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MICROBIOLOGY

M protein gene (*emm* type) analysis of group A *Streptococcus* isolates recovered during an acute glomerulonephritis outbreak in northern Western Australia

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Summary

Certain M protein types of group A streptococcus (GAS) are known to cause acute post-streptococcal glomerulonephritis (APSGN). Outbreaks of APSGN can occur regularly in tropical regions but the *emm* types responsible are geographically and temporally diverse. GAS isolates from Western Australia (WA) were analysed for *emm* type and *emm* cluster during the period of increased APSGN activity in the tropical northern Kimberley region of WA. Although *emm* types 49, 75 and 108 and corresponding *emm* clusters E3, E6 and D4 were more common in WA during the outbreak there was no predominant circulating *emm* type or cluster found to correspond to the APSGN activity. This is consistent with the high diversity of GAS strains found during APSGN outbreaks in other countries. Potential vaccine coverage of the new 30-valent M-protein GAS vaccine was 70%.

Key words: *emm* type; *emm* cluster; *Streptococcus pyogenes*; glomerulonephritis.

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INTRODUCTION

Acute post-streptococcal glomerulonephritis (APSGN) is an acute nephritic syndrome following group A streptococcus (GAS) infection of the throat or skin. Although the prevalence of APSGN has decreased overall in developed countries, the incidence in developing countries remains high.[1] In the tropical north of Australia sporadic cases of APSGN are reported annually, more commonly following skin infections than after pharyngitis and associated with scabies infestation.[2]

Lancefield M protein serotyping was the gold standard typing schema for the study of GAS epidemiology. Certain M protein serotypes of GAS are more commonly found in the tropics compared to temperate regions, with some M types associated with APSGN, including types 1, 2, 4, 12, 25, 49, 57, 59, 60, and 61.[1] M serotyping has been replaced with the M protein gene (*emm*) molecular typing system which is based on sequence analysis of polymerase chain reaction (PCR) products of the N-terminal hypervariable region of the gene. This method provides a simple typing scheme, with over 200 *emm* types now described, which is highly concordant with M serotyping and has allowed the classification of previously unknown GAS types, especially those from tropical regions.[3,4] The *emm* types can also be grouped into 48 discrete *emm* clusters,[5] based on shared binding and structural properties of the M proteins. This method generates *emm* clusters of *emm* types which are both genetically and functionally similar and have been correlated with tissue tropism (pharyngitis for cluster A–C, impetigo for cluster D, and both for cluster E).[6] The *emm*-cluster typing system has been used for prediction of the M protein antigen content for GAS vaccines.[7]

In May 2014 an increased incidence of APSGN was noted in the tropical northern Kimberley region of Western Australia (WA), commencing late 2013. In mid-2015 a second increased incidence of APSGN was recognised in the Mid-West region of WA. We report the molecular *emm* typing and *emm* clusters of GAS isolates recovered from the skin, throat and other sites from APSGN cases, those linked to APSGN cases, and those not linked to APSGN cases during these periods to ascertain the predominant circulating *emm* types and *emm* clusters.

METHODS

This was a retrospective audit of stored GAS isolates from specimens received by PathWest Laboratory Medicine WA (PathWest) through routine diagnostic testing procedures. PathWest is the single public pathology provider in WA servicing the metropolitan and all regional areas of WA. Local medical practitioners in the Kimberley region of WA were requested to swab infected skin lesions from suspected APSGN cases and their contacts by the regional Public Health Unit during an increased incidence of APSGN. If no skin lesions were present a throat swab was recommended. A suspect case was defined as any two clinical criteria of moderate haematuria on bed-side testing, hypertension (systolic blood pressure >95th centile for age and sex), and facial or peripheral oedema combined with all of laboratory confirmed haematuria, low complement 3 level and a recent GAS infection confirmed by either culture or raised anti-streptolysin O titre (ASOT) and/or antiDNAse B antibody level. All cases were then recommended to receive IM benzathine penicillin regardless of whether skin sores or pharyngitis was evident. Contacts were defined as family, household or other close contacts

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who had resided in the household in the 2 weeks prior to the onset of APSGN in the case.

GAS isolates were obtained over a 10 month period from May 2014 to February 2015 during the increased APSGN activity in the Kimberley region and over a 3 month period from July to September 2015 during increased APSGN activity in the Mid-West region of WA. In addition GAS isolates from routine diagnostic testing of unrelated cases from the Kimberley region and from other regions of WA were also included. Information regarding APSGN diagnosis or contact was obtained retrospectively from the laboratory request form submitted with the original specimen.

The GAS isolates, identified through standard laboratory practice and confirmed by Lancefield typing, were stored in brain heart infusion broth with 15% glycerol (Excel Laboratory Products, Australia), then subcultured and *emm* typed in batches using the established *emm* gene sequencing protocol.[8] Sequences were trimmed and edited (Chromas Lite 2.0) then submitted to the National Centers for Disease Control Blast-*emm* server (http://www2a.cdc.gov/ncidod/biotech/strepblast.asp) to assign an *emm* type and subtype. *emm* clusters were assigned as described by Sanderson-Smith *et al.*[5] Statistical analyses were performed using StatsDirect version 3.0.177 (StatsDirect, UK).

