Molecular epidemiology of group A streptococcus from pharyngeal isolates in Auckland, New Zealand, 2013

Deborah A Williamson, Nicole J Moreland, Philip Carter, Arlo Upton, Julie Morgan, Thomas Proft, Diana Lennon, Michael G Baker, Rod Dunbar, John D Fraser

Abstract

Aims To describe the molecular epidemiology of emm types associated with circulating pharyngeal group A streptococcus (GAS) isolates in Auckland, New Zealand.

Methods GAS isolates were collected over a 10-day period from a community pathology provider in Auckland. PCR analysis and sequencing of the emm gene was performed at the Institute of Environmental Science and Research.

Results A total of 52 emm types were identified from 278 GAS isolates. The three most common emm types were emm1, emm89 and emm12. Overall, the experimental 30-valent GAS M protein vaccine covered 19 / 52 (37%) of emm types in our study.

Discussion Our study provides baseline data on the circulating pharyngeal GAS emm types in Auckland. Future clinical and molecular surveillance of GAS pharyngitis is essential in the context of ongoing GAS vaccine development.

Group A streptococcus (GAS) is a major human pathogen and is responsible for considerable morbidity and mortality. GAS infections cause a range of acute clinical manifestations, including pharyngitis, skin and soft tissue infection (SSTI), and serious invasive disease such as bacteraemia, necrotising fasciitis and streptococcal toxic shock syndrome. Moreover, the non-suppurative sequelae of GAS infection (rheumatic fever and post-streptococcal glomerulonephritis) result in a substantial clinical and economic burden.

New Zealand has one of the highest reported incidence rates of rheumatic fever, with significant sociodemographic disparity. Consequently, a number of initiatives designed to reduce the incidence of rheumatic fever have recently been implemented in New Zealand. These include measures to: (i) improve housing conditions; (ii) systematically identify and treat childhood sore throats in the school and primary care settings; and (iii) improve patient health literacy. In addition to these public health approaches, there has been renewed interest in developing an effective vaccine to prevent GAS infections and their consequences.

Recently, an international workshop was held in Auckland to assess potential GAS vaccine candidates [Moreland NJ et al, manuscript in draft]. Although several GAS vaccines are currently in development, only two have reached clinical trial stage—the most advanced being a multivalent vaccine based on the GAS M protein, encoded by the emm gene. As such, knowledge of the locally circulating GAS emm types is a prerequisite when considering the potential effectiveness of this vaccine in a specific population.
To date, there are limited contemporary data on the circulating GAS emm types in New Zealand. In this context, and to inform discussion for the above workshop, we performed a ‘snapshot’ survey of the molecular epidemiology of circulating GAS emm types in Auckland.

Methods
LabTests Auckland (LTA) provides the majority of community diagnostic microbiology services to the 1.4 million population of the greater Auckland region. This includes all referrals from primary care, such as general practitioners, midwives, and the school-based throat-swatting programme. Over a 10-day period in January 2013, all non-duplicate group A streptococcus isolates growing from throat swabs were collected. Throat swabs were plated onto tryptic soy sheep blood agar and incubated in 5% CO2 overnight at 37°C. GAS isolates were identified using a MALDI-TOF MS Biotyper (Bruker, Germany) and purity plated onto nutrient agar slopes.

All GAS isolates were forwarded to the Invasive Pathogens Laboratory at the Institute of Environmental Science and Research (ESR) for further analysis. Polymerase chain reaction (PCR) analysis and DNA sequencing of the emm gene was performed using previously described methods. Simpson’s index of diversity was used to assess variation in emm types. This index indicates the probability that two emm types randomly selected are of different types – i.e. the higher the index, the greater the diversity of emm types in a particular population. 95% confidence intervals (CI) for the Simpson’s index were calculated as previously described.

Results
Between the 7th and 16th of January 2013, 1418 throat swabs were received at LTA. Of these, 282/1418 (19.8%) specimens grew GAS. The median age of the patients with GAS isolated was 12 years (range 3–69 years), and 120/282 (43%) of patients were male. Of the 282 GAS isolates, 278 were emm typed. Overall, a total of 52 different emm types were identified (Figure 1).

A relatively small number of emm types predominated, such that six emm types (emm1; emm89; emm12; emm28; emm75; and emm22) together accounted for 59% of all isolates. The Simpson’s index of diversity was 0.904 (95% CI, 0.883–0.924). Overall, 19/52 (37%) emm types were represented in the experimental 30-valent M protein vaccine (Figure 1). These emm types included 17/30 (57%) of the most common circulating emm types (Figure 1).

Recent data suggest the 30-valent M-protein vaccine evokes cross-opsonic antibodies against non-vaccine emm types. When the putative effect of cross-opsonic antibodies against other emm types was considered, the potential vaccine coverage increased to 29/52 (56%) of all emm types, and 21/30 (70%) of the 30 most common emm types (Figure 1).
Figure 1. Thirty most common \textit{emm} types from group A streptococcal pharyngeal isolates in Auckland, New Zealand, January 2013 (*denotes potential effect of cross-opsonic antibodies)

Conclusions

Our study provides a contemporary ‘snapshot’ of the circulating pharyngeal GAS \textit{emm} types in Auckland, New Zealand. Although we found substantial diversity in \textit{emm} types, only a few types predominated.

