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

X V Khosa,1 MSc (Med Microbiol); O Kgasha,1 MSc (Med); H Mabhuza,2 BTh, MB ChB, MFamMed, FCFP (SA); M Moshe,2 MB ChB, FC Paeds (SA); Cert Cardiol Paeds (SA); K Engel,4 BSc (Biotechnol); M Nchabeleng1,5 MB ChB; MMed (Microbiol)

1 Department of Microbiology, School of Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa
2 Department of Family Medicine, School of Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa
3 Department of Paediatrics, School of Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa
4 Department of Medicine, Faculty of Health Sciences, University of Cape Town, South Africa
5 Dr George Mukhari Tertiary Laboratory, National Health Laboratory Service, Pretoria, South Africa

Corresponding author: X V Khosa (xongeekay@gmail.com)

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. Emn 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.
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 35°C (2°C). 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 -70°C.

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 94°C for 15 seconds, annealing at 47°C for 30 seconds and an extension at 75°C for 75 seconds. PCR products were sequenced at Inqaba Biotechnologies and the sequences generated were analysed using BioEdit v7.1.1 (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).](image)

![Fig. 2. Age distribution of patients whose clinical isolates were collected from the Dr George Mukhari laboratory (N=35).](image)
RESEARCH

The main objective of this study was to determine the \textit{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.\textsuperscript{[1-3]} Pharyngitis is prevalent in children aged 5 - 15 years and rarely occurs in children aged <3 years.\textsuperscript{[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 \textit{emm} types among 43 isolates, the most prevalent type being \textit{emm} 92, which is a vaccine type. This \textit{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,\textsuperscript{[21]} and from patients with invasive disease.\textsuperscript{[22]} Of the GAS \textit{emm} types commonly associated with pharyngitis in previous studies, we identified \textit{emm} 3 (5\%) and 6 (2\%), also from throat swabs. These \textit{emm} types are commonly associated with pharyngitis, and they are also labelled as rheumatogenic owing to their association with ARF.\textsuperscript{[23]}

Several studies have shown that \textit{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.\textsuperscript{[16]} reported 26 different \textit{emm} types among 157 GAS isolates. The most prevalent \textit{emm} type in Cape Town was 48, which is also a vaccine type.\textsuperscript{[16]} In a study conducted in Mali, 67 \textit{emm} types among 372 GAS isolates were reported by Tapia et al.\textsuperscript{[24]} in 2015. Only 18 of the 67 types (27\%) were included in the 30-valent vaccine under development.\textsuperscript{[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 \textit{emm} types.\textsuperscript{[4,15]} Although our sample size is small, our study suggests that this might not be the case. We reported a high diversity of \textit{emm} types, only 5 (33\%) of which are covered in the 30-valent vaccine under development.\textsuperscript{[8,15,16]}

Lack of information regarding \textit{emm} type distribution in most parts of SA is a major challenge for vaccine development.\textsuperscript{[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 \textit{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 \textit{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 common 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,[16,18] 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.

Acknowledgements. We thank the DGM laboratory management for granting us permission to collect GAS clinical isolates. We gratefully acknowledge Dr Mark E Engel, principal investigator of the AFROStrep Registry project based at the University of Cape Town, for collaboration and for the training provided for the molecular work. We also acknowledge the dedication of the research nurse. Lastly, we would like to acknowledge the late Prof. Bongani Mayosi, who was involved in the conceptualisation of this study.

Author contributions. XVK, the MSc student, performed experiments, analysed data and drafted the manuscript. OK was involved in data analysis and interpretation 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.

Funding. The work reported was made possible through funding by the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development under the internship scholarship programme (XVK). The content of this article is the sole responsibility of the authors and does not necessarily represent the official views of the SAMRC.

Conflicts of interest. None.

