# Molecular characterisation of group A streptococcus isolates recovered from the north-west of Pretoria, South Africa

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**Background.** Group A streptococcus (GAS) is a human pathogen responsible for a wide range of invasive and non-invasive infections. Pharyngitis caused by GAS may have complications such as acute rheumatic fever subsequently leading to rheumatic heart disease (RHD). RHD continues to have high morbidity and mortality and affects millions of children and young adults, mostly in developing countries. An effective preventive vaccine against GAS may reduce the morbidity and mortality. A 30-valent M-protein-based vaccine is currently at the clinical trials stage of development. Potential vaccine coverage will depend on the geographical distribution of GAS *emm* (M protein) types.
**Objectives.** To determine the *emm* types of GAS isolates circulating in the north-west of Pretoria, South Africa.
**Methods.** Throat swabs were collected from patients aged 3 - 20 years presenting with pharyngitis at one local clinic. In addition, GAS clinical isolates were collected from the National Health Laboratory Service diagnostic laboratory. *Emm* genotyping was done on the GAS isolates by amplification of the *emm* gene followed by sequencing of the 5′ portion of the gene. The *emm* types were correlated with the types in the vaccine.
**Results.** A total of 54 GAS isolates were collected, comprising 19 pharyngitis and 35 clinical isolates. We found 15 different *emm* types among the 43 GAS isolates that were successfully sequenced. Eleven isolates (20%) could not be typed. The most prevalent *emm* type was 92 (26%), which is part of the 30-valent vaccine. This was followed by *emm* 25 and 75, each accounting for 12% of the isolates. Up to 67% of the *emm* types are not covered in the 30-valent vaccine.
**Conclusions.** Fifteen *emm* types were identified, of which 92 was the most prevalent. It is concerning that 67% of the *emm* types are not covered in the vaccine currently under development. It is recommended that surveillance studies be extended to include other parts of the country in order to expand knowledge of the circulating *emm* types.

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Group A streptococcus (GAS) is a pathogen responsible for a wide range of invasive and non-invasive infections.[1-3] Pharyngitis caused by GAS may have complications such as acute rheumatic fever (ARF), which may subsequently lead to rheumatic heart disease (RHD).[4,5] RHD affects millions of children and young adults in developing countries and continues to result in high morbidity and mortality, with 349 000 deaths estimated annually worldwide.[6]

Antibiotics alone, although GAS remains susceptible to them, fail to control the burden of RHD, so other strategies such as preventive vaccines are required.[7-9] Various GAS vaccine candidates are being developed.[10] These are broadly divided into M-protein-based and non-M-protein-based vaccines.[10] M-protein is a major virulence factor of GAS, encoded by the *emm* gene.[11] A 30-valent (SteptAnova) vaccine currently at clinical trials stage consists of 4 recombinant subunits each containing 7 or 8 N-terminal fused peptides of 30 different *emm* types.[8] However, the major drawback is that there are more than 220 *emm* types worldwide, and a single vaccine design with all *emm* types is not practical.[8] Potential vaccine coverage will rely on geographical molecular information of the GAS *emm* types circulating.[8,10,12,13] There are currently limited data on the circulating *emm* types in South Africa (SA).

GAS *emm* typing technique has helped to identify and estimate the diversity of GAS strains that are circulating.[13] This technique is based on amplification of the *emm* gene, followed by sequencing of 160 - 600 bases from the 5′ portion of the gene.[13] The molecular epidemiology of GAS *emm* types is the information required for the development of an effective globally relevant vaccine.[8,14,15]

## Objectives

To characterise the GAS isolates and determine the *emm* types circulating in north-west Pretoria, SA, in order to assess the local relevance of the 30-valent vaccine currently under development. This was an extension of a study conducted in Vanguard in Cape Town by Engel *et al.*[16]

## Methods

### Ethical considerations

The study was approved by the Sefako Makgatho Health Sciences University Research and Ethics Committee (ref. no. SMUREC/M/154/2017: PG) and the Gauteng Provincial Ethics Committee. Permission was obtained from the management of the Dr George Mukhari (DGM) tertiary diagnostic laboratory, National Health Laboratory Service. Consent was obtained from the patients, parents and patient carers where necessary. Assent was obtained from the patients, where possible.