The three predominant \textit{emm} types in our region (\textit{emm}1; \textit{emm}89 and \textit{emm}12) are similar to those described from GAS pharyngeal isolates in other developed countries. For example, Shulman et al analysed over 7000 GAS pharyngeal isolates in North America over a 7-year period from 2000–2007.\textsuperscript{19} In both the United States and Canada, the two predominant \textit{emm} types were \textit{emm}1 and \textit{emm}12. Similar to our setting, they found that a relatively small number of \textit{emm} types predominated, such that, in their study, 10 \textit{emm} types accounted for approximately 90% of all isolates.\textsuperscript{19} Moreover, Steer et al performed a systematic review of global \textit{emm} types,\textsuperscript{20} and found that in high-income countries, the two most common \textit{emm} types were \textit{emm}1 and \textit{emm}12.

Interestingly, a recent study described the emergence of \textit{emm}89 (the second most common \textit{emm} type in our study) as a major \textit{emm} type in a Canadian population, increasing from 2.7% of GAS isolates in 2002 to 14.7% in 2010.\textsuperscript{21} Of note, the third most common \textit{emm} type in our study (\textit{emm}12) was not represented in the 25 most common \textit{emm} types in a previous study in the Auckland region.\textsuperscript{22}
This study assessed the emm types associated with invasive GAS disease in Auckland from January 2005 to December 2006. However, temporal variation in circulating GAS emm types is well described, as are differences in emm types according to clinical syndrome.

Despite the short sampling frame in our study, we observed considerable diversity in the circulating GAS pharyngeal emm types in Auckland. We found that the experimental 30-valent M protein vaccine covered only 37% of emm types in our sample, although this coverage increased to 57% when only the 30 most common emm types were considered.

Our estimated vaccine coverage is higher than that calculated in the previous Auckland study of emm types associated with invasive GAS disease, where only 17/58 (29%) emm types were covered by the 30-valent vaccine. However, the recent demonstration that immune sera evoked by the 30-valent vaccine contains significant levels of bactericidal antibodies to 24 of 40 non-vaccine serotype indicates the coverage of the multivalent M-protein vaccine may be greater than originally predicted.

When this additional potential coverage was extrapolated to our study sample, vaccine coverage increased to 56% of all strains and 70% when only the 30 most common emm types were considered. Despite this, ongoing questions remain around the widespread usage of M-protein based vaccines, including coverage of emm types in less developed parts of the world, and the theoretical potential for serotype replacement, resulting in the emergence of non-vaccine serotypes.

There were several limitations in our study. We did not have clinical information relating to each patient and as such, were unable to distinguish colonizing and infecting GAS pharyngeal isolates. However, given that each patient had a throat swab taken as part of a primary care consultation it is probable that these patients had pre-test clinical symptoms suggestive of pharyngitis.

A further limitation was our short sampling frame, which meant we were unable to assess longitudinal changes in the molecular epidemiology of circulating emm types. In addition, our sampling frame was during the 2012–2013 school summer holidays, and as such would not have included children presenting as part of the school-based throat swabbing programme.

In summary, our study provides baseline information on the molecular epidemiology of GAS pharyngeal isolates in Auckland, New Zealand. Despite high national rates of rheumatic fever, and ongoing work around sore throat prevention in schools and primary care, there is no formal system of surveillance of GAS pharyngitis in New Zealand.

Future work should aim to systematically assess the national clinical and molecular epidemiology of this significant disease burden.
Competing interests: Nil.

Author information: Deborah A Williamson, Clinical Microbiologist and Research Fellow¹,²,⁴, Nicole J Moreland, Senior Research Fellow³; Philip Carter, Scientist, ²; Arlo Upton, Clinical Microbiologist⁴; Julie Morgan, Senior Technician; Thomas Proft, Associate Professor¹; Diana Lennon, Professor¹; Michael G Baker, Professor⁵; Rod Dunbar, Professor³; John D Fraser, Professor

1. Faculty of Medical and Health Sciences, University of Auckland
2. Institute of Environmental Science and Research, Wellington
3. Maurice Wilkins Centre and School of Biological Sciences, University of Auckland
4. LabTests Pathology, Auckland
5. Department of Public Health, University of Otago, Wellington

Acknowledgements: We thank the laboratory staff at LabTests Auckland for help with collecting isolates. This work was funded by a grant from the Maurice Wilkins Centre for Biodiscovery, University of Auckland. DAW is a Clinical Research Training Fellow of the Health Research Council of New Zealand.

Correspondence: Dr Deborah Williamson, Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand. Email: deb.williamson@auckland.ac.nz

References: