and agreed with the final decision to submit for publication.

Declaration of interests

All authors declare no competing interests.

Data sharing

Reads are available on the NCBI Sequence Read Archive Bioproject PRJNA879913. 22 of the whole-genome sequences in this study, including the closed genome NS4972, have been previously published[25] under Bioproject PRJEB2232.

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