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Group A Streptococcal *emm* Type Prevalence among Symptomatic Children in Cape Town and Potential Vaccine Coverage

Mark E. Engel, PhD^{1,*}, Babu Muhamed, MS^{1,*}, Andrew C. Whitelaw, PhD^{2,**}, Munyaradzi Musvosvi, BSc², Bongani M. Mayosi, MD¹, and James B. Dale, MD³

¹Department of Medicine, Groote Schuur Hospital and University of Cape Town Cape Town, South Africa

²Division of Microbiology, Department of Clinical Laboratory Sciences, National Health Service and University of Cape Town, Cape Town, South Africa

³University of Tennessee Health Science Center, Memphis, TN, USA

Abstract

The molecular epidemiology of group A streptococcal pharyngeal infections in children in the Vanguard Community of Cape Town revealed 26 *emm* types among 157 isolates from 742 subjects. Coverage of a 30-valent vaccine is predicted to be 95% of pharyngitis cases in this population at high risk of rheumatic fever.

Keywords

streptococcal pharyngitis; *emm* types; vaccine coverage; rheumatic heart disease

Introduction

Group A streptococcus (GAS, *Streptococcus pyogenes*) causes a wide spectrum of clinical illnesses, ranging from uncomplicated pharyngitis and pyoderma, to serious infections, including necrotizing fasciitis, toxic shock syndrome, and sepsis. GAS is the most common bacterial cause of pharyngitis, which in some cases can trigger the onset of acute rheumatic fever (ARF) and rheumatic heart disease (RHD) (1). RHD accounts for the greatest global burden of GAS disease, as measured by morbidity and mortality, with a prevalence estimated to be over 15 million cases and 349,000 deaths annually (2). 95% of the disease burden from RHD is in low- and middle-income countries (3) and in disadvantaged populations in high-income countries (4, 5) where it continues to have a significant impact on the health of children and young adults. In a recent study in Soweto, South Africa the

Corresponding author: James B. Dale, MD, University of Tennessee Health Science Center, 956 Court Avenue, Room E332, Memphis, TN 38163, PH: (901) 448-2101, FAX: (901) 448-8231, jbdale@uthsc.edu.

*MEE and BM contributed equally to this work.

**New address: Division of Microbiology, National Health Laboratory Service and Stellenbosch University, Tygerberg, South Africa

Conflicts of Interest: J.B.D. is the inventor of certain technologies related to the development of GAS vaccines. The University of Tennessee Research Corporation has licensed the technology to Vaxent, LLC. J.B.D. serves as the Chief Scientific Officer of Vaxent and has a financial interest in the LLC.

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incidence of new cases of RHD in individuals over 14 years of age was calculated to be 23.5 cases/100 000 per annum (6). Programs designed to control the incidence of ARF are based on the administration of antibiotics to prevent GAS pharyngitis in individuals that have previously experienced a bout of ARF (secondary prophylaxis). Antibiotic prophylaxis programs have largely been ineffective in resource-poor countries because of inadequate medical infrastructure and the personnel costs associated with maintaining ARF registries (7).

Introduction of safe, effective and affordable vaccines to prevent GAS infections may be the most cost-effective method of primary prevention of ARF/RHD (8). One of the vaccines in early development is based on the variable N-terminal regions of the surface M protein of GAS (9). M-protein is an important virulence determinant of GAS that also serves as a major protective antigen. Multivalent M protein-based vaccines have been developed that contain up to 30 different M protein peptides expressed as components of recombinant hybrid vaccine proteins (9). Potential vaccine coverage in different geographic regions, especially those with high rates of ARF/RHD, requires a detailed understanding of the molecular epidemiology of GAS infections and the prevalent *emm* types circulating in the community. Currently there is no information regarding the *emm* types of GAS in the South African population, which experiences very high prevalence rates of RHD (6). The current study was undertaken to identify the *emm* types of GAS causing symptomatic pharyngeal infections in children in the Vanguard region of Cape Town and to predict the potential coverage an M protein-based vaccine that is in early clinical development.

Materials and Methods

Pharyngeal isolates of GAS were collected during a study of the molecular epidemiology of GAS pharyngitis in the Vanguard community (Bonteheuwel/Langa) of Cape Town, South Africa. The isolates were from children between the ages of 3 and 15 years presenting with sore throat to the Vanguard Community Health Center, Langa and Netreg Clinics. The details of the epidemiology, clinical characteristics, and demographics, as well as temporal variations in GAS *emm* type prevalence are reported elsewhere (10). After obtaining informed consent from parents or legal guardians, throat swabs were performed and submitted to the Microbiology Laboratory of the National Health Laboratory Service at Groote Schuur Hospital for culture and isolation of GAS. This study was approved by the University of Cape Town Human Research Ethics Committee.

Swabs were streaked onto 2% blood agar and incubated in the presence of 5% CO₂ for 48 hours at 37°C. Beta haemolytic colonies were grouped by the Microbiology Laboratory and isolates belonging to Lancefield groups A, C or G were stored in a -70°C. Only isolates belonging to Lancefield group A were investigated further. GAS isolates were sub-cultured from frozen stocks, DNA was extracted and the 5' portion of the *emm* gene was amplified by PCR using standardized protocols developed at the Centers for Disease Control, Atlanta, GA (11). Purified PCR products were sequenced at Stellenbosch University, South Africa and the sequences generated were analysed using BioEdit v7.0.9 (Ibis Biosciences, USA). The *emm* sequences were submitted electronically to the *S. pyogenes emm* sequence database center at the CDC which assigned all the *emm* types and subtypes (11).