### Sampling

This was a quantitative cross-sectional study. Throat swabs were collected by a research nurse from patients aged between 3 and 20 years presenting with pharyngitis at Soshanguve 3 clinic in north-western Pretoria. The standard demographic data of the patients were recorded. In addition, GAS clinical isolates were collected from the DGM laboratory from May 2017 to October 2018. Both throat swabs and clinical isolates were transported to the research laboratory for further testing.

### Phenotypic identification of GAS

On arrival at the research laboratory, the throat swabs were cultured on 5% sheep blood agar (DMP Diagnostics, SA) and incubated for 18 - 24 hours at a mean (standard deviation (SD)) of 35ºC (2ºC). The presence of GAS was confirmed by standard microbiology tests including haemolysis on 5% sheep blood agar, a catalase test and bacitracin susceptibility testing. Streptex (Thermofisher Scientific, UK) was also used to confirm the presence of the Lancefield group A antigen. The GAS isolates were stored at –70ºC.

### *Emm* typing

*Emm* typing was done according to the guidelines of Beall *et al.*[17] and the Centers for Disease Control and Prevention.[18] Genomic GAS DNA was extracted using the boiling method as described by Dashti *et al.*[19] The 5′ portion of the *emm* gene was amplified using a conventional polymerase chain reaction (PCR). Primer 1 (forward) and primer 2 (reverse) were provided by Inqaba Biotechnologies (SA). The reaction conditions began with an initial denaturation step at 94ºC for 15 seconds, annealing at 47ºC for 30 seconds and an extension at 75ºC for 75 seconds. PCR products were sequenced at Inqaba Biotechnologies and the sequences generated were analysed using BioEdit v71.1 (Biosciences, USA). The resultant sequences were subjected to homology searches on the National Centre of Biotechnology Information (https://www.ncbi.nlm.nih.gov/) basic local alignment search tool (BLAST).[20]

## Results

During the 9-month study period, a total of 114 throat swabs were collected from the patients who presented with pharyngitis at the clinic. Only 19 (17%) were culture positive for GAS. The age range of the culture-positive patients was 4 - 20 years, with the mean (SD) age 11 (7) years (Fig. 1). In addition, a total of 35 clinical GAS isolates were collected from the DGM laboratory, with the ages of the patients ranging from 9 months to 83 years (Fig. 2). The isolates were recovered from various specimen types, with pus swabs being the most common (67%), followed by sputum (19%) (Fig. 3). In total, 54 GAS isolates were available for further testing.

The results of *emm* sequence analysis of 43 GAS isolates are shown in Table 1. We observed 15 different *emm* types, the most prevalent being 92 (26%) (Table 1). The second most prevalent *emm* types were 25 and 75, each accounting for 12% of the isolates. *Emm* 6 and two of its subtypes, *emm* 6.63 and 6.92, together accounted for 14% of the isolates. Of the 15 *emm* types found, only 5 (33%) are covered in the 30-valent vaccine. Eleven isolates (20%) could not be sequenced.

## Discussion

To our knowledge, this is the first study to characterise the GAS *emm* types circulating in the north-western Pretoria region. Data on the molecular epidemiology of GAS in developing countries are limited. There are several vaccine candidates that are currently in the preclinical and clinical phases of

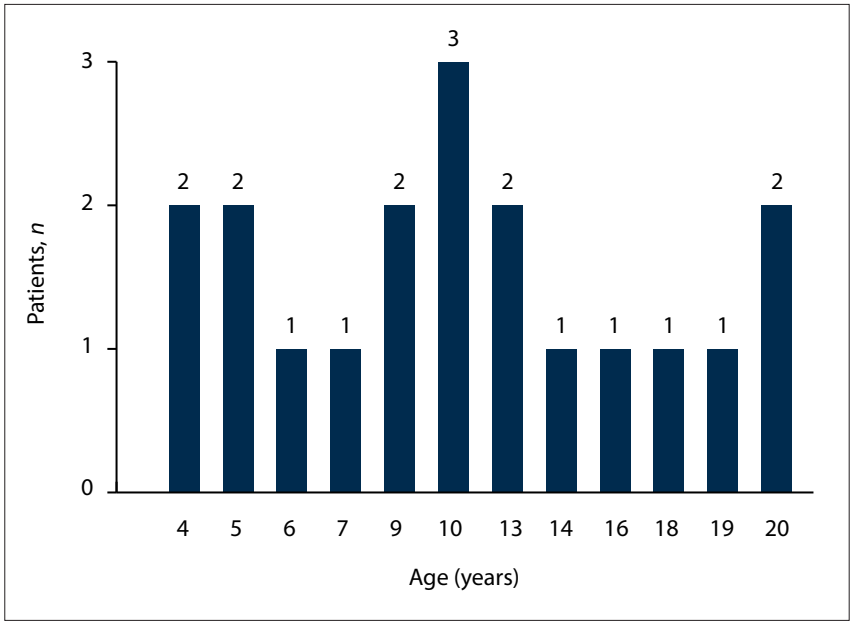


*Fig. 1. Age distribution of patients with pharyngitis who were culture positive for group A streptococcus (N=19).*

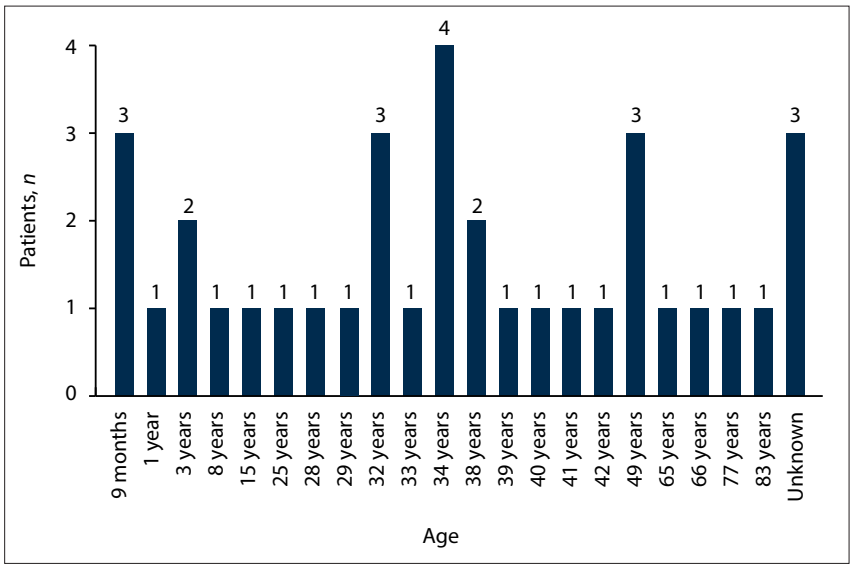


*Fig. 2. Age distribution of patients whose clinical isolates were collected from the Dr George Mukhari laboratory (N=35).*

**Table 1. Summary of *emm* type distribution and vaccine coverage of the isolates (*N*=43)**

| *Emm* type | Isolates, *n* (%) | Site(s) isolated | Vaccine coverage |
|---|---|---|---|
| 92 | 11 (26) | Pus, sputum | VT |
| 25 | 5 (12) | Abscess, pus, throat swab | NVT |
| 75 | 5 (12) | Throat swab, wound swab | VT |
| 6.63 | 4 (9) | Throat swab | NVT |
| 52 | 4 (9) | Pus swab | NVT |
| 3 | 2 (5) | Throat swab | VT |
| 70 | 2 (5) | Throat swab | NVT |
| st3735.0 | 2 (5) | Pus swab | NVT |
| st2147.0 | 2 (5) | Blood culture, sputum | NVT |
| 6 | 1 (2) | Throat swab | VT |
| 6.92 | 1 (2) | Throat swab | NVT |
| 53.1 | 1 (2) | Throat swab | NVT |
| 58 | 1 (2) | Throat swab | VT |
| 80 | 1 (2) | Throat swab | NVT |
| stG245.0 | 1 (2) | Throat swab | NVT |

VT = vaccine type; NVT = non-vaccine type.

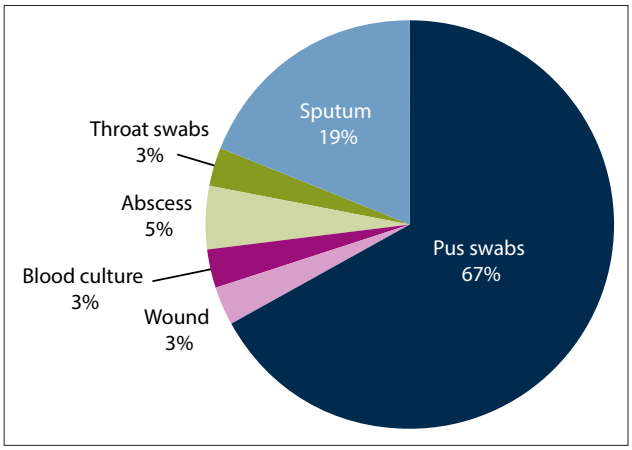


*Fig. 3. Specimen type distribution of the clinical group A streptococcus isolates.*

development, including the 30-valent vaccine. The main objective of this study was to determine the *emm* types circulating in this region, with a view to assessing the potential coverage of the 30-valent vaccine currently at clinical trials stage of development.

