Group A *Streptococcus* (GAS): its surface phosphoglycerate kinase (PGK) and recent epidemiology in Alberta

by

Faisal Hussein Hirji

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Molecular Pathology

Department of Laboratory Medicine and Pathology
University of Alberta

Abstract

Streptococcus pyogenes, also known as Group A *Streptococcus* (GAS) is a bacterium that causes diseases such as pharyngitis, acute rheumatic fever and invasive disease. In Alberta, the invasive GAS incidence rate has increased from 4.77/100 000 (2010) to 7.69/100 000 (2016). GAS *emm* 1 and 59 were the most common *emm* types associated with invasive disease in 2004-2011. However, a more in-depth insight into recent *emm* types and epidemiological trends correlated with invasive GAS has not been conducted. In addition, the theoretical coverage of invasive GAS with the 26-valent and 30-valent vaccines, two GAS vaccine candidates, in Alberta has not been assessed. With regards to the 26-valent and 30-valent vaccines, an issue that arises may be their limited coverage. To improve the coverage, GAS phosphoglycerate kinase (PGK) could be added to the candidates or be an alternative to them. GAS PGK is a glycolytic enzyme that is present on the bacterial surface and reacts with human immunoglobulin. Although PGK has been demonstrated on the surface of a few GAS *emm* types, an extensive assessment of its surface presence has not been conducted. In addition, the mechanisms behind PGK surface expression on GAS have not been explored either. The objectives of this research project are to investigate epidemiological trends associated with invasive GAS in Alberta in 2013- 2016, provide an assessment of the theoretical coverage of the 26-valent and 30-valent vaccines in Alberta for invasive GAS and determine the extent of surface PGK presence in GAS *emm* types tested using enzyme linked immunosorbent assay.

Between 2013- 2016, a total of 1 085 invasive GAS cases were selected for analysis. The top five invasive GAS *emm* types in Alberta during this period, in order of prevalence, were *emm* 1 (204 cases), 82 (71 cases), 28 (70 cases), 101 (66 cases) and 41

(62 cases). Other interesting features were that invasive GAS *emm* 74 was first detected in Alberta (fifteen cases in 2016) and GAS *emm* 59 invasive cases increased from five cases (2013) to 20 (2016), possibly indicating its reemergence in the province. Invasive GAS in Alberta also shows seasonality, affects males (incidence rate of 9.9/100 000 population in 2016) more than females (6.1/100 000 population) and seems to mainly target 51->80 year olds based on specific laboratory-acquired data. According to the theoretical coverage assessment for the 26-valent and 30-valent vaccines, the 30-valent vaccine had better coverage than the 26-valent vaccine in combating recent invasive GAS in Alberta. However, the non-coverage of both vaccine candidates seems to be gradually worsening in Alberta over the years assessed. Therefore, the GAS PGK antigen may help to increase the coverage for the 30-valent vaccine in Alberta.

Twenty-one GAS strains were tested for surface PGK. Out of these 21, 19 demonstrated the surface protein. These 19 strains consisted of 17 different M/*emm* types, of which 14 had previously not been known to possess surface PGK. In addition, the surface expression of PGK on GAS was not apparently affected by the tissue microenvironment. Rather, surface PGK expression was GAS strain-specific. The comparison of GAS strains with detectable and non-detectable surface PGK may lead to a better understanding as to how this protein is surface expressed, as well as its additional roles in GAS pathogenesis.

I dedicate this thesis to my mother Shakila Mithani-Hirji. Thank you for always picking me up when I was down.

Acknowledgements

There are many individuals and organizations without whom my research would not have been possible. I would like to thank my supervisor Dr. Gregory Tyrrell for his support and providing me with the 2014 revised data for analysis, as well as my committee members Dr. Carmen Charlton and Dr. Xing-fang Li for their expert insight and feedback in different aspects of my research. I want to thank the Bacterial Typing Unit, especially Donna Hurteau and Jocelyne Kakulphimp, for working-up and providing the data used in my epidemiological analysis, as well as supplying the requested bacteria used in my experiments. I would like to acknowledge Brad Jansen and the Mycology team for allowing me to use their microplate reader, as well as answering my questions related to reader. I want to thank Danuta Sereda and the Media and Glassware team for the various services they provided, such as supplying skim milk and washing glassware. Within the Tyrrell research lab, I would especially like to acknowledge Areej Alhhazmi for training me to use the reader, allowing me to use her reagents/equipment, providing her expert opinion on my research and being a good friend and colleague. In addition, I want to thank LeeAnn Turnbull for her willingness to always help me out and Maram Hulbah. Finally, I would like to thank Cassandra Feader for her administrative assistance during my program.

The completion of my research would not have been possible without funding. Therefore, I would like to acknowledge the following current or past funding sources: Motyl Graduate Studentship in Cardiac Sciences, DynaLife Dx Major Incentive Award, Queen Elizabeth II Graduate Scholarship – Master's Level, Queen Elizabeth II Graduate Scholarship (Doctoral Level), Alexander Graham Bell Canada Graduate Scholarship – Master's (from NSERC), Walter H. Johns Graduate Fellowship, HE Bell Scholarship, Medical Sciences Graduate Program Scholarship and the University of Alberta Doctoral Recruitment Scholarship. The attainment of these scholarships/awards could not have been made possible without assistance from my supervisor, Cheryl Titus and Dr. Monika Keelan. I would also like to thank Cheryl Titus and Dr. Keelan for their advice and insight into my graduate program.

Finally, I would like to thank my parents Murad Hirji and Shakila Mithani-Hirji for their constant support and encouragement, as well as my aunt Dr. Siddika Mithani for her

guidance, advice and encouragement. Without all of you, I would not have been able to complete this endeavor. Thank you for always being there when I need you!

Table of Contents

List of Tables

List of Figures

List of Abbreviations

A_{405} – Absorbance at 405 nm

A_{600} – Absorbance at 600 nm

Anti-DNASE B – Anti-deoxyribonuclease B

AP1 – Ancillary pilin 1

AP2 – Ancillary pilin 2

ARF – Acute rheumatic fever

ASO – Anti-streptolysin O

BP – Backbone pilin

C4BP – C4b-binding protein

CovR/S - Cluster of virulence responder/sensor

CRP – C-reactive protein

CvfA – Conserved virulence factor A

DNAse – Deoxyribonuclease

DNAse B – Deoxyribonuclease B

ELISA – Enzyme linked immunosorbent assay

ESR – Erythrocyte sedimentation rate

FCT – Fibronectin-binding, collagen-binding T antigen

GAPDH – Glyceraldehyde-3-phosphate dehydrogenase

GAS – Group A *Streptococcus*/Group A streptococcal/Group A streptococci/*Streptococcus pyogenes*

GBS – Group B streptococcus/*Streptococcus agalactiae*

IgA – Immunoglobulin A

iGAS – Invasive GAS

IgG – Immunoglobulin G

Mrp – M related protein

N/A – Not applicable

NR – Not reported

OCD – Obsessive-compulsive disorder

Mga – Multigene activator

MLST – Multilocus sequence typing

NF – Necrotizing fasciitis

PANDAS – Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections

PGK – Phosphoglycerate kinase

PSGN – Post streptococcal glomerulonephritis

PYR – L-pyrrolidonyl-β-naphthylamide

RADT – Rapid antigen detection test

RHD – Rheumatic heart disease

SC – Sydenham's chorea

SF – Sub-family

SOF – Serum opacity factor

SLO – Streptolysin O

SLS – Streptolysin S

Spa – Streptococcal protective antigen

speB – Streptococcal pyrogenic exotoxin B

SpyCEP – *S. pyogenes* cell envelope proteinase

ST – Sequence type

STSS – Streptococcal toxic shock syndrome

TBS – Tris buffered saline

THY – Todd Hewitt and yeast extract

uPAR – urokinase plasminogen activator receptor (CD87)

Chapter 1: Introduction

Streptococcus pyogenes/group A streptococcus (GAS) is a Gram positive bacterium (1-2) that causes an estimated >500 000 deaths/year worldwide (3). It is responsible for a variety of diseases, such as pharyngitis, acute rheumatic fever (ARF), bacteremia, necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS) (1, 4-5). Although antibiotics can be used to combat GAS diseases (5-7), a vaccine is needed to prevent further disease (3, 5, 8). Although there is no licensed vaccine for GAS available, there are candidates in development (8). Examples of these candidates are the 30-valent vaccine, 26-valent vaccine and GAS phosphoglycerate kinase (PGK) (8-12). The 30-valent and 26-valent vaccines are mainly derived from the M protein, a virulence factor and epidemiological marker of GAS (9, 11, 13). GAS PGK is an antigenic glycolytic enzyme that reacts with pooled human sera/immunoglobulin (10, 12, 14-15). It can be surface-bound, present in the cytoplasm or detected in the environment. When present in the environment, GAS PGK stimulates neutrophil activation, which may be responsible for the hyperinflammatory response correlated with invasive GAS infection (10, 16). However, the role of PGK on the GAS surface is unknown. This chapter will provide an in-depth exploration of GAS diseases, epidemiology, virulence factors, vaccine candidates and glycolytic enzymes that will be necessary to understand this thesis work. The objectives of this project are:

a) To provide a recent in-depth epidemiological analysis of invasive GAS in Alberta.
b) To assess the theoretical coverage of invasive cases with the 30-valent and 26-valent vaccines in Alberta.
c) To determine the extent of PGK surface expression in GAS strains tested, in order to gain insight into its potential function in GAS virulence.

1.1 GAS diseases

1.1.1 Pharyngitis

GAS pharyngitis is a superficial infection (17) responsible for approximately greater than 600 million global symptomatic cases per year in individuals more than four years of age (3). The condition mainly affects 5-15 year olds (18) and those in lesser

developed countries (3) and commonly arises in early spring and winter within temperate climates (18). Although transmission of GAS pharyngitis mostly seems to be respiratory-related, foodborne transmission is also possible (19). Presentation of GAS pharyngitis includes fever, sore throat of rapid emergence, vomiting and tonsillopharyngeal inflammation for example (18).

Although GAS pharyngitis is self-limiting, there are benefits in treatment (18). These include lowering GAS transmission to others, alleviating the condition quicker and halting the development of acute rheumatic fever/ARF (discussed further in section 1.1.4). The recommended treatment for GAS pharyngitis is antibiotics (18). However in order to be effective, GAS diagnosis must be timely and accurate. This will respectively prevent ARF development and unnecessary use of antibiotics in non-GAS pharyngitis individuals leading to increasing antibiotic resistance, for example.

1.1.2 Nonbullous impetigo

GAS nonbullous impetigo or GAS impetigo contagiosa is a superficial GAS infection of the skin (17, 20-21) transmitted by direct contact and fomites (6, 20). It is an example of a GAS pyoderma or pus-generating skin infection (20-22), which accounts for greater than 111 million cases in <15 year olds in lesser developed countries (3). GAS nonbullous impetigo particularly affects 2-5 year olds and occurs in humid and warm climates (20-21). Systemic ailments, malnutrition and previous skin disruption are examples of risk factors for GAS nonbullous impetigo. The condition can occur together with impetigo caused by *Staphylococcus aureus* as well (6, 20). GAS nonbullous impetigo is characterized by maculopapular lesions, which become rupturing thin-walled vesicles (20-21). These vesicles generate superficial erosions that are masked by honey-coloured crusts. The erosions can be painful/pruritic. However, the crusts eventually dry and healing of the remainder area occurs. The diagnosis of impetigo is usually clinically-based (21).

Similar to GAS pharyngitis, GAS nonbullous impetigo is self-limiting (18, 20-21). However once again, there are benefits to antibiotics. These include decreased transmission within and between individuals, more rapidly alleviating the condition and

halting GAS nonbullous impetigo reoccurrence (6, 20-21). However, antibiotics seem to be ineffective in halting the progression of GAS nonbullous impetigo to acute poststreptococcal glomerulonephritis (discussed further in section 1.1.5) or PSGN (20-21, 23). Therefore, it is only through proper hygiene that GAS nonbullous impetigo and acute PSGN can be prevented.

1.1.3 Erysipelas

GAS erysipelas is a superficial skin infection typically of the lower limb (20, 24). It typically affects males, those with predisposing /underlying conditions and ≈ 60 year olds (25), children and neonates (26). Risk factors for erysipelas include obesity, impetigo, edema, ulceration and eczema (20, 27). GAS erysipelas displays an upper dermis inflammation with a demarcated erythema, chills, pain and fever (24-25). Left untreated, the condition may develop into necrotizing fasciitis (NF; discussed further in section 1.1.8.3) (4, 20, 27).

1.1.4 Acute rheumatic fever (ARF)

The sequelae ARF occurs 2-4 weeks following GAS pharyngitis that is not treated (28). It affects greater than 471 000 individuals globally each year (3, 28) and is predominant in resource-limited countries (28). In addition, the risk of adults acquiring ARF is low (18, 29). ARF manifests as malaise, weight loss and gradual fever initially (28). Major manifestations of the condition are carditis, Sydenham's chorea (SC), polyarthritis, erythema marginatum and subcutaneous nodules for example (28, 30).