Results

A total of 742 subjects were enrolled in the study between May, 2008-September, 2011. GAS was recovered from 160 participants, yielding a culture-positive rate of 21.6%. Of the 157 GAS isolates available for typing, twenty-six different *emm* types were recovered (See Table, Supplemental Digital Content 1, <http://links.lww.com/INF/B670>). The most prevalent

emm type was *emm48*, which accounted for 15% of the total isolates. The nine most prevalent *emm* types (*emm48*, *emm89*, *emm4*, *emm12*, *emm75*, *emm1*, *emm94*, *emm22*, *emm9*) accounted for 73% of the 157 isolates (See Table, Supplemental Digital Content 1, <http://links.lww.com/INF/B670>). There were eleven additional *emm* types comprising 36 isolates representing 23% of the collection. The remaining six *emm* types were represented by only one isolate. Four *emm* types were represented by two types/subtypes: *emm9/9.2*, *emm48/48.1*, *emm82/82.1* and *116/116.1* (See Table, Supplemental Digital Content 1, <http://links.lww.com/INF/B670>). All of the *emm* types observed in the Cape Town cohort had validated standard reference *emm* sequences. One isolate, which was initially recognized as *st2002.2*, was later validated as *emm240*.

Of the twenty-six *emm* types represented in the Cape Town collection, seventeen (65%) were vaccine types (See Table, Supplemental Digital Content 1, <http://links.lww.com/INF/B670>) that accounted for 63% of the pharyngitis cases in the study (Fig. 1). An additional six *emm* types (23%) have previously been shown to be cross-opsonized and killed (See Table, Supplemental Digital Content 1, <http://links.lww.com/INF/B670>) by rabbit antisera against the 30-valent vaccine (defined as killing ≥ 50% (9, 12). These non-vaccine *emm* types also accounted for 32% of the infections in the study participants (Fig. 1). Two *emm* types were not killed at all in previous bactericidal tests using the 30-valent antiserum and one *emm* type had not been previously tested. Taken together and using the data available to date, the potential coverage of the 30-valent vaccine is approximately 95.5% of all cases of pharyngitis in the study population (Fig. 1) and 88% of the *emm* types represented in the collection of clinical isolates.

Discussion

The African continent has one of the highest prevalence rates of ARF/RHD (3), yet there is a paucity of data related to the molecular epidemiology of GAS causing symptomatic pharyngitis (13). The overall goal of the current study was to document the prevalence of GAS *emm* types in a population at high risk for ARF/RHD and to assess the potential efficacy of an M protein-based vaccine that is under development (9, 12). Previous reports have underscored the complexity of the epidemiology of GAS infections in low- and middle-income countries where RHD is common, indicating that multivalent M protein-based vaccines may provide insufficient coverage against the high number of circulating *emm* types (13). Indeed, a recent study in Bamako, Mali (12) showed that a collection of 372 pharyngeal GAS isolates from symptomatic children contained 67 different *emm* types. Eighteen of the 67 *emm* types (27%) were represented in the 30-valent vaccine. However, functional bactericidal assays using a subset of the most prevalent *emm* types indicated that the 30-valent vaccine could potentially cover 84% of the infections in this population. By comparison, the collection of 157 pharyngeal GAS isolates from Cape Town contained only 26 different *emm* types and 17/26 *emm* types (65%) were vaccine types. The lower diversity of *emm* types in the Cape Town collection more closely resembles the epidemiology of GAS infections in North America and Europe than that observed in tropical/subtropical environments (13). Based on previously published results of bactericidal tests using the *emm* types in the Cape Town collection, we would predict that the 30-valent vaccine could cover ~95% of the cases of pharyngitis in the school age population.

Vaccine prevention of GAS infections that may trigger ARF has been a goal for many decades. Although there are several potential vaccine candidates in various stages of development, studies in animals and humans have shown that M antibodies provide protection against infection with the same serotype of GAS (14). Our recent findings that the 30-valent vaccine evokes cross-reactive bactericidal antibodies against many non-vaccine types indicate that vaccine coverage may be much broader than originally predicted. We

recognize that a human immune correlate of protection against GAS infection has not yet been established. The absolute level of bactericidal activity (50%) used in this and previous studies to define potential “coverage” will need to be determined during future vaccine efficacy trials. However, the concept of “type-specific” immunity is being re-defined based on in vitro functional assays using multivalent vaccine antisera and collections of clinical isolates of GAS (9, 12). The results of the current study suggest that the potential efficacy of highly complex multivalent M protein-based vaccines may be sufficient in populations at high risk of ARF/RHD. This information is a necessary prerequisite for eventual clinical trials designed to measure the impact of vaccines on the incidence of pharyngitis as well as ARF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Summary of 157 GAS *emm* types recovered from symptomatic children in the Vanguard Community of Cape Town and an estimate of potential coverage of a 30-valent M protein-based vaccine. A total of 26 different *emm* types were represented in the collection, 17 of which were vaccine types (black bars) and 6 of which have previously been shown to be cross-opsonized (>50% killing in indirect bactericidal tests) by rabbit antisera against the vaccine (gray bars). Two of the *emm* types were not killed in previous studies (hatched bars) and one was not tested (white bar). Overall potential coverage of the vaccine is predicted to be 95% of all cases of pharyngitis (solid line).