Research ethics

Only stored bacterial isolates originating from clinical specimens were used. The use of human data in this study did not require an approval from the ethics committee as only subject data already obtained for diagnostic testing were used, and all data were de-identified. The study was registered as a research project through the Sir Charles Gairdner Hospital (SCGH) Governance, Evidence, Knowledge, Outcomes (GEKO) System (ID14528) and the SCGH Ethics Committee notified of the intent to publish.

RESULTS

Acute rheumatic fever is notifiable to the WA Health Department but not GAS infection or APSGN. The number of acute rheumatic fever cases in WA decreased from 60 cases in 2013 to 27 cases in 2014 and nine cases in 2015.[9] As APSGN is not notifiable in WA annual surveillance data are not available; however, in May 2014 four APSGN cases were diagnosed within one week in the Kimberley region following two cases in March 2014. The last known increase in APSGN cases in the Kimberley was in 2005 when four cases were diagnosed over a 6 week period. Anecdotally, the baseline incidence of APSGN in the Kimberley is most commonly one to two cases per year. In total, 89 cases of APSGN were reported from the Kimberley region from late 2013 until July 2015.

Recommendations for APSGN surveillance, contact tracing, microbiological sampling and GAS infection treatment were published and disseminated to all clinicians in the region.[10] These measures also guided public health responses in other areas of the state. In addition there was a media release from the WA Health Department to raise awareness to the outbreak.[11]

A total of 148 GAS isolates were obtained from the Kimberley region of WA from May 2014 to February 2015, 17 isolates were collected from the Mid-West region of WA during July, August and September 2015, and a further 72 isolates were collected from other parts of Western Australia (Fig. 1). There were 16 GAS isolates from APSGN cases, 18 isolates from APSGN contacts and 204 isolates from unrelated cases. Due to the retrospective nature of this study it was not possible to link individual contacts to their respective cases. GAS isolates preceding May 2014 were not available.

The majority of isolates were from skin swabs (183, 77%), followed by throat swabs (35, 15%), with the remainder (19, 8%) from a range of other sites. The male to female ratio was approximately equal (126 males, 52.5%) and 90% of samples were from patients aged 1–55 years.

Forty-one *emm* types were identified from 216 of 237 GAS isolates (Fig. 2). Diversity as calculated by Simpson's diversity index (D) was 0.95 [95% confidence interval (CI) 0.94–0.96] for all *emm* types and 0.90 (95% CI 0.83–0.98) for only APSGN-linked *emm* types. *emm*49 was most commonly identified, followed by *emm*75 and *emm*108. Cases of *emm*49 were more common in APSGN-linked regions than non-linked regions ($p = 0.0147$). The only *emm* type in APSGN-linked cases found in both the Kimberley and the Mid-West regions was *emm*49; there were eight *emm*49 isolates linked to APSGN from the Kimberley and one linked to APSGN from the Mid-West. In addition *emm*49 was more commonly found in APSGN cases (50%) than their contacts (11%) ($p = 0.02$). Together, *emm*49, *emm*75 and *emm*108 accounted for 29% of all GAS in this study and were isolated from APSGN-linked cases, with nine of 28 (32%) *emm*49 cases APSGN-linked. Fifteen different *emm* types were found in the 30 APSGN-linked cases. Of the 173 skin isolates *emm*49 (25 isolates), *emm*75 and *emm*108 (15 isolates each)

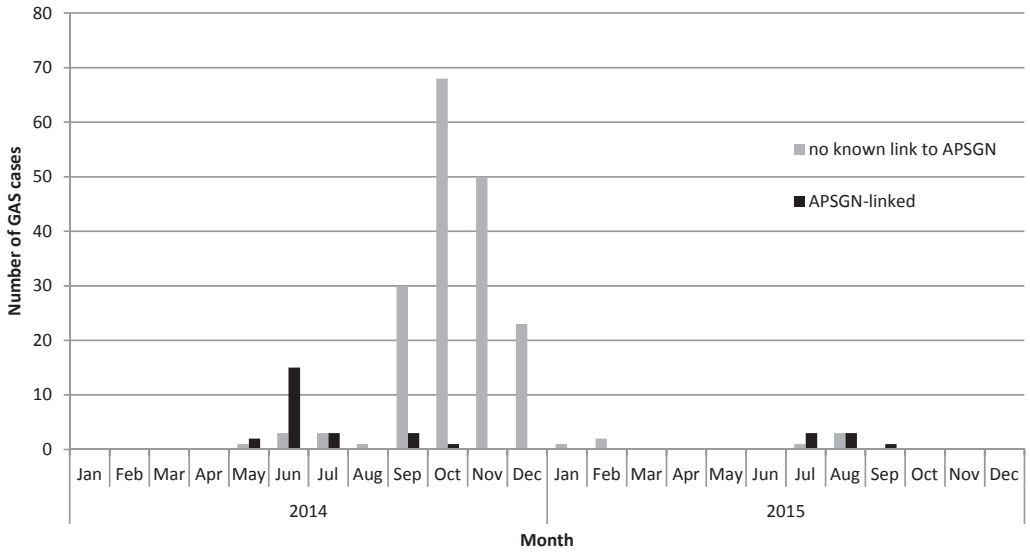

Fig 1 Collection timeline of 216 acute post-streptococcal glomerulonephritis-linked and non-linked group A streptococcus isolates from all regions.

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