As mentioned in section 1.1.1, antibiotics against GAS pharyngitis halt primary ARF development (18, 29). However for patients with ARF already, antibiotics can be used to prevent recurrent ARF episodes that may lead to RHD (28-29). RHD occurs in 60% of ARF cases and causes an estimated 233 000 deaths/year worldwide (3, 28, 31). It affects the aortic and mitral heart valves typically and can cause valve stenosis, regurgitation or both (32). In addition, RHD can result in stroke and infective endocarditis (3). Antibiotics can prevent RHD or worsening RHD by dealing with recurrent GAS pharyngitis episodes that lead to recurrent ARF (28-29). Therefore, administration of

antibiotics in this case must be long-term and continuous, in order to combat asymptomatic GAS pharyngitis episodes and the fact that recurrent ARF can happen even if GAS pharyngitis is treated. Other treatments for ARF are anti-inflammatory therapy and supportive care (28). For RHD, surgery may be warranted to replace or repair damaged valves (28, 32).

1.1.5 Acute PSGN

Acute PSGN is a post-infection condition that is secondary to GAS pharyngitis and pyoderma (17, 23). It affects an estimated greater than 450 000 individuals/year globally and is prevalent in developing countries, 4-12 year olds and indigenous communities (23). In temperate areas, acute PSGN cases associated with GAS pharyngitis occur primarily in spring and winter, while cases of GAS pyoderma occur predominantly in fall and summer, and no seasonality is noted for tropical climates (23). Acute PSGN symptoms occur 3-6 weeks following GAS skin infections or 1-2 weeks following GAS pharyngitis in most circumstances (23). The condition may be clinical or subclinical. In clinical acute PSGN, hypertension, edema and gross hematuria typically occur and last for a small time period. In subclinical acute PSGN, blood pressure may be normal to mildly increased and microscopic asymptomatic hematuria is present.

Unlike ARF, there is no adequate proof to suggest that early administration of antibiotics prevent acute PSGN development (18, 23, 29). However, antibiotics should still be used to limit transmission of GAS infection that leads to acute PSGN (23). Treatment of acute PSGN focuses on alleviating typically short-term hypertension, edema and hyperkalemia associated with the disease. Long-term prognosis of acute PSGN is generally good, with recurrence being rare (23).

1.1.6 Scarlet fever

Scarlet fever is commonly associated with GAS pharyngitis (20, 33) and rarely with GAS infection of the uterus, surgical wounds or skin and soft tissue (20, 33-35). It mostly occurs in 2-10 year olds in school and can be transmitted by fomites, skin contact and aerosols (35). Typical associated complications of scarlet fever are osteomyelitis,

pneumonia, otitis media, septicaemia, acute glomerulonephritis and rheumatic fever (36). Scarlet fever manifests typically in children as a sore throat and fever, followed by a rash 1-2 days later (37). Except for the soles and palms, the rash distributes throughout the rest of the body. In addition, sandpaper-like papules and erythematous macules similar to sunburn are characteristic of the rash. After the fading of the rash, skin desquamation occurs and lasts 4-6 weeks. In the 19th and early 20th centuries, scarlet fever was responsible for major mortality and morbidity in children (38). Because of antibiotics and improvements to conditions socioeconomically, mortality and morbidity have declined (34, 38). However, scarlet fever cases have increased in England and Wales (39) and outbreaks have occurred in Hong Kong and mainland China (38).

1.1.7 Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS)

PANDAS is a condition that is thought to have an autoimmune nature and is associated with GAS infection (35, 40-41). It occurs prior to adolescence (40) and in individuals with a tic disorder or obsessive-compulsive disorder/OCD (41). PANDAS results in an acute onset of tics and/or OCD symptoms (40). Other abnormalities of the condition include choreiform movements and motor hyperactivity. PANDAS seems to remit and relapse and this nature is correlated with exacerbating GAS infection. There is questionability as to whether PANDAS is a distinct clinical condition, as opposed to a coincidental phenomenon with GAS infection (35, 41). Therefore, therapies for PANDAS cannot be recommended without doing further research on the condition (41).

1.1.8 Invasive disease

Invasive disease occurs upon GAS colonization and penetration of the skin and oropharynx epithelia (42). According to the Alberta Health Notifiable Disease Management Guidelines, a confirmed invasive disease case is defined by GAS isolation from a sterile site, such as blood or peritoneal fluid (43). Invasive disease results in approximately >660 000 cases per year and causes an estimated >160 000 deaths per year worldwide (3). Invasive disease is predominant and causes more deaths in less developed

countries, compared to more developed ones. According to the Active Bacterial Core Surveillance (ABCs), a Centers for Disease Control and Prevention system that investigates invasive GAS cases in ten sites throughout the United States, invasive GAS infections are predominant in early spring and winter (44). In addition, from the same eight year ABCs study, it was reported that <2 year olds, ≥50 year olds and non-whites had higher incidence of invasive infections compared with other ages and whites. In Canada, a national surveillance report showed a similar result to the ABCs study in that <1 year olds and 60+ year olds had a higher prevalence of invasive GAS than other age groups (44-45).

There are many risk factors associated with invasive disease (44). Examples of these include heart disease, diabetes meillitus, smoking (44), pregnancy, usage of injection drugs (4), presence of a nonsurgical/surgical wound, alcohol abuse (13) and infection with varicella virus (4) or influenza virus (46). In addition invasive disease has many forms (4), some of which are described below.

1.1.8.1 Bacteremia

In Alberta, bacteremia has generally been the most common form of invasive disease reported (4, 13). Its severity can range from life-threatening condition to febrile illness (47). GAS bacteremia has an association with cellulitis, toxic shock syndrome and necrotizing fasciitis. These diseases are described below.

1.1.8.2 Cellulitis

In general, cellulitis is the second most common invasive disease form in Alberta (4, 13). It is described as a skin infection that can start from impetigo, extends deeper compared with erysipelas and involves subcutaneous tissue (20, 27). The manifestations of cellulitis are similar to erysipelas in that an orange peel-like skin, skin petechiae/ecchymosis, lymphangitis and fever can develop, for example. Recommended diagnosis of cellulitis involves culture of skin biopsy, tissue aspirate or blood when patients have malignancy, neutropenia and diabetes mellitus for example (7, 20, 27).

1.1.8.3 Necrotizing fasciitis (NF)

NF is another invasive disease form reported in Alberta (4, 13, 43). It spreads from skin lesions and affects the superficial fascia, which refers to tissue between the underlying muscles and skin, and the lower limb extremities mostly (4, 7, 20, 27). NF manifests initially as cellulitis and progresses to involve lethargy, high temperature and disorientation. In addition, cutaneous inflammation, gangrene, edema and anesthesia are commonly present at local sites. NF diagnosis requires good clinical judgment. Examples of findings that suggest the condition include skin necrosis, firm underlying tissue and antibiotic failure towards cellulitis. A more definitive diagnosis is established during surgery, which is also a major treatment method. During surgery, fascia appears dull gray and swollen and possesses stringy necrotic areas. In addition, dissection of the tissue planes occurs readily with blunt instrumentation or a gloved finger. To best establish GAS as the cause of NF, a positive blood culture or Gram stain and culture of deep tissue acquired during surgery is needed.

Surgical debridement as a treatment for NF is carried out until it is no longer required (4, 7, 20, 27). Debridement is coupled with antimicrobial therapy. Antimicrobial therapy is also continued until the patient clinically improves and after 48-72 hours fever is no longer present.

1.1.8.4 Streptococcal Toxic Shock Syndrome (STSS)

STSS is an invasive disease form present in Alberta (4, 13) and associates with GAS cellulitis, NF and bacteremia (4, 42). STSS can manifest as hypotension, disseminated intravascular coagulation, renal impairment, erythematous macular rash which could undergo desquamation and adult respiratory distress syndrome for example (4, 48). Treatment for STSS involves antimicrobial therapy similar to NF (4, 7).

1.2 GAS laboratory diagnosis

1.2.1 Culture and identification testing

To detect GAS, clinical specimens are first cultured on blood agar plates, which are used to screen for colonies displaying β-hemolysis (1, 49). In GAS, streptolysin O (SLO) and streptolysin S (SLS) are responsible for β-hemolysis (49-50). GAS SLO is a secreted pore-forming toxin (50-51) that contributes to hemolysis beneath the blood agar surface, because it is oxygen labile (50). It has been linked to neutrophil apoptosis, suppression of oxidative burst by neutrophils (51), prevention of GAS trafficking to lysosomes and decreased GAS internalization by human oropharyngeal keratinocytes (52) for example. Therefore, SLO may contribute to the survival and persistence of GAS in causing disease (50-52). SLS is an oxygen stable cytotoxin that can be cell-bound or an exotoxin (50, 53). It mainly contributes to β-hemolysis and is active on the blood agar surface (49-50). SLS may be involved in neutrophil death and paracellular translocation of GAS across epithelial barriers for example (54). Ultimately, these potential effects of SLS may contribute to severe GAS invasive disease development.

After screening for β-hemolytic colonies suggestive of GAS, the catalase test is conducted to confirm that streptococci are present (1, 49). Next, species identification tests such as the bacitracin susceptibility, L-pyrrolidonyl-β-naphthylamide (PYR) and Lancefield antigen determination, are performed. The bacitracin susceptibility test involves placing a 0.04 U bacitracin disk on a sheep blood agar plate containing a pure streptococcal β-hemolytic strain and incubating the plate at 35°C overnight in 5% carbon dioxide. The presence of a zone of inhibition around the disc possibly indicates that *S. pyogenes* is present. PYR testing involves detecting the enzyme pyrrolidonyl aminopeptidase, which is present in *S. pyogenes*. Pyrrolidonyl aminopeptidase can hydrolyze PYR to β-naphthylamide. Upon addition of a cinnamaldehyde reagent, β-naphthylamide generates a red colour that indicates the presence of the enzyme. The first Lancefield antigen determination method was developed by Rebecca Lancefield (1, 49, 55). Today, the method involves the use of commercial kits that contain substrates that extract Lancefield antigens located on the streptococcal surface (1, 49). These antigens are combined with antibodies typically against Lancefield antigens G, F, C, B and A. The

detection of the Group A antigen can indicate the presence of *S. pyogenes*. In *S. pyogenes*, the Group A antigen is a cell wall carbohydrate composed of a polyrhamnose backbone and N-acetylglucosamine side chain (49, 56). In addition to its uses in diagnostic testing, N-acetylglucosamine may protect *S. pyogenes* against neutrophil killing and antimicrobials from platelets (1, 56). N-acetylglucosamine has also been implicated in the development of ARF and RHD through its possible role in molecular mimicry (28, 56). With regards to the identification tests described, using at least two tests gives reliable results (49).

An identification test that does not rely on culturing of GAS is the rapid antigen detection test (RADT) (1, 18, 49). The RADT detects the group A carbohydrate directly in throat swabs via immunoassay or agglutination methods. Negative RADT results should be confirmed with a throat culture in adolescents and children. However, negative RADT results in adults do not require a throat culture, because GAS pharyngitis incidence and ARF risk is low (18, 28).

1.2.2 Serologic Testing

Anti-streptolysin O (ASO) and anti-deoxyribonuclease B/anti-DNAse B tests can be used to confirm a recent history of GAS pharyngitis and therefore aid in the diagnosis of acute PSGN and ARF (23, 28, 30). The ASO test determines the titers of antibodies targeting SLO (49-50). These titers can be measured using a neutralization assay, whereby patient serum containing antibodies against SLO are exposed to SLO.

GAS DNAses are secreted proteins produced by all strains (57). They seem to be significant in the development of GAS pharyngitis and protect against killing by polymorphonuclear leukocytes. DNAse B is a DNAse specific to *S. pyogenes* that produces a consistent host immune response (1, 49). Compared with ASO titers, anti-DNAse B titers more accurately confirm the presence of a previous GAS skin infection and can be elevated for a longer period. Similar to ASO titers, anti-DNAse B assays can be of the neutralization variety, where nuclease inhibition in the presence of patient serum is determined.

1.3 GAS typing

Reference laboratories primarily conduct GAS typing (1, 49). Typing is used in outbreak settings and epidemiologic surveys and does not immediately impact therapeutic or diagnostic decisions related to GAS. A few typing systems for GAS are described below.

1.3.1 M/emm typing

1.3.1.1 emm pattern groups

There are five *emm* pattern groups correlated with GAS (1, 58). These group designations are based on the arrangement of three genes in the GAS chromosome designated *mrp*, *enn* and *emm* (Figure 1.1, 1, 58). The *mrp* gene is located upstream from the *emm* and *enn* genes. The *mrp* gene encodes for the M-related protein (Mrp), a GAS surface protein that can bind to human immunoglobulin G (IgG) and fibrinogen (59-60). The interaction of GAS with fibrinogen through Mrp may aid the bacterium in avoiding phagocytosis by halting complement deposition (1, 61). Similarly, the binding of the Fc region of IgG to Mrp may also provide phagocytosis resistance (1, 62). The *mrp* gene is present in particular GAS strains and has a 3'peptidoglycan cell-wall spanning domain that exists in a genetic form referred to as sub-family (SF)-4 (Figure 1.1, 1, 58). The *enn* gene is located downstream from the *emm* and *mrp* genes. The M-like protein is produced from the *enn* gene (1, 58, 62), located on the GAS surface and binds to the immunoglobulin A (IgA) Fc region (60, 63). The *enn* gene is highly prevalent in GAS strains and similar to the *mrp* gene, has a 3'peptidoglycan cell-wall spanning domain (Figure 1.1, 1, 58). The genetic forms of this domain in the *enn* gene can be either SF-3 or SF-1. The *emm* gene encodes the M protein (discussed further in section 1.3.1.2) and can be present between the *mrp* and *enn* genes (Figure 1.1, 1, 58, 62). The 3'peptidoglycan domain of the *emm* gene has two forms referred to as SF-2 and SF-1.