GAS is among the most prevalent bacterial childhood infections, constituting 20 - 40% of pharyngitis cases.[1-3] Pharyngitis is prevalent in children aged 5 - 15 years and rarely occurs in children aged <3 years.[1] We managed to collect a total of 149 samples, including 114 swabs collected from patients aged 4 - 20 years. From the 149 samples, we identified 15 *emm* types among 43 isolates, the most prevalent type being *emm* 92, which is a vaccine type. This *emm* type was recovered from pus swabs and sputum samples, which are from non-invasive sites. GAS isolates have previously been isolated from sputum samples associated with pneumonia,[21] and from patients with invasive disease.[22] Of the GAS *emm* types commonly associated with pharyngitis in previous studies, we identified *emm* 3 (5%) and 6 (2%), also from throat swabs. These *emm* types are commonly associated with pharyngitis, and they are also labelled as rheumatogenic owing to their association with ARF.[23]

Several studies have shown that *emm* type distribution may vary in different geographical regions. In a study similar to ours, conducted in Cape Town, SA, in 2014, our collaborators Engel *et al.*[16] reported 26 different *emm* types among 157 GAS isolates. The most prevalent *emm* type in Cape Town was 48, which is also a vaccine type.[16] In a study conducted in Mali, 67 *emm* types among 372 GAS isolates were reported by Tapia *et al.*[24] in 2015. Only 18 of the 67 types (27%) were included in the 30-valent vaccine under development.[8,15,24]

It would be ideal for the developed vaccine to be global, providing sufficient coverage in both developed and developing countries based on the prevalent *emm* types.[8,15] Although our sample size is small, our study suggests that this might not be the case. We reported a high diversity of *emm* types, only 5 (33%) of which are covered in the 30-valent vaccine under development.

Lack of information regarding *emm* type distribution in most parts of SA is a major challenge for vaccine development.[8,15,16] There will therefore be a need for surveillance studies to include other parts of the country in order to expand the knowledge of the *emm* types circulating in the country as a whole. The present study provides important baseline information, but owing to the small study sample size it does not allow robust conclusions. Future studies are warranted to expand the data.

## Study limitations

The present study has limitations. These include the small sample size, which makes it impractical to draw conclusions on the most prevalent isolates in this region. Another limitation is the fact that pharyngitis patients were recruited from a single clinic in the area, and the isolates found may therefore not be representative of the whole region. The fact that the clinical isolates were isolated from the laboratory before a clinical diagnosis was made is another limitation. Lastly, 11 of the 54 isolates could not be sequenced owing to technical issues.

## Recommendations

It is recommended that surveillance studies be done to include other parts of the country in order to expand the knowledge of the *emm* types circulating in SA. This particular study should be continued in order to increase the sample size, reach better conclusions and make statistical inferences.

## Conclusions

To our knowledge, this is the first study to determine the molecular characteristics of GAS in this region. The most prevalent *emm* type is 92 (26%), which was isolated from pus swabs and sputum samples. From the throat swabs, the most commom *emm* type was 6.63 at 9%. Of concern is the fact that 67% of the *emm* types recovered are not covered in the 30-valent vaccine. These data, together with findings from the Cape Town group,[16] provide important information on the circulating *emm* types and form the basis for a vaccine that should provide sufficient coverage in the country. This is a preliminary study, and it will be expanded on.

**Declaration.** The research for this study was done in partial fulfilment of XVK's MSc (Medical Microbiology) degree at Sefako Makgatho Health Sciences University.

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**Author contributions.** XVK, the MSc student, performed experiments, analysed data and drafted the manuscript. OK was involved in data analysis and interpretion as well drafting of the manuscript. HM and MM were involved in throat swab sample collection and patient recruitment. MN was involved in the design of the local study, modifying the UCT protocol, as well as the critical revision of the manuscript. KE and the RHD team at the University of Cape Town shared their study protocol as well as training in and assisting with the optimisation of the *emm* typing technique and data interpretation.

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**Conflicts of interest.** None.

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