1. Cunningham MM. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2008;21(3):470-501. https://doi.org/10.1128/CMR.00036-08
2. Waller M, Ramsay T, McArthur D, et al. Disease manifestation and pathogenic mechanism of group A streptococcus. Clin Microbiol Rev 2014;27(2):426-301. https://doi.org/10.1128/CMR.00005-13
3. Bessen D, McShan WM, Nguyen, et al. Molecular epidemiology and genomics of group A streptococcus. Infect Genet Evol 2013;23:399-418. https://doi.org/10.1016/j.meegid.2013.01.011
4. Carpoitte RJ, Baxton A, Cunningham MPH, et al. Acute rheumatic fever and rheumatic heart disease. Nat Rev Dis Primers 2016;4:12(1):1-8. https://doi.org/10.1038/nrdpp.2015.84
5. Zühlke L, Betton A, Enger M, et al. Group A streptococcus: Acute rheumatic fever and rheumatic heart disease: Epidemiology and clinical considerations. Curr Treat Options Cardiovasc Med 2017;19(2):15.
6. Charan B, Bar R, Edelman M, Krenn Y, Gal V, Colombier R. Susceptibility of group A streptococcus to antibiotics in northern Israel: A surveillance study. Microb Drug Resist 2015;21(3):551-555. https://doi.org/10.1089/mdr.2015.0040
7. Horn DL, Zahrude M, Austin R, et al. Why have group A streptococcus remained susceptible to penicillin? Report on a symposium. Clin Infect Dis 1999;29(6):1341-1345. https://doi.org/10.1086/316375
8. Dale J, Pentoud T, Tamborini R, et al. Potential coverage of a multivalent M protein-based group A streptococcus vaccine. Vaccine 2013;31(12):1576-1581. https://doi.org/10.1016/j.vaccine.2013.01.019
9. Imou M, Linden M. Antimicrobial susceptibility of invasive Streptococcus pyogenes isolates in Germany during 2003–2011. Plos ONE 2015;10(9):1-8. https://doi.org/10.1371/journal.pone.0137183
10. Suer AG, Carpoitte C, Dale JB, et al. Status of research for the development of vaccines for Streptococcus pyogenes. Vaccine 2016;34(26):2953-2958. https://doi.org/10.1016/j.vaccine.2016.05.073
11. Metzger D, Zampoli A. The M protein of group A streptococcus is a key virulence factor and a clinically relevant strain identification marker. Virulence 2011;2(5):402-412. https://doi.org/10.4161/viru.2.5.16542
12. Sniekers PJF, McMillan DJ, Sriprakah K. The Streptococcus pyogenes M protein: A highly versatile molecule. Trends Microbiol 2016;24(2):273-282. https://doi.org/10.1016/j.tim.2015.10.007
13. Bessen DE, McGregor KE, Whittome AM. Relationships between emm and multilocus sequence types within a global collection of Streptococcus pyogenes. BMC Microbiol 2008;8(59):1-15. https://doi.org/10.1186/1471-2180-8-59
14. Fleminglin A, Chioretti E, Gillen CM, et al. Conserved anchorage surface proteins as group A streptococcal vaccine candidates. J Mol Med (Berl) 2012;110(11):1197-1207. https://doi.org/10.1007/s00109-012-1087-9
15. Dashti A, Bashoff MB, Chary PP, et al. Current approaches to group A streptococcus vaccine development. In: Ferretti JJ, Stevens DL, Fischer VA, eds. Streptococcus pyogenes: Basic Biology to Clinical Manifestations. Oklahoma City: University of Oklahoma Health Sciences Center, 2016:1-68. https://www.ncbi.nlm.nih.gov/books/NBK134113/ (accessed January 2018)
16. Enger M, Mahmut R, Whitcomb A, Muroff M, Mayosi BM, Dale J. Group A streptococcus emm type prevalence among symptomatic children in Cape Town and potential of vaccine coverage. Pediatr Infect Dis 2014;33(2):208-210. https://doi.org/10.1097/INF.0b013e3182a3ea2a
17. Buafi B, Faulkner R, Thompson T. Sequencing emm-specific PCR products for routine and accurate typing of invasive group A streptococcus. J Clin Microbiol 1999;37(4):1109-1112. https://doi.org/10.1128/JCM.37.4.1109-1112.1999
18. Centers for Disease Control and Prevention (CDC). Streptococcus Laboratory. Last reviewed 15 June 2018. https://www.cdc.gov/streptlab/index.html (accessed August 2018).
19. Dauder A, Jarema M, Abdallaam A, Dauder H. Heat treatment of bacteria: A simple method of DNA extraction for molecular techniques. J Environ Anal 2009;42(2):117-122.
20. Alsheikh SG, Miller W, Myers DW, Lyman, DJ. Basic local alignment search tool (BLAST). J Mol Biol 1990;221(3):403-410. https://doi.org/10.1016/0022-2836(90)90329-1
21. Mori N, Hassou S, Oymadice Y, et al. Characteristics of mucoid Streptococcus pyogenes isolated from two patients with pneumonia in a local community. IDCases 2008;6:43-46. https://doi.org/10.1016/j.idcases.2008.02.002
22. Eisenberg J, Luca-Harari B, Jaffe A, Sanders A, Peterson H. Molecular and clinical characteristics of invasive group A streptococcal infections in Sweden. Clin Infect Dis 2007;45(4):458-458. https://doi.org/10.1086/516375
23. Deuschel J, Baxton A, Cunningham MPH, et al. Acute rheumatic fever and rheumatic heart disease. Nat Rev Dis Primers 2015;4:12(1):1-8. https://doi.org/10.1038/nrdpp.2015.84
24. Tapia MD, Sow SO, Tamboura B, et al. Streptococcal pharyngitis in schoolchildren in Bamako, Mali. Pediatr Infect Dis J 2012;34(5):463-469. https://doi.org/10.1097/INF.0b013e3182500608

Accepted 25 November 2020.