The five *emm* pattern groups of GAS are known as *emm* pattern A-E (Figure 1.1, 1, 58). *Emm* pattern A is defined by the presence of an SF-1 *emm* gene only. *Emm* patterns B and C are similar in that both possess the SF-1 *emm* gene as well. However, *emm* pattern

B also contains an SF-1 *enn* gene, while *emm* pattern C has an additional SF-3 *enn* gene. Because *emm* patterns B and C are rarely seen, they are combined with the *emm* pattern A group to form the *emm* pattern A-C group. *Emm* pattern D contains an SF-4 *mrp* gene, SF-1 *emm* gene and SF-3 *enn* gene. *Emm* pattern E possess the same gene forms for *mrp* and *enn*, as *emm* pattern D. However, *emm* pattern E has a SF-2 *emm* gene, rather than a SF-1 *emm* gene.

Although exceptions exist, *emm* pattern groups generally correlate with GAS tissue tropism (1, 58, 64). *Emm* pattern A-C is correlated with throat infections, while *emm* pattern D is linked to superficial skin infections. *Emm* pattern E has associations with both superficial skin and throat infections.

1.3.1.2 M protein and other Mga-regulated proteins

The M protein was identified by Lancefield in the 1920s (65-66). It is a fibrillar α-helical coiled-coil dimer and extends from the GAS cell wall (1, 64, 67-68). The length of the M protein is related to the *emm* pattern (Figure 1.2, 64). In the McMillan *et al* study, *emm* pattern A-C was predicted to have the longest M proteins while *emm* pattern E should possess the shortest. The N-terminal of the M protein is surrounded by the extracellular environment and is thought to consist of variable and conserved amino acid regions (Figure 1.2, 1, 49, 64, 68). The heterogenic sequence of amino acids in the N-terminal gives rise to the antigenic diversity associated with the M protein. In contrast, the conserved M protein C-terminal is present in the cytoplasm.

The M protein N-terminal is thought to consist of non-repeat and repeat amino acid sequences (Figure 1.2, 1, 49, 64, 68). These repeat sequences are designated A, B, C and D. In the McMillan *et al*. study, the A repeats are predicted to be located in the variable region and were more common for *emm* pattern A-C than D or E (Figure 1.2, 1, 49, 64, 68). The B repeats are present in the variable region as well and are more commonly associated with *emm* pattern A-C and D. In addition, although most M proteins that possess B repeats contain two tandem repeat units, a greater number of units are correlated with *emm* pattern A-C. The C repeat region consists of C repeat units and seems to be present in all M proteins. The units are located in the conserved region and there are 35

conserved amino acids per unit. Most M proteins contain three C repeat units and sometimes these units are separated by C repeat linkers. The D repeats are located in the conserved region and closest to the M protein cell wall spanning domain. A non-helical region seems to be prevalent in 80% of M proteins and located in the variable region preceding the repeat and non-repeat regions.

The M protein is transcriptionally controlled by a positive single-component transcriptional regulator known as the multigene activator or Mga (62-63). The *mga* gene is located upstream from the *emm* or *mrp* gene depending on the *emm* pattern considered and regulates other genes besides the *emm* gene (58, 62-63). Examples of these other genes include *mrp*, *enn*, *sof* and *scpA*. The *sof* gene produces serum opacity factor (SOF), a surface and released protein that possess two important functional domains (1, 62, 69-70). The first domain is an opacification domain that interacts with high-density lipoprotein and ultimately results in host serum opacification (1, 70). This opacification is inhibited with antisera targeting a SOF type-specific determinant (1, 58, 69, 71). This opacification inhibition forms the basis of the historical SOF typing method. Another method to determine SOF types involves sequencing the *sof* gene's 5'end (58, 62, 71). Besides its role in typing, the opacification domain seems to inhibit GAS beta-hemolysis, participate in invasion of epithelial cells, bind fibulin-1 and enhance GAS virulence in mouse models for invasive infection (1, 58, 70, 72-73). Fibulin-1 is located in human plasma and the extracellular matrix (73). It is a protein that seems to be involved in such host functions as tissue organization or blood vessel maintenance (1, 73). Fibulin-1 also binds to extracellular matrix components such as fibronectin, fibrinogen and laminin. The recruitment of fibulin-1 by GAS via SOF may aid in adhesion of GAS to the extracellular matrix and the initiation of infection. The second domain of SOF is a fibronectin-binding one that interacts with fibrinogen and fibronectin of the host (1, 70). Binding to fibronectin via SOF may allow GAS to adhere to epithelial cells as well as invade them through fibronectin-α5β1 integrin receptor interactions (72).

The *scpA* gene produces the surface endopeptidase known as GAS C5a peptidase (1, 74). GAS C5a peptidase cleaves a complement system anaphylatoxin known as C5a (1, 74-75). C5a is a macrophage and neutrophil chemoattractant (75). In addition, it activates interleukin-8, interleukin-6, interleukin-1β and tumor necrosis factor-α expression from

endothelial cells and macrophages. The cleavage of C5a and removal of its leukocyte-binding site by GAS C5a peptidase halts chemotaxis and ultimately delays GAS host clearance (1, 74). In addition to its role in C5a cleavage, GAS C5a peptidase seems to be involved in fibronectin binding and epithelial cell invasion.

The M protein binds to a variety of host proteins (60). These include IgG, IgA, fibrinogen, plasminogen, C4b-binding protein (C4BP) and albumin (60, 76). However, binding to these host proteins does not appear to be consistent across different M proteins (13, 49, 60, 76). In addition, binding seems to be localized to different regions of the M protein (13, 49, 60, 64). For example, C4BP binding is specific to the variable N-terminus, while albumin binding activity can be present in the C repeat domain.

Plasminogen is a serine protease acquired by GAS via plasminogen or fibrinogen receptors (77). The protein can undergo conversion to plasmin via the host plasminogen activator urokinase or the GAS-secreted plasminogen activator streptokinase for instance (77-79). When bound to GAS, plasmin remains active and is not affected by plasmin inhibitors (ex: α-2-antiplasmin) (77-78). Plasmin degrades extracellular matrix constituents and results in tissue barrier removal. This ultimately allows GAS to migrate to other areas of the body in invasive infections.

C4BP is a plasma protein that inhibits activation of the classical complement pathway (76, 80). M protein-bound C4BP may aid in phagocytosis resistance of GAS by preventing the deposition of complement on its surface (1, 60, 76, 80). On the other hand, albumin is a plasma protein that carries fatty acids (81). The albumin-binding abilities of M protein and M-like protein may allow GAS to use host fatty acids for its metabolism, as opposed to wasting energy in synthesizing its own fatty acids (1, 81). Finally, similar to group A carbohydrate, M proteins may play a role in the development of ARF possibly via molecular mimicry (28, 56, 80).

1.3.1.3 M typing

Lancefield described a GAS typing system in 1928 based on the M protein (65, 82). As discussed in section 1.3.1.2, the M protein N-terminus demonstrates a heterogenic amino acid sequence that provides antigenic specificity (49, 64). This concept allowed

Lancefield to identify M types 1 to 50 from years 1928 to the late 1950s using type-specific antisera (49, 64, 82). Another thirty-one M types known as M51 to M81 were then described from 1965-1976 (49, 82). By the late 1980s, it was clear that the antisera available could not assign M types to many GAS isolates. To extend the Lancefield M typing system, an *emm* typing system was developed (82). As previously described in section 1.3.1.1, the *emm* gene encodes the M protein (49). By amplifying and sequencing the 90 nucleotides in the *emm* gene corresponding to 30 amino acids in the M protein N-terminus, an *emm* type can be assigned to GAS (49, 82). This *emm* type corresponds to the M type (49, 71, 82). In addition, an *emm* subtype is obtained when 150 nucleotides correlating with 50 amino acids in the M protein N-terminus are sequenced (49, 82). There are 234 different *emm* types and ≈1200 *emm* subtypes that have been identified (58).

There is an association between M/*emm* type and GAS disease (13). For instance, GAS pediatric pharyngitis has been correlated with *emm* 1-4, 12 and 28 in the United States and Canada (13, 83), while ARF can be associated with M29, 27, 24, 19, 18, 14, 6, 5, 3 and 1 (13, 28, 84). With regards to the latter types, other characteristics that are correlated with their rheumatogenicity (ability to progress to ARF) include no SOF generation, high M protein quantities and hyaluronate capsules that are large (13, 84). Hyaluronic acid capsule is an antiphagocytic glucuronic-β-1,3-N-acetylglucosamine polymer present on the GAS surface (85). It aids GAS in avoiding host immune system detection by mimicking mammalian polysaccharides. In addition, hyaluronic acid capsule may promote GAS survival within neutrophil extracellular traps, which are chromatin fibers containing antimicrobial proteins produced by dead neutrophils that immobilize and kill pathogens (85-86).

Acute PSGN is correlated with SOF positive GAS strains (23, 70). These nephritogenic strains can be subcategorized into pharyngitis and skin infection-associated M types. M25, 12, 4 and 1 are linked to pharyngitis-associated nephritogenic strains (23, 49). On the other hand, M60, 57, 56, 49, 42 and 2 are linked to skin infection-associated nephritogenic strains. In Alberta, invasive GAS has been associated with M1, M3, M11, M12, M28, M59 and M91for instance (4, 13, 49). In particular, M1 has been correlated with NF, STSS and pneumonia. In addition to its relationship with GAS disease, *emm* type is also correlated with *emm* pattern (Table 1.1, 13, 49, 60, 64).

1.3.2 Multilocus sequence typing (MLST)

MLST is used to determine the presence of GAS clones (a common ancestor's descendants) (49, 87). It involves sequencing the nucleotides of fragments from seven housekeeping gene loci (87-88). These loci are *yqiL* (acetoacetyl-CoA thiolase), *xpt* (xanthine phosphoribosyl-transferase), *recP* (transketolase), *mutS* (DNA mismatch repair protein), *murI* (glutamate racemase), *gtr* (glutamine transport ATP-binding protein) and *gki* (glucose kinase). An allele number is designated at each locus depending on the sequence present and together the numbers create an allelic profile of seven integers. These allelic profiles correspond to a sequence type (ST). Different STs can be associated with a particular *emm* type, as may be seen with *emm* pattern D and E (58, 64, 89). On the other hand, in *emm* pattern A-C, *emm* types correlate to the same ST (clone) mostly (58, 64, 87, 89). Disease-affiliated GAS clones wax and wane (13, 90). This cycling seems to be dependent on clonal fitness, herd immunity and virulence.

1.3.3 T typing

GAS pili/fimbriae are hair-like surface structures involved in biofilm generation and keratinocyte and pharyngeal cell adherence (91). They consist of ancillary pilin 1 (AP1), ancillary pilin 2 (AP2) and backbone pilin (BP) (91-93). AP1 generally interacts with extracellular matrix proteins (ex: collagen) (91) and is located at the tip of the pilus (91-93). AP2 is found at the base of the pilus and is involved in anchoring the pilus to the cell wall (91, 93). BP is variable antigenically (91) and upon polymerization, forms the fibre of the pilus (91, 93). The genes for AP1, AP2, BP and the sortases involved in the assembly of the pilus can be found in an operon contained in the fibronectin-binding, collagen-binding T antigen (FCT) region (64, 91-93, 94). There are nine FCT regions that have been identified for GAS based on gene organization and size (designated as FCT1-6/9) or variation in single genes (designated as FCT7/8) within the region (93). The FCT region correlates with M/*emm* type and may contribute to the tissue tropisms seen with GAS (64, 93-94).

The classification of GAS based on serum detection of the T antigen was conducted by Lancefield and colleagues (95-96). The T antigen correlates with the BP of

GAS pili and forms the basis for T serological typing (91, 96). There are 21 T serotypes that can be identified (49, 93, 96). However, similar to *emm* typing, T serotyping can be substituted with *bp*/*tee* gene typing (49, 93, 97).

1.4 GAS vaccine candidates

Ideally, a vaccine will prevent GAS infections and sequelae (8, 23, 80, 98). In addition, it may decrease the need for treatment (see section 1.1) in combating GAS. Although a licensed GAS vaccine is unavailable, the development of candidates is occurring (8). Some of these are described below.

1.4.1 26-valent M protein-based vaccine

The 26-valent M protein-based vaccine has reached the phase II developmental stage (8-9). Also known as StreptAvax, the formulation of the vaccine consists of four recombinant fusion proteins that adhere to aluminum hydroxide (9, 99). These fusion proteins, in turn, are composed of N-terminal peptides from 26 distinct M proteins as well as an N-terminal peptide from the surface protein, streptococcal protective antigen (Spa) (Table 1.2, 9, 13, 49, 68, 99-100). The 26 M types/subtypes selected for the vaccine reflect rheumatogenic, sterile site-associated and pharyngitis-linked GAS (9, 13, 49, 84, 99-100). On the other hand, the N-terminal peptide from Spa of M18 specifically targets this M type (13, 49, 68, 80).

The 26-valent vaccine generates mainly mild and self-limiting adverse reactions, such as headache and pain upon arm movement, in the adult subjects studied in a phase I trial (9). In addition, the development of ARF, glomerulonephritis and cross-reactive antibodies against human tissue are not evident with vaccine administration (9, 28). The 26-valent vaccine demonstrates bactericidal antibodies not only against M types/subtypes contained within it, but also M4, a non-26-valent vaccine M type (9, 13, 49, 99-100).

1.4.2 30-valent M protein-based vaccine

The 30-valent M protein-based vaccine is in the phase I developmental stage (8, 11, 101). Its formulation consists of four fusion proteins complexed with alum (5, 11). The

four proteins, in turn, are composed of N-terminal peptides from 30 distinct M proteins and Spa from M18 (Table 1.2, 11, 13, 49, 68, 100). The 30 M subtypes chosen for use in the vaccine reflect rheumatogenic, invasive and pharyngitis-associated GAS (11, 13, 49, 83-84, 100). The 30-valent vaccine is able to generate bactericidal antibodies against GAS types represented in the vaccine, as well as a number of non-vaccine types (Table 2.3, 1, 5, 11, 13, 49).

1.4.3 J8 vaccine

The J8 peptide consists of p145 and non-M protein amino acids (102). The p145 is a peptide located in the C-repeat region of the M protein (64, 102). Antibodies to p145 have been found in humans and result in the opsonization of multiple strains of GAS (80, 102). The twelve amino acids from p145 selected in the J8 peptide reflect the minimum epitopes needed to stimulate an immune response without autoreactivity being a concern. The non-M protein amino acids surround the twelve p145 amino acids and contribute to the antigenicity and helical folding of J8.

When combined with the diphtheria toxoid carrier protein, the J8 peptide seems to protect against GAS bacteremia and pyoderma in a mouse model (103). However, the same cannot be said for GAS cluster of virulence responder/sensor (CovR/S) mutants (1, 103). CovR/S is a two-component regulatory system where membrane-associated CovS senses alterations in the environment, such as low oxygen tension or high temperature, and phosphorylates/dephosphorylates CovR (103-104). The intracellular protein CovR then modulates transcriptional derepression or repression of other GAS genes. The CovR/S system regulates ≈10-15% of the genome, including such genes as those for SLO and hyaluronic acid capsule (52, 103-104). During invasive infections, some GAS develop inactivating *covR* or *covS* gene mutations at particular stages to aid in the infectious process (103-104). An upregulated virulence factor in CovR/S mutants is *S. pyogenes* cell envelope proteinase (SpyCEP). SpyCEP cleaves the IL-8 chemokine and prevents chemotaxis of neutrophils to the infectious site (103). The incorporation of a SpyCEP fragment into the J8-diphtheria toxoid vaccine helps to combat GAS CovR/S mutants. In 2013, the J8 vaccine reached a phase I trial (8).

1.4.4 StreptInCor vaccine

StreptInCor is a 55 amino acid peptide that consists of a 25 amino acid T epitope, 22 amino acid B epitope and eight amino acid region linking the two epitopes (105). It is derived from the C2 and C3 units in the C repeat region of GAS M5 (1, 49, 64, 105). Antibodies against StreptInCor seem to result in GAS M87, M22, M12, M5 and M1 opsonization (1, 49, 80, 105). In addition, no indications for autoimmunity with human heart components have been demonstrated with the vaccine. In 2016, StreptInCor was planned to undergo phase I assays (8).

1.4.5 Pre-clinical candidates

There are many GAS vaccine candidates in the pre-clinical developmental stage (8). Examples of these include C5a peptidase (8, 106), group A carbohydrate possessing the polyrhamnose backbone but lacking N-acetylglucosamine (implicated in autoimmunity) (8, 49, 56, 80), SOF(8, 49, 69, 80), BP (2, 8) and glycolytic enzymes (8, 10, 14-15, 80). The rest of this review will focus on the GAS glycolytic enzymes (10, 15).

1.5 GAS glycolytic enzymes

GAS relies mainly on glucose as its carbon-based energy source (Figure 1.3, 1, 15). The glucose is metabolized by the Embden-Meyerhof-Parnas glycolytic pathway to generate two $NADH_2$ and two ATP. GAS also possesses the alternate Entner-Doudoroff glycolytic pathway and tricarboxylic acid pathway. However, these pathways are incomplete and have no known significance for GAS. In the Embden-Meyerhof-Parnas pathway, the glycolytic enzymes for metabolism can be localized to the cytoplasm. However, nine of these twelve enzymes have been shown to be associated with the GAS cell wall and secreted in the bacterial environment as well (1,10, 15). These are pyruvate kinase, phosphoglycerate mutase, triose phosphate isomerase, fructose-bisphosphate aldolase, 6-phosphofructokinase, enolase, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK) (1, 10, 12, 15). The nine enzymes demonstrate reactivity with pooled sera or immunoglobulin from humans (1, 10, 12, 14-15, 80). However, the

reactivity for each enzyme seems to differ depending on the GAS strain considered. In the Reglinski *et al*. study, PGK from 18 of 20 GAS strains tested are reactive with pooled immunoglobulin (1, 12, 14, 80). In the same study, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase shows reactivity with only one of the 20 GAS strains. The next sections focus specifically on GAS GAPDH, enolase and PGK (10, 12).

1.5.1 GAPDH

GAS GAPDH binds to a variety of proteins (10, 75, 78, 107-108). These can include lysozyme, actin, myosin, fibronectin (10, 107-108), plasmin (10, 78), plasminogen (10, 109), C5a (10, 75) and urokinase plasminogen activator receptor (uPAR or CD87) (10, 110). The binding of GAPDH to the innate immune component lysozyme may aid GAS in suppressing and thus avoiding the immune response (10, 80, 107-108). GAPDH interaction with actin and myosin may allow GAS to colonize injured tissue. C5a binding to GAS GAPDH may inhibit C5a function in two distinct mechanisms (10, 75). The first mechanism involves GAS GAPDH in the surrounding environment interacting with C5a and halting its properties of chemotaxis. The second mechanism involves surface GAPDH binding to C5a for cleavage by GAS C5a peptidase. Finally, uPAR is present on the pharyngeal cell surface (110). Therefore, interaction of GAPDH with uPAR may aid GAS adherence to pharyngeal cells (10, 110).

Besides its ability to bind proteins (10, 107-108), GAS GAPDH is associated with mouse macrophage apoptosis (1, 111), ADP-ribosylation (10, 112), histone H3 phosphorylation in pharyngeal cells (10, 107, 113) and regulation of other GAS genes (10, 49, 114). Mono-ADP-ribosylation is a post-translational process where ADP-ribose is covalently attached to a substrate (112). Cell wall GAS GAPDH undergoes mono-ADP-ribosylation at its own cysteine amino acid (10, 112). The significance of this auto-ADP ribosylation is that it may allow GAS to regulate its own internal processes through signal transduction. Besides its auto-ADP-ribosylation ability, GAS GAPDH can ADP-ribosylate cysteine amino acids that are not its own. This may allow GAS to modify epithelial cell surface components for infection/colonization.

Histone H3 is a nucleosome inner core protein that aids in coiling DNA (113). The phosphorylation of histone H3 possibly via signal transduction mechanisms that are triggered when GAS GAPDH binds to pharyngeal cell membrane proteins may modify chromatin structure and allow GAS to adhere and invade pharyngeal cells (10, 107, 113).

GAS contains only one GAPDH gene and one pathway (Embden-Meyerhof-Parnas) that primarily metabolizes glucose (1, 15, 75, 109). Because of this, the development of a GAPDH knockout mutant to study GAPDH function, especially on the GAS surface, has been unsuccessful. Boël *et al*. has constructed a GAS mutant that possesses a GAPDH with a 12 amino acid hydrophobic C-terminal tail (10, 49, 109). This mutant shows less GAPDH in its cell wall fraction and more GAPDH in its cytoplasmic fraction. In addition, the GAPDH mutant demonstrates upregulation of 64 genes related to different functions and downregulation of 128 genes (10, 49, 109, 114). Eighteen of the 192 total genes undergoing significant transcriptional changes compared to the wild type are related to virulence. Examples of these virulence genes include streptokinase A, *emm*1, SLS and SLO (13, 49-50, 109, 114). The phenomena observed with the GAS mutant imply that GAPDH is a regulator of many genes.

1.5.2 Enolase and PGK

GAS enolase interacts with conserved virulence factor A (CvfA) (1, 115), plasminogen and plasmin (1, 116). CvfA is a putative membrane-associated ribonuclease that seems to regulate GAS genes related to virulence for instance (1, 115). When the concentration of carbohydrates in the environment is low, the repression of such virulence genes as streptokinase and *emm* by CvfA occurs. On the other hand, the streptococcal pyrogenic exotoxin B (discussed further below) or *speB* gene is upregulated by CvfA when the concentration of peptides in the environment is low. The potential role of GAS enolase in its interaction with CvfA is that it may sense the GAS nutritional status and regulate the activity of CvfA accordingly.

In addition to its binding capabilities (1, 115-116), GAS enolase may be implicated in ARF development (1, 80, 109, 117). In the Fontán *et al*. study, enolase from B lymphocytes, T lymphocytes, neutrophils and monocytes react with anti-GAS enolase

antibodies (1, 80, 109, 117). In addition, anti-GAS enolase antibodies are found in ARF patients at higher levels than healthy subjects, as well as in pharyngitis patients at levels lower than ARF patients. In contrast, GAS GAPDH antibody levels remain consistent between healthy, pharyngitis and ARF subjects in the study. The Fontán *et al*. study raises concerns regarding autoimmunity should GAS glycolytic enzymes be used as vaccine candidates (1, 8, 10, 14-15, 80, 109, 117-118). To overcome this issue, a region of the glycolytic enzyme specific for GAS should be used in a vaccine.

SpeB is a surface-associated and secreted protein of GAS (1, 119). It is a cysteine protease involved, for instance, in the degradation of fibronectin, cleavage of C3b from complement, induction of epithelial cell and macrophage apoptosis, modification of SLO and M protein (80, 120) and binding to laminin (1, 119). Laminin is a glycosylated protein of the extracellular matrix (121). By interacting with laminin, SpeB may be able to aid in GAS adhesion to host cells (1, 119, 122). In addition to the prior properties (1, 119-120), SpeB has been implicated as an antigen that may be responsible for the generation of immune complexes in glomeruli that lead to acute PSGN (23, 123). Another antigen that may be involved in immune complex generation is GAS GAPDH. Although the *speB* gene is possessed by nearly all GAS strains, there is variation in its expression (1, 119-120). SpeB expression is regulated by *mga*, *covRS* and the transcriptional factor *ropB* for instance.

In a study by Uhlmann *et al*., speB negative GAS supernatants seemed to activate neutrophils more strongly than speB positive ones (1, 16). After investigation of the speB negative supernatants, it was revealed that GAS PGK was a neutrophil activator. This implies that GAS PGK may contribute to a hyperinflammatory response that causes tissue damage and are correlated with NF and STSS (1, 4, 16). In addition, GAS PGK is susceptible to degradation by SpeB.

1.5.3 Surface expression of glycolytic enzymes

Surface glycolytic enzymes in prokaryotes are anchorless surface proteins (77, 109). This means that these enzymes do not possess an N-terminal signal peptide necessary for protein exportation and C-terminal LPXTG or choline-binding repeat for

surface anchoring (77, 109, 124). Each glycolytic enzyme seems to have a different mechanism for surface expression between and within the same bacteria (109, 111, 118, 125-127). For instance, Group B streptococcus (GBS) GAPDH, a glycolytic enzyme shown to induce mouse macrophage apoptosis (111), increase levels of the immunosuppressive cytokine IL-10 in mice (128) and interact with fibrinogen, actin, glu-plasminogen and lys-plasminogen (128-129), is released into the supernatant via lysis of bacteria (111, 118, 124). Once released, GAPDH may reassociate non-specifically with the GBS surface. A similar phenomenon is demonstrated with *Streptococcus pneumoniae* GAPDH, a glycolytic enzyme that binds C1q for possible inactivation (127), hemoglobin, heme (130), plasmin and plasminogen (127, 131), in terms of the fact that cell lysis correlates with surface GAPDH expression (111, 118, 124, 127). However, *S. pneumoniae* GAPDH may reassociate with the bacterial surface by attaching to peptidoglycan (124, 127).

On the other hand, GBS PGK is a glycolytic enzyme that binds to fibrin, fibrinogen, actin and plasminogen (118). In addition, the enzyme may be involved in GBS invasion of epithelial cells (132). GBS PGK seems to be secreted outside the bacteria possibly via SecA2 and SecA for surface attachment (109, 118, 124). SecA2 has been shown to also be involved in enolase surface expression in *Listeria monocytogenes* (133). Once outside the bacteria, PGK may reassociate with the GBS surface by attaching to lipoteichoic acid, Sag 1003 protein and Sag 912 protein possibly (109, 118, 124, 134). The binding of glycolytic enzymes to lipoteichoic acid has also been demonstrated for surface GAPDH and enolase, which are involved in interacting with plasminogen in *Lactobacillus crispatus* (118, 126). The PGK surface expression in GBS also seems to involve Srr1, *sag0979* gene and the pH of the medium (118).

In GAS, GAPDH seems to be the only glycolytic enzyme so far where information regarding its surface expression is known (10, 15, 109, 124-125, 127). Based on the fact that the addition of a C-terminal hydrophobic tail decreased GAPDH expression on the GAS surface, it can be inferred that GAPDH is secreted by GAS through an unknown mechanism (10, 109, 124, 127). Once secreted, GAPDH may reassociate with the GAS surface by interacting with M protein and Mrp (10, 62, 109, 124-125, 127). The regulation of GAS GAPDH surface expression involves the *mga* gene (10, 125).

1.6 Research rationale

In Alberta, the incidence rate of invasive GAS is generally increasing (Table 2.1, 4, 13, 135). In 2010, the incidence rate was 4.77/100 000 while in 2016, incidence rate was 7.69/100 000. In addition in 2004-2011, the predominant *emm* types in Alberta were *emm* 1 and 59 (13, 43). A Canadian national surveillance study has shown that although some epidemiological aspects of invasive GAS remain consistent throughout the years, others change (4, 13, 45, 49, 136). This may apply to Alberta, where a recent in-depth epidemiological data analysis regarding invasive GAS has not been conducted (Table 2.1, 4, 13, 43, 45, 49, 136). In addition to outdated information, the theoretical coverage of invasive GAS with a 30-valent or 26-valent vaccine has not been explored for Alberta yet (Table 2.1, 4-5, 8-9, 11, 13, 43, 45, 49, 99, 136).

Although the 30-valent and 26-valent vaccines are in phase I and phase II trials respectively, a disadvantage of these vaccines may be their limited coverage (5, 8-9, 11, 49, 80, 99, 101). In order to overcome this disadvantage, alternatives to these vaccines or addition of antigens to increase their coverage should be considered. One of these alternatives/antigens may be GAS PGK (5, 8-12, 49, 80, 99). GAS PGK is a glycolytic enzyme located in the bacterial cytoplasm and cell wall, as well as outside the bacteria (1, 10, 15). It is associated with neutrophil activation, which may result in hyperinflammatory responses that cause tissue injury (10, 16). In a study by Reglinski *et al*., 18 of 20 GAS strains showed pooled human immunoglobulin reactivity with surface PGK (1, 12, 14). This was the greatest degree of reactivity demonstrated amongst the GAS glycolytic enzymes detected (1, 12, 14-15). Although PGK has been detected on the surface of GAS already, the testing has been limited to a few M/*emm* types (10, 12, 98). Before GAS PGK should be explored as a potential vaccine component, its prevalence as a surface expressed protein in a wide variety of GAS types should be determined (5, 8-12, 49, 80, 98-99).

While much work has been done regarding the surface expression of GAS GAPDH, the same cannot be said for GAS PGK (10, 12, 109, 118, 124-125, 127).

1.7 Objectives and Hypotheses

Objective One – To collate the seasonality and M/*emm* types associated with invasive GAS in Alberta for 2013-2016 and to determine the incidence rate for invasive GAS based on gender and age in 2016.

Hypothesis One – Invasive GAS incidence rates are rising and this increase can be attributed to particular M/*emm* types causing specific invasive diseases.

Objective Two – To determine the theoretical coverage of the 26-valent and 30-valent vaccines in Alberta for 2013-2016.

Hypothesis Two – Theoretical coverage with the 26-valent and 30-valent vaccines is greater than 50% in each year examined.

Objective Three – To explore PGK surface expression on a variety of GAS M/*emm* types and investigate the relationship between *emm* pattern, GAS tissue source and surface PGK expression.

Hypothesis Three – GAS PGK is present on the surface of all GAS strains, regardless of M/*emm* type and disease type.

1.8 References

1. Fittipaldi N, Beres SB, Olsen RJ, Kapur V, Shea PR, Watkins ME, Cantu CC, Laucirica DR, Jenkins L, Flores AR, Lovgren M, Ardanuy C, Liñares J, Low DE, Tyrrell GJ, Musser JM. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A *streptococcus*. Am J Pathol. 2012 Apr;180(4):1522–34.
2. Young PG, Moreland NJ, Loh JM, Bell A, Atatoa Carr P, Proft T, Baker EN. Structural conservation, variability, and immunogenicity of the T6 backbone pilin of serotype M6 *Streptococcus pyogenes*. Infect Immun. 2014 Jul;82 (7):2949–57.
3. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005 Nov; 5(11):685-94.
4. Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. Invasive group A streptococcal disease in Alberta, Canada (2000 to 2002). J Clin Microbiol. 2005 Apr; 43(4):1678–83.
5. Dale JB, Penfound TA, Tamboura B, Sow SO, Nataro JP, Tapia M, Kotloff KL. Potential coverage of a multivalent M protein-based group A streptococcal vaccine. Vaccine. 2013 Mar 15;31(12):1576–81.
6. Cole C, Gazewood J. Diagnosis and treatment of impetigo. Am Fam Physician. 2007 Mar 15;75(6):859–64.
7. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan SL, Montoya JG, Wade JC, Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis. 2014 Jul;59(2):e10-52. Erratum in: Clin Infect Dis. 2014;59:147-50.; Clin Infect Dis. 2015.
8. Steer AC, Carapetis JR, Dale JB, Fraser JD, Good MF, Guilherme L, Moreland NJ, Mulholland EK, Schodel F, Smeesters PR. Status of research and development of vaccines for *Streptococcus pyogenes*. Vaccine. 2016 Jun 3;34(26): 2953–8.
9. McNeil SA, Halperin SA, Langley JM, Smith B, Warren A, Sharratt GP, Baxendale DM, Reddish MA, Hu MC, Stroop SD, Linden J, Fries LF, Vink PE, Dale JB. Safety and immunogenicity of 26-valent group A *streptococcus* vaccine in healthy adult volunteers. Clin Infect Dis. 2005 Oct 15;41(8):1114–22.

10. Cole JN, Ramirez RD, Currie BJ, Cordwell SJ, Djordjevic SP, Walker MJ. Surface analyses and immune reactivities of major cell wall-associated proteins of group A streptococcus. Infect Immun. 2005 May;73(5):3137–46.
11. Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine. 2011 Oct 26;29(46): 8175–8.
12. Siddiqua K. Mutant group B streptococcus surface expressed phosphoglycerate kinase (PGK) with reduced plasminogen binding [master's thesis]. [Edmonton(AB)]: University of Alberta; 2014. 108 p.
13. Tyrrell GJ, Lovgren M, St. Jean T, Hoang L, Patrick DM, Horsman G, Van Caeseele P, Sieswerda LE, McGeer A, Laurence RA, Bourgault A-M, Low DE. Epidemic of group A *streptococcus* M/emm59 causing invasive disease in Canada. Clin Infect Dis. 2010 Dec 1;51(11):1290–7.
14. Reglinski M, Gierula M, Lynskey NN, Edwards RJ, Sriskandan S. Identification of the *Streptococcus pyogenes* surface antigens recognised by pooled human immunoglobulin. Sci Rep. 2015;5:15825.
15. Pancholi V, Caparon M. *Streptococcus pyogenes* metabolism. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
16. Uhlmann J, Siemens N, Kai-Larsen Y, Fiedler T, Bergman P, Johansson L, Norrby-Teglund A. Phosphoglycerate kinase – a novel streptococcal factor involved in neutrophil activation and degranulation. J Infect Dis. 2016 Dec 15; 214(12):1876–83.
17. Barnett TC, Liebl D, Seymour LM, Gillen CM, Lim JY, LaRock CN, Davies MR, Schulz BL, Nizet V, Teasdale RD, Walker MJ. The globally disseminated M1T1 clone of group A streptococcus evades autophagy for intracellular replication. Cell Host Microbe. 2013 Dec 11;14(6):675–82.
18. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the infectious diseases society of America. Clin Infect Dis. 2012 Nov 15;55(10):1–17. Erratum in: Clin Infect Dis. 2014 May;58(10):1496.

19. Kemble SK, Westbrook A, Lynfield R, Bogard A, Koktavy N, Gall K, Lappi V, DeVries AS, Kaplan E, Smith KE. Foodborne outbreak of group A streptococcus pharyngitis associated with a high school dance team banquet - Minnesota, 2012. Clin Infect Dis. 2013 Sep;57(5):648–54.
20. Guarner J, Sumner J, Paddock CD, Shieh WJ, Greer PW, Reagan S, Fischer M, Van Beneden CA, Zaki SR. Diagnosis of invasive group A streptococcal infections by using immunohistochemical and molecular assays. Am J Clin Pathol. 2006 Jul; 126(1):148–55.
21. Hartman-Adams H, Banvard C, Juckett G. Impetigo: diagnosis and treatment. Am Fam Physician. 2014 Aug 15;90(4):229–35.
22. Bowen AC, Mahé A, Hay RJ, Andrews RM, Steer AC, Tong SY, Carapetis JR. The global epidemiology of impetigo: a systematic review of the population prevalence of impetigo and pyoderma. PLoS One. 2015 Aug 28; 10(8):e0136789.
23. VanDeVoorde RG 3rd. Acute poststreptococcal glomerulonephritis: the most common acute glomerulonephritis. Pediatr Rev. 2015 Jan; 36(1): 3-12; quiz 13.
24. Inghammar M, Rasmussen M, Linder A. Recurrent erysipelas - risk factors and clinical presentation. BMC Infect Dis. 2014 May 18;14:270.
25. Bläckberg A, Trell K, Rasmussen M. Erysipelas, a large retrospective study of aetiology and clinical presentation. BMC Infect Dis. 2015 Sep 30; 15:402.
26. Henningham A, Barnett TC, Maamary PG, Walker MJ. Pathogenesis of group A streptococcal infections. Discov Med. 2012 May; 13(72): 329-42.
27. Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, Wade JC, Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin Infect Dis. 2005 Nov 15;41(10):1373–406. Erratum in: Clin Infect Dis. 2005 Dec 15;41(12):1830.; Clin Infect Dis. 2006 Apr 15;42(8): 1219.
28. Bono-Neri F. Acute rheumatic fever: global persistence of a preventable disease. J Pediatr Health Care. 2017 May-Jun;31(3):275-84.
29. Armstrong C. Practice guidelines: AHA guidelines on prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. Am Fam Physician. 2010 Feb 1; 81(3):346-59.

30. Gewitz MH, Baltimore RS, Tani LY, Sable CA, Shulman ST, Carapetis J, Remenyi B, Taubert KA, Bolger AF, Beerman L, Mayosi BM, Beaton A, Pandian NG, Kaplan EL, American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young. Revision of the Jones criteria for the diagnosis of acute rheumatic fever in the era of Dopper echocardiography: a scientific statement from the American Heart Association. Circulation. 2015 May 19;131(20):1806-18.
31. Martins CO, Demarchi L, Ferreira FM, Pomerantzeff PM, Brandao C, Sampaio RO, Spina GS, Kalil J, Cunha-Neto E, Guilherme L. Rheumatic heart disease and myxomatous degeneration: differences and similarities of valve damage resulting from autoimmune reactions and matrix disorganization. PLoS One. 2017 Jan 25; 12(1):e0170191.
32. Russell EA, Tran L, Baker RA, Bennetts JS, Brown A, Reid CM, Tam R, Walsh WF, Maguire GP. A review of valve surgery for rheumatic heart disease in Australia. BMC Cardiovasc Disord. 2014 Oct 2; 14:134.
33. Wessels MR. Pharyngitis and scarlet fever. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
34. Gidaris D, Zafeiriou D, Mavridis P, Gombakis N. Scarlet fever and hepatitis: a case report. Hippokratia. 2008 Jul-Sep;12(3):186-7.
35. Mahara G, Wan C, Yang K, Chen S, Guo J, Gao Q, Wang W, Wang Q, Guo X. The association between environmental factors and scarlet fever incidence in Beijing region: using GIS and spacial regression models. Int J Environ Res Public Health. 2016 Nov;13(11):1083.
36. Parvez Y, Thomas SV. Acute cholecystitis in a child with scarlet fever: a rare association. S Afr J Child Health. 2016;10(3):188-9.
37. Allmon A, Deane K, Martin KL. Common skin rashes in children. Am Fam Physician. 2015 Aug 1;92(3):211-6.
38. Ben Zakour NL, Davies MR, You Y, Chen JH, Forde BM, Stanton-Cook M, Yang R, Cui Y, Barnett TC, Venturini C, Ong CL, Tse H, Dougan G, Zhang J, Yuen KY, Beatson SA, Walker MJ. Transfer of scarlet fever-associated elements into the group A

streptococcus M1T1 clone. Sci Rep. 2015 Nov 2;5:15877.

39. Chalker V, Jironkin A, Coelho J, Al-Shahib A, Platt S, Kapatai G, Daniel R, Dhami C, Laranjeira M, Chambers T, Guy R, Lamagni T, Harrison T, Chand M, Johnson AP, Underwood A, Scarlet Fever Incident Management Team. Genome analysis following a national increase in scarlet fever in England 2014. BMC Genomics. 2017 Mar 10; 18(1):224.
40. Baytunca MB, Donuk T, Erermiş S. [Evaluation of a Neuropsychiatric Disorder: From PANDAS to PANS and CANS]. Turk Psikiyatri Derg. 2016;27(2). Turkish.
41. Tan J, Smith CH, Goldman RD. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. Can Fam Physician. 2012 Sep;58(9):957-9.
42. Cole JN, Barnett TC, Nizet V, Walker MJ. Molecular insight into invasive group A streptococcal disease. Nat Rev Microbiol. 2011 Sep 16;9(10):724-36.
43. Government of Alberta [Internet]. Alberta Government. Streptococcal disease – group A, invasive; c2003-2013 [revised 2013 Jun; cited 20 Aug 2017]; [about 17 p]. Available from: https://open.alberta.ca/dataset/b6405d01-6ffd-4b8a-9762-2d6c29772f9c/resource/0fc41de9-1b42-427d-914c-0c687eca0652/download/Guidelines-Streptococcal-A-Invasive-2013.pdf
44. Nelson GE, Pondo T, Toews KA, Farley MM, Lindegren ML, Lynfield R, Aragon D, Zansky SM, Watt JP, Cieslak PR, Angeles K, Harrison LH, Petit S, Beall B, Van Beneden CA. Epidemiology of invasive group A streptococcal infections in the United States, 2005-2012. Clin Infect Dis. 2016 Aug 15;63(4):478-86.
45. Demczuk WHB, Griffith A, Singh R, Montes K, Sawatsky P, Martin I, Mulvey M. National laboratory surveillance of invasive streptococcal disease in Canada: annual summary 2014 [Internet]. Winnipeg: Public Health Agency of Canada; 2015 Dec [cited 2017 Jul 21]. Available from: http://www.healthycanadians.gc.ca/publications/drugs-products-medicaments-produits/2014-streptococcus/alt/surveillance-streptococca-eng.pdf
46. Darenberg J, Henriques-Normark B, Lepp T, Tegmark-Wisell K, Tegnell A, Widgren K. Increased incidence of invasive group A streptococcal infections in Sweden, January 2012-February 2013. Euro Surveill. 2013 Apr 4;18(14):20443.
47. Megged O, Yinnon AM, Raveh D, Rudensky B, Schlesinger Y. Group A streptococcus

bacteraemia: comparison of adults and children in a single medical centre. Clin Microbiol Infect. 2006 Feb;12(2):156-62.

48. Allen UD, Moore DL. Invasive group A streptococcal disease: Management and chemoprophylaxis. Paediatr Child Health. 2010;15(5):295-302.
49. Spellerberg B, Brandt C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci). In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
50. Molloy EM, Cotter PD, Hill C, Mitchell DA, Ross RP. Streptolysin S-like virulence factors: the continuing sagA. Nat Rev Microbiol. 2011 Aug 8;9(9):670-81.
51. Uchiyama S, Döhrmann S, Timmer AM, Dixit N, Ghochani M, Bhandari T, Timmer JC, Sprague K, Bubeck-Wardenburg J, Sinom SI, Nizet V. Streptolysin O rapidly impairs neutrophil oxidative burst and antibacterial responses to group A streptococcus. Front Immunol. 2015 Nov 16;6:581.
52. Logsdon LK, Håkansson AP, Cortés G, Wessels MR. Streptolysin O inhibits clathrin-dependent internalization of group A *streptococcus*. MBio. 2011 Feb 15;2(1):e00332-10.
53. Miyoshi-Akiyama T, Takamatsu D, Koyanagi M, Zhao J, Imanishi K, Uchiyama T. Cytocidal effect of *Streptococcus pyogenes* on mouse neutrophils in vivo and the critical role of streptolysin S. J Infect Dis. 2005 Jul 1;192(1):107-16.
54. Sumitomo T, Nakata M, Higashino M, Jin Y, Terao Y, Fujinaga Y, Kawabata S. Streptolysin S contributes to group A streptococcal translocation across an epithelial barrier. J Biol Chem. 2011 Jan 28;286(4):2750-61.
55. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. J Exp Med. 1933 Mar 31;57(4):571-95.
56. van Sorge NM, Cole JN, Kuipers K, Henningham A, Aziz RK, Kasirer-Friede A, Lin L, Berends ETM, Davies MR, Dougan G, Zhang F, Dahesh S, Shaw L, Gin J, Cunningham M, Merriman JA, Hütter J, Lepenies B, Rooijakkers SHM, Malley R, Walker MJ, Shattil SJ, Schlievert PM, Choudhury B, Nizet V. The classical Lancefield antigen of group A *streptococcus* is a virulence determinant with implications for vaccine design. Cell Host Microbe. 2014 Jun 11;15(6):729-40.

57. Sumby P, Barbian KD, Gardner DJ, Whitney AR, Welty DM, Long RD, Bailey JR, Parnell MJ, Hoe NP, Adams GG, Deleo FR, Musser JM. Extracellular deoxyribonuclease made by group A streptococcus assists pathogenesis by enhancing evasion of the innate immune response. Proc Natl Acad Sci USA. 2005 Feb 1; 102(5):1679-84.
58. Bessen DE. Molecular basis of serotyping and the underlying genetic organization of *Streptococcus pyogenes*. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
59. Dale JB, Niedermeyer SE, Agbaosi T, Hysmith ND, Penfound TA, Hogn CM, Pullen M, Bright MI, Murrell DS, Shenep LE, Courtney HS. Protective immunogenicity of group A streptococcal M-related proteins. Clin Vaccine Immunol. 2015 Mar; 22(3):344-50.
60. Sanderson-Smith M, De Oliveira DM, Guglielmini J, McMillan DJ, Vu T, Holien JK, Henningham A, Steer AC, Bessen DE, Dale JB, Curtis N, Beall BW, Walker MJ, Parker MW, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR, M Protein Study Group. A systematic and functional classification of *Streptococcus pyogenes* that serves as a new tool for molecular typing and vaccine development. J Infect Dis. 2014 Oct 15; 210(8):1325–38.
61. Li Y, Courtney HS. Promotion of phagocytosis of *Streptococcus pyogenes* in human blood by a fibrinogen-binding peptide. Microbes Infect. 2011 Apr;13(4):413-8.
62. Courtney HS, Li Y. Non-immune binding of human IgG to M-related proteins confers resistance to phagocytosis of group A streptococci in blood. PLoS One. 2013 Oct 25;8(10):e78719.
63. Liang Z, Zhang Y, Agrahari G, Chandrahas V, Glinton K, Donahue DL, Balsara RD, Ploplis VA, Castellino FJ. A natural inactivating mutation in the CovS component of the CovRS regulatory operon in a pattern D *Streptococcal pyogenes* strain influences virulence-associated genes. J Biol Chem. 2013 Mar 1;288(9):6561-73.
64. McMillan DJ, Drèze PA, Vu T, Bessen DE, Guglielmini J, Steer AC, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR. Updated model of group A streptococcus M proteins based on a comprehensive worldwide study. Clin Microbiol Infect.

2013 May;19(5):E222-9.

65. Lancefield RC. The antigenic complex of Streptococcus haemolyticus: I. demonstration of a type-specific substance in extracts of Streptococcus haemolyticus. J Exp Med. 1928 Jan 1;47(1):91-103.
66. Fischetti VA. M protein and other surface proteins on streptococci. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
67. Phillips GN Jr, Flicker PF, Cohen C, Manjula BN, Fischetti VA. Streptococcal M protein: alpha-helical coiled-coil structure and arrangement on the cell surface. Proc Natl Acad Sci USA. 1981 Aug;78(8):4689-93.
68. Dale JB, Chiang EY, Liu S, Courtney HS, Hasty DL. New protective antigen of group A streptococci. J Clin Invest. 1999 May 1;103(9):1261–8.
69. Courtney HS, Hasty DL, Dale JB. Serum opacity factor (SOF) of *Streptococcus pyogenes* evokes antibodies that opsonize homologous and heterologous SOF-positive serotypes of group A streptococci. Infect Immun. 2003 Sep;71(9):5097-103.
70. Zhu L, Olsen RJ, Musser JM. Opacification domain of serum opacity factor inhibits beta-hemolysis and contributes to virulence of *Streptococcus pyogenes*. mSphere. 2017 Mar-Apr;2(2):e00147-17.
71. Beall B, Gherardi G, Lovgren M, Facklam RR, Forwick BA, Tyrrell GJ. *Emm* and *sof* gene sequence variation in relation to serological typing of opacity-factor-positive group A streptococci. Microbiology. 2000 May;146(Pt 5):1195-209.
72. Gillen CM, Courtney HS, Schulze K, Rohde M, Wilson MR, Timmer AM, Guzman CA, Nizet V, Chhatwal GS, Walker MJ. Opacity factor activity and epithelial cell binding by the serum opacity factor protein of *Streptococcus pyogenes* are functionally discrete. J Biol Chem. 2008 Mar 7;283(10):6359-66.
73. Courtney HS, Li Y, Twal WO, Argraves WS. Serum opacity factor is a streptococcal receptor for the extracellular matrix protein fibulin-1. J Biol Chem. 2009 May 8;284(19):12966-71.
74. Shet A, Kaplan EL, Johnson DR, Cleary PP. Immune response to group A streptococcal C5a peptidase in children: implications for vaccine development. J Infect Dis. 2003 Sept 15;188(6):809-17.

75. Terao Y, Yamaguchi M, Hamada S, Kawabata S. Multifunctional glyceraldehyde-3-phosphate dehydrogenase of *Streptococcus pyogenes* is essential for evasion from neutrophils. J Biol Chem. 2006 May 19;281(20):14215-23.
76. André I, Persson J, Blom AM, Nilsson H, Drakenberg T, Lindahl G, Linse S. Streptococcal M protein: structural studies of the hypervariable region, free and bound to human C4BP. Biochemistry. 2006 Apr 11;45(14):4559-68.
77. Cork AJ, Ericsson DJ, Law RH, Casey LW, Valkov E, Bertozzi C, Stamp A, Jovcevski B, Aquilina JA, Whisstock JC, Walker MJ, Kobe B. Stability of the octameric structure affects plasminogen-binding capacity of streptococcal enolase. PLoS One. 2015 Mar 25;10(3):e0121764.
78. Winram SB, Lottenberg R. The plasmin-binding protein Plr of group A streptococci is identified as glyceraldehyde-3-phosphate dehydrogenase. Microbiology. 1996 Aug;142(Pt 8): 2311–20.
79. Khil J, Im M, Heath A, Ringdahl U, Mundada L, Carey Engleberg N, Fay WP. Plasminogen enhances virulence of group A streptococci by streptokinase-dependent and streptokinase—independent mechanisms. J Infect Dis. 2003 Aug 15;188(4):497-505.
80. Sompayrac L. How the immune system works. 3rd ed. Malden (MA): Blackwell Publishing; 2008.
81. Malmström J, Karlsson C, Nordenfelt P, Ossola R, Weisser H, Quandt A, Hansson K, Aebersold R, Malmström L, Björck L. *Streptococcus pyogenes* in human plasma: *adaptive mechanisms analyzed by mass spectrometry-based proteomics*. J Biol Chem. 2012 Jan 6;287(2):1415-25.
82. Facklam RF, Martin DR, Lovgren M, Johnson DR, Efstratiou A, Thompson TA, Gowan S, Kriz P, Tyrrell GJ, Kaplan E, Beall B. Extension of the Lancefield classification for group A streptococi by addition of 22 new M protein gene sequence types from clinical isolates: *emm*103 to *emm*124. Clin Infect Dis. 2002 Jan 1;34(1):28-38.
83. Shulman ST, Tanz RR, Dale JB, Beall B, Kabat W, Kabat K, Cederlund E, Patel D, Rippe J, Li Z, Sakota V, North American Streptococcal Pharyngitis Surveillance Group. Seven-year surveillance of North American pediatric group A streptococcal

pharyngitis isolates. Clin Infect Dis. 2009 Jul 1;49(1):78–84.

84. Shulman ST, Stollerman G, Beall B, Dale JB, Tanz RR. Temporal changes in streptococcal M protein types and the near-disappearance of acute rheumatic fever in the United States. Clin Infect Dis. 2006 Feb 15;42(4):441-7.
85. Cole JN, Pence MA, von Köckritz-Blickwede M, Hollands A, Gallo RL, Walker MJ, Nizet V. M protein and hyaluronic acid capsule are essential for *in vivo* selection of *covRS* mutations characteristic of invasive serotype M1T1 group A *streptococcus*. MBio. 2010 Sep-Oct;1(4): e00191-10.
86. Liu S, Su X, Pan P, Zhang L, Hu Y, Tan H, Wu D, Liu B, Li H, Li H, Li Y, Dai M, Li Y, Hu C, Tsung A. Neutrophil extracellular traps are indirectly triggered by lipopolysaccharide and contribute to acute lung inury. Sci Rep. 2016 Nov 16;6:37252.
87. Enright MC, Spratt BG, Kalia A, Cross JH, Bessen DE. Multilocus sequence typing of *Streptococcus pyogenes* and the relationships between *emm* type and clone. Infect Immun. 2001 Apr;69(4):2416-27.
88. McGregor KF, Spratt BG, Kalia A, Bennett A, Bilek N, Beall B, Bessen DE. Multilocus sequence typing of *Streptococcus pyogenes* representing most known *emm* types and distinctions among subpopulation genetic structures. J Bacteriol. 2004 Jul; 186(13):4285-94.
89. Bessen DE, McGregor KF, Whatmore AM. Relationships between *emm* and multilocus sequence types within a global collection of *Streptococcus pyogenes*. BMC Microbiol. 2008 Apr 11;8:59.
90. Athey TBT, Teatero S, Sieswerda LE, Gubbay JB, Marchand-Austin A, Li A, Wasserscheid J, Dewar K, McGeer A, Williams D, Fittipaldi N. High incidence of invasive group A *streptococcus* disease caused by strains of uncommon *emm* types in Thunder Bay, Ontario, Canada. J Clin Microbiol. 2016 Jan;54(1):83-92.
91. Tsai JC, Loh JM, Clow F, Lorenz N, Proft T. The group A streptococcus serotype M2 pilus plays a role in host cell adhesion and immune evasion. Mol Microbiol. 2017 Jan;103(2):282-98.
92. Linke-Winnebeck C, Paterson NG, Young PG, Middleditch MJ, Greenwood DR, Witte G, Baker EN. Structural model for covalent adhesion of the *Streptococcus pyogenes* pilus through a thioester bond. J Biol Chem. 2014 Jan 3;289(1):177-89.

93. Steemson JD, Moreland NJ, Williamson D, Morgan J, Carter PE, Proft T. Survey of the bp/tee genes from clinical group A streptococcus isolates in New Zealand - implications for vaccine development. J Med Microbiol. 2014 Dec;63(Pt 12):1670–8.
94. Kratovac Z, Manoharan A, Luo F, Lizano S, Bessen DE. Population genetics and linkage analysis of loci within the FCT region of *Streptococcus pyogenes*. J Bacteriol. 2007 Feb;189(4):1299-310.
95. Lancefield RC, Dole VP. The properties of T antigens extracted from group A hemolytic streptococci. J Exp Med. 1946 Oct 31;84(5):449-71.
96. Mora M, Bensi G, Capo S, Falugi F, Zingaretti C, Manetti AG, Maggi T, Taddei AR, Grandi G, Telford JL. Group A streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. Proc Natl Acad Sci USA. 2005 Oct 25;102(43):15641-6.
97. Falugi F, Zingretti C, Pinto V, Mariani M, Amodeo L, Manetti AG, Capo S, Musser JM, Orefici G, Margarit I, Telford JL, Grandi G, Mora M. Sequence variation in group A *streptococcus* pili and association of pilus backbone types with Lancefield T serotypes. J Infect Dis. 2008 Dec 15;198(12):1834-41.
98. Severin A, Nickbarg E, Wooters J, Quazi SA, Matsuka YV, Murphy E, Moutsatsos IK, Zagursky RJ, Olmsted SB. Proteomic analysis and identification of *Streptococcus pyogenes* surface-associated proteins. J Bacteriol. 2007 Mar;189(5):1514–22.
99. Hu MC, Walls MA, Stroop SD, Reddish MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. Infect Immun. 2002 Apr;70(4):2171–7.
100. CDC *Streptococcus* laboratory: about the *Streptococcus* laboratory [Internet]. Atlanta: CDC; [updated 2016 Aug 17]. Assigning *emm* types and subtypes; [updated/reviewed 2007 Dec 19; cited 2017 Jul 21]; [about 51 lines]. Available from: https://www.cdc.gov/streplab/assigning.html
101. Excler JL, Kim JH. Accelerating the development of a group A *streptococcus* vaccine: an urgent public health need. Clin Exp Vaccine Res. 2016 Jul;5(2):101-7.
102. Batzloff MR, Hayman WA, Davies MR, Zeng M, Pruksakorn S, Brandt ER, Good MF. Protection against group A streptococcus by immunization with J8-diphtheria toxoid: contribution of J8- and diphtheria toxoid-specific antibodies to protection. J Infect Dis. 2003 May 15;187(10):1598-608.

103. Pandey M, Langshaw E, Hartas J, Lam A, Batzloff MR, Good MF. A synthetic M protein peptide syngergizes with a CXC chemokine protease to induce vaccine-mediated protection against virulence streptococcal pyoderma and bacteremia. J Immunol. 2015 Jun 15;194(12):5915-25.

104. Agrahari G, Liang Z, Mayfield JA, Balsara RD, Ploplis VA, Castellino FJ. Complement-mediated opsonization of invasive group A *Streptococcus pyogenes* strain AP53 is regulated by the bacterial two-component cluster of virulence responder/sensor (CovRS) system. J Biol Chem. 2013 Sep 20;288(38):27494-504.

105. De Amicis KM, Freschi de Barros S, Alencar RE, Postól E, Martins Cde O, Arcuri HA, Goulart C, Kalil J, Guilherme L. Analysis of the coverage capacity of the StreptInCor candidate vaccine against *Streptococcus pyogenes*. Vaccine. 2014 Jul 7;32(32):4104-10.

106. Cleary PP, Matsuka YV, Huynh T, Lam H, Olmsted SB. Immunization with C5a peptidase from either group A or B streptococci enhances clearance of group A streptococci from intranasally infected mice. Vaccine. 2004 Oct 22;22(31-32):4332-41.

107. Pancholi V, Fischetti VA. A major surface protein on group A streptococci is a glyceraldehyde-3-phosphate-dehydrogenase with multiple binding activity. J Exp Med. 1992 Aug 1;176(2):415–26.

108. Seidler KA, Seidler NW. Role of extracellular GAPDH in *Streptococcus pyogenes* virulence. Mo Med. May-June 2013;110(3):236-40.

109. Boël G, Jin H, Pancholi V. Inhibition of cell surface export of group A streptococcal anchorless surface dehydrogenase affects bacterial adherence and antiphagocytic properties. Infect Immun. 2005 Oct;73(10):6237–48.

110. Jin H, Song YP, Boel G, Kochar J, Pancholi V. Group A streptococcal surface GAPDH, SDH, recognizes uPAR/CD87 as its receptor on the human pharyngeal cell and mediates bacterial adherence to host cells. J Mol Biol. 2005 Jul 1;350(1):27–41.

111. Oliveira L, Madureira P, Andrade EB, Bouaboud A, Morello E, Ferreira P, Poyart C, Trieu-Cuot P, Dramsi S. Group B streptococcus GAPDH is released upon cell lysis, associates with bacterial surface, and induces apoptosis in murine macrophages. PLoS One. 2012;7(1):e29963.

112. Pancholi V, Fischetti VA. Glyceraldehyde-3-phosphate dehydrogenase on the surface of group A streptococci is also an ADP-ribosylating enzyme. Proc Natl Acad Sci USA. 1993 Sept 1;90(17):8154-8.

113. Pancholi V, Fischetti VA. Regulation of the phosphorylation of human pharyngeal cell proteins by group A streptococcal surface dehydrogenase: signal transduction between streptococci and pharyngeal cells. J Exp Med. 1997 Nov 17;186(10):1633–43.

114. Jin H, Agarwal S, Agarwal S, Pancholi V. Surface export of GAPDH/SDH, a glycolytic enzyme, is essential for *Streptococcus pyogenes* virulence. MBio. 2011 May 31;2(3):e00068-11.

115. Kang SO, Caparon MG, Cho KH. Virulence gene regulation by CvfA, a putative RNase: the CvfA-enolase complex in *Streptococcus pyogenes* links nutritional stress, growth-phase control, and virulence gene expression. Infect Immun. 2010 Jun;78(6):2754-67.

116. Pancholi V, Fischetti VA. α-Enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. J Biol Chem. 1998 Jun 5;273(23):14503-15.

117. Fontán PA, Pancholi V, Nociari MM, Fischetti VA. Antibodies to streptococcal surface enolase react with human alpha-enolase: implications in poststreptococcal sequelae. J Infect Dis. 2000 Dec;182(6):1712-21.

118. Boone TJ. Characterization of phosphoglycerate kinase expressed on the surface of group B streptococcus [PhD's thesis]. [Edmonton (AB)]: University of Alberta; 2012. 249 p.

119. Hytönen J, Haataja S, Gerlach D, Podbielski A, Finne J. The SpeB virulence factor of *Streptococcus pyogenes*, a multifunctional secreted and cell surface molecule with strepadhesin, laminin-binding and cysteine protease activity. Mol Microbiol. 2001 Jan;39(2):512-9.

120. Olsen RJ, Raghuram A, Cantu C, Hartman MH, Jimenez FE, Lee S, Jgo A, Rice KA, Saddington D, Spillman H, Valson C, Flores AR, Beres SB, Long W, Nasser W, Musser JM. The majority of 9,729 group A streptococcus strains causing disease secrete SpeB cysteine protease: pathogenesis implications. Infect Immun. 2015 Dec;83(12):4750-8.

121. Linke C, Caradoc-Davies TT, Young PG, Proft T, Baker EN. The laminin-binding protein Lbp from *Streptococcus pyogenes* is a zinc receptor. J Bacteriol. 2009 Sep;191(18):5814-23.

122. Terao Y, Kawabata S, Kunitomo E, Nakagawa I, Hamada S. Novel laminin-binding protein of *Streptococcus pyogenes*, Lbp, is involved in adhesion to epithelial cells. Infect Immun. 2002 Feb;70(2):993-7.

123. Batsford SR, Mezzano S, Mihatsch M, Schiltz E, Rodríguez-Iturbe B. Is the nephritogenic antigen in post-streptococcal glomerulonephritis pyrogenic exotoxin B (SPEB) or GAPDH? Kidney Int. 2005 Sep;68(3):1120-9.

124. Chhatwal GS. Anchorless adhesins and invasins of Gram-positive bacteria: a new class of virulence factors. Trends Microbiol. 2002 May;10(5):205-8.

125. D'Costa SS, Romer TG, Boyle MD. Analysis of expression of a cytosolic enzyme on the surface of *Streptococcus pyogenes*. Biochem Biophys Res Commun. 2000 Nov 30;278(3):826-32.

126. Antikainen J, Kuparinen V, Lähteenmäki K, Korhonen TK. pH-dependent asssociation of enolase and glyceraldehyde-3-phosphate dehydrogenase of *Lactobacillus crispatus* with the cell wall and lipoteichoic acids. J Bacteriol. 2007 Jun;189(12):4539-43.

127. Terrasse R, Amoroso A, Vernet T, Di Guilmi AM. *Streptococcus pneumoniae* GAPDH is released by cell lysis and interacts with peptidoglycan. PLoS One. 2015 Apr 30; 10(4):e0125377.

128. Madureira P, Baptista M, Vieira M, Magalhães V, Camelo A, Oliveira L, Ribeiro A, Tavares D, Trieu-Cuot P, Vilanova M, Ferreira P. *Streptococcus agalactiae* GAPDH is a virulence-associated immunomodulatory protein. J Immunol. 2007 Feb 1;178(3):1379-87.

129. Seifert KN, McArthur WP, Bleiweis AS, Brady LJ. Characterization of group B streptococcal glyceraldehyde-3-phosphate dehydrogenase: surface localization, enzymatic activity, and protein-protein interactions. Can J Microbiol. 2003 May;49(5):350-6.

130. Vázquez-Zamorano ZE, González-Lóopez MA, Romero-Espejel ME, Azuara-Liceaga El, López-Casamichana M, Olivares-Trejo Jde J. *Streptococcus pneumoniae* secretes a glyceraldehyde-3-phosphate dehydrogenase, which binds haemoglobin and haem. Biometals. 2014 Aug;27(4):683-93.

131. Bergmann S, Rohde M, Hammerschmidt S. Glyceraldehyde-3-phosphate dehydrogenase of Streptococcus pneumoniae is a surface-displayed plasminogen-binding protein. Infect Immun. 2004 Apr;72(4):2416-9.

132. Burnham CA, Shokoples SE, Tyrrell GJ. Phosphoglycerate kinase inhibits epithelial cell invasion by group B streptococci. Microb Pathog. 2005 May-Jun;38(5-6):189-200.

133. Lenz LL, Mohammadi S, Geissler A, Portnoy DA. SecA2-dependent secretion of autolytic enzymes promotes *Listeria monocytogenes* pathogenesis. Proc Natl Acad Sci USA. 2003 Oct 14;100(21):12432-7.

134. Hulbah MJ, Tyrrell GJ. Identification of a gene in group B streptococcus involved in the surface expression of GBS-phosphoglycerate kinase. Poster session presented at: Woman and Children's Health Research Institute Research Day;2015 Oct 28; Edmonton,AB.

135. CDC. Principles of epidemiology in public health practice: an introduction to applied epidemiology and biostatistics [Internet]. 3rd ed. Atlanta: CDC; 2006 Oct [updated 2012 May, cited 2017 Jul 21]. Available from: https://www.cdc.gov/ophss/csels/dsepd/ss1978/SS1978.pdf

136. Health topics: epidemiology [Internet]. WHO;2017 [cited 2017 Jul 21]. Available from: http://www.who.int/topics/epidemiology/en/

137. Tyrrell GJ, Lovgren M, Forwick B, Hoe NP, Musser JM, Talbot JA. M types of group A streptococcal isolates submitted to the National Centre for Streptococcus (Canada) from 1993 to 1999. J Clin Microbiol. 2002 Dec;40(12):4466–71.

Table 1.1. GAS *emm* patterns and examples of their associated *emm* types*

emm pattern	*emm* type
A-C	*emm* 1
	emm 3
	emm 5
	emm 6
	emm 12
D	*emm* 33
	emm 41
	emm 53
	emm 59
	emm 64
	emm 74
	emm 80
	emm 81
	emm 83
	emm 91
	emm 101
	emm 115
	emm 182
E	*emm* 2
	emm 4
	emm 9
	emm 11
	emm 22
	emm 25
	emm 28
	emm 44
	emm 68
	emm 73
	emm 75
	emm 77
	emm 82
	emm 87
	emm 89
	emm 94
	emm 102
	emm 104
	emm 114
	emm 118

*Note:

The selection of *emm* types for each *emm* pattern is based on Table 2.3 and Table 3.1

(49, 60, 64).

Table 1.2. Components of the 26-valent and 30-valent vaccines.

Vaccine type	Proteins from which N-terminal components derived	References
26-valent M protein-based vaccine	M1.0 M1.2 M2 M3 M5 M6 M11 M12 M14 M18 M19 M22 M24 M28 M29 M33 M43 M59 M75 M76 M77 M89 M92 M94 M101 M114 Spa from M18	9, 49, 68, 99-100
30-valent M protein-based vaccine	M1.0 M2.0 M3.1 M4.0 M5.14 M6.4 M11.0 M12.0 M14.3 M18.0 M19.0 M22.0 M24.0 M28.0 M29.2	9, 11, 49, 68, 100

M44.0
M49.0
M58.0
M73.0
M75.0
M77.0
M78.0
M81.0
M82.0
M83.1
M87.0
M89.0
M92.0
M114.0
M118.0
Spa from M18

*Notes regarding coverage assessments (section 2.2.2):

a) The M serotypes considered are those that demonstrated any level of killing in bactericidal assays involving the vaccines (5, 9, 11).

b) For the 30-valent vaccine study, bactericidal assays considered M types not subtypes (11, 49, 100, 137).

c) Streptococcal protective antigen (Spa) (68), whose amino-terminal is a component of the 30-valent vaccine (11, 68) and 26-valent vaccine (9, 68), targets M18 (9, 11, 68).

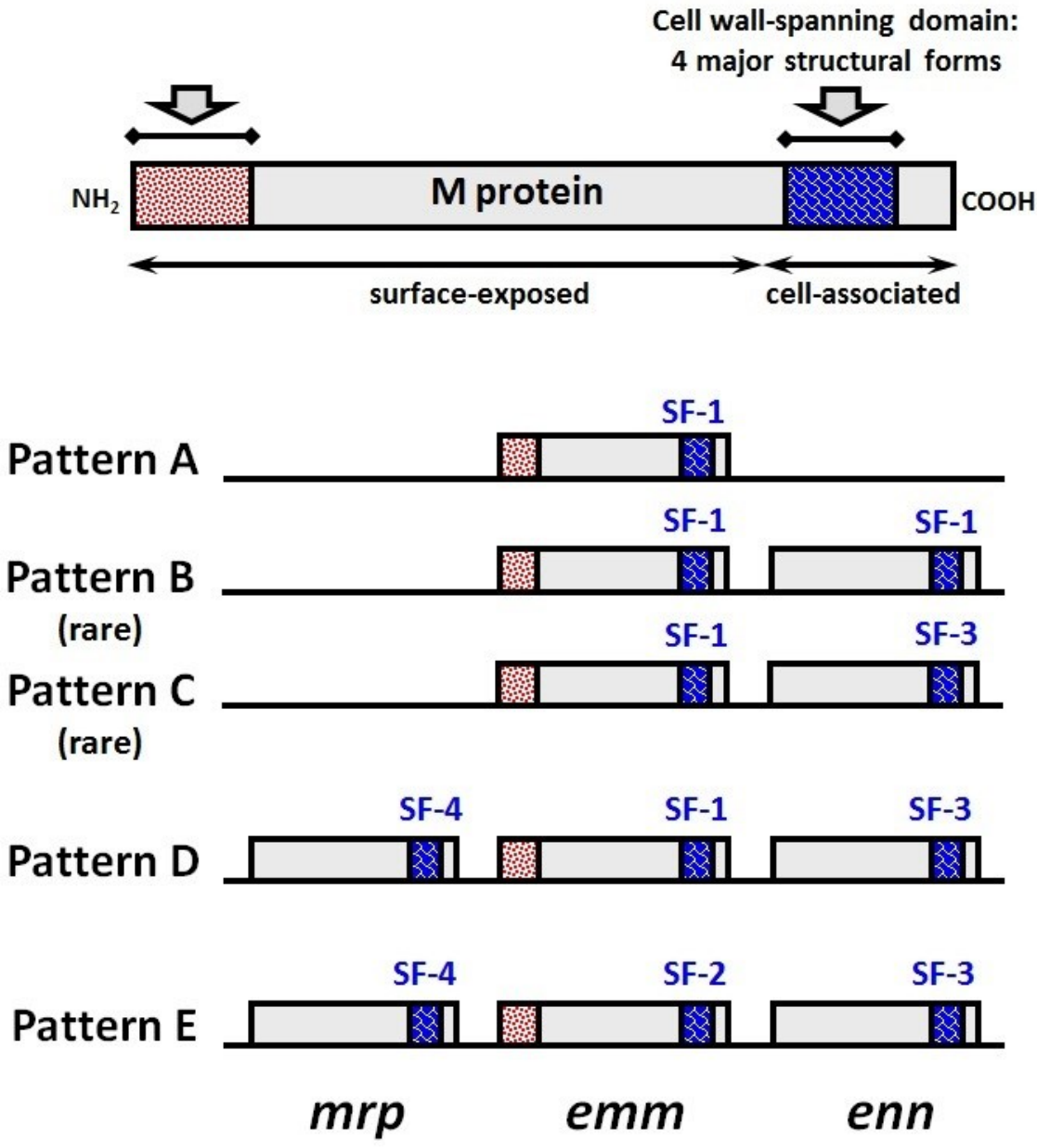


Figure 1.1. The arrangement and presence/absence of *emm*-like (*mrp* and *enn* genes) and *emm* genes that define the *emm* patterns of GAS (58, 64)[1]. SF refers to the subfamily forms at the 3' portion of *emm*-like and *emm* genes (58).

[1]Taken from Bessen, DE, Molecular Basis of Serotyping and the Underlying Genetic Organization of *Streptococcus pyogenes*, in Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: Basic Biology to Clinical Manifestations [Internet]. Oklahoma City (OK): with permission from the publisher: University of Oklahoma Health Sciences Center; 2016-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK333424/

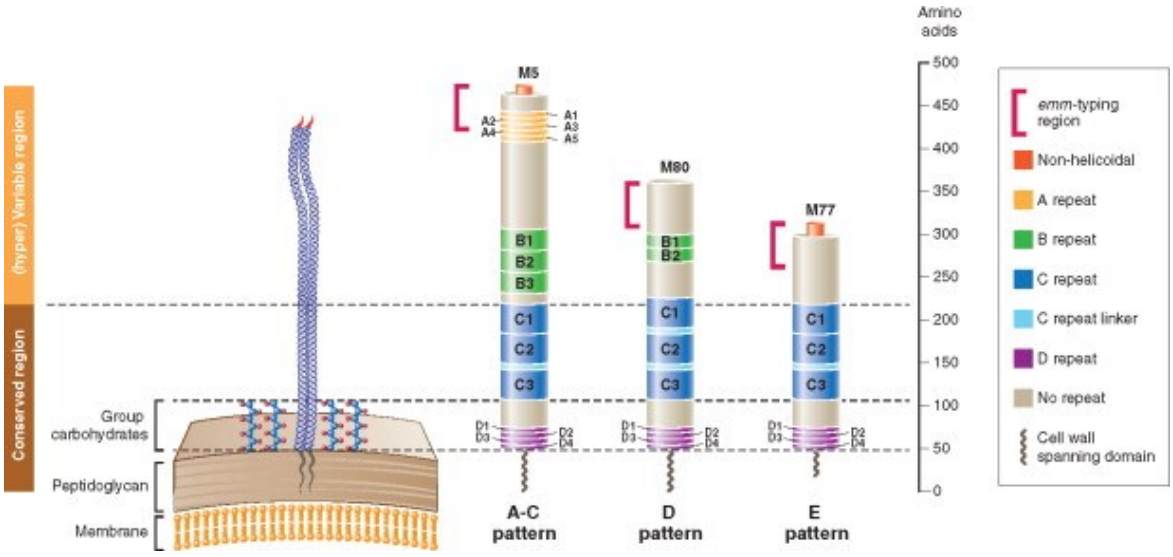


Figure 1.2. Different M protein structural models according to their *emm* pattern (64)[2]. The GAS M types of M80, M77 and M5 represent prototypical structures for each *emm* pattern (13, 49, 64).

[2]Reprinted from Clinical Microbiology and Infection, Vol 19, McMillan DJ, -A. Drèze P, Vu T, Bessen DE, Guglielmini J, Steer AC, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR, Updated model of group A *Streptococcus* M proteins based on a comprehensive worldwide study, E222-E229, Copyright 2017, with permission from Elsevier

Fig.1

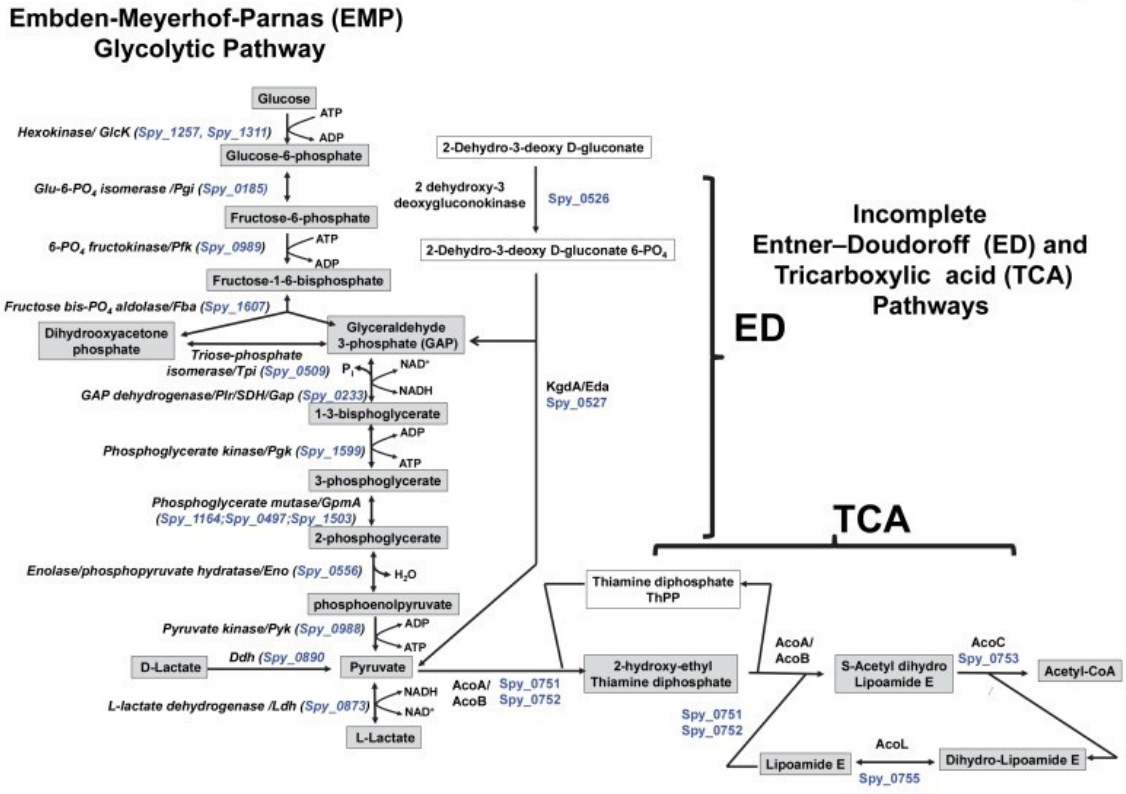


Figure 1.3. The Entner-Doudoroff, tricarboxylic acid and Embden-Meyerhof-Parnas pathways in GAS (1, 15)[3]. The genes producing the glycolytic enzymes are presented in the blue font and are from GAS M1 MGAS5005 (1, 15, 49).

[3]Taken from Pancholi, V, Chaparon, M, *Streptococcus pyogenes* metabolism, in Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: Basic Biology to Clinical Manifestations [Internet]. Oklahoma City (OK): with permission from the publisher: University of Oklahoma Health Sciences Center; 2016-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK333424/

Chapter 2: Epidemiology of invasive GAS in Alberta based on laboratory data

2.1 Introduction

Group A *Streptococcus* (GAS) invasive disease comes in many forms (1-2). Examples include cellulitis, bacteremia, necrotizing fasciitis (NF), pneumonia and streptococcal toxic shock syndrome (STSS). It is estimated that GAS invasive disease affects >660 000 individuals annually and causes >160 000 deaths per year worldwide (3). Therefore, the province of Alberta in Canada is no exception to encounters with GAS invasive disease (1, 3). Since August 1999, GAS invasive disease has been under surveillance within Alberta. In addition, the Alberta Provincial Laboratory for Public Health (ProvLab Alberta) conducts serotyping for invasive GAS received (4-6). In Alberta, incidence rates for invasive disease have increased from 5.0/100 000 in 2000 to 7.69/100 000 in 2016 (Table 2.1, 1, 7). According to the Tyrrell *et al*., 2005 study, the disease is predominant in the metropolitan areas of Edmonton and Calgary and affects mainly < l year olds and ≥65 year olds (1). In addition, most invasive disease cases in Alberta were shown to occur in winter and early spring.

GAS typing is useful for understanding the evolutionary relationship of GAS strains (2, 4). It is conducted in outbreak settings and epidemiologic surveys. An example of a GAS typing system involves sequencing the 90 nucleotides of the *emm* gene that corresponds with the N-terminus of the GAS M protein virulence factor and assigning the sequence to an *emm* type. These *emm* types correlate with the GAS M serotypes, which are derived through a typing system involving M-protein specific antisera. In addition to assigning *emm* types, an *emm* subtype can be designated for GAS by sequencing the 150 *emm* gene nucleotides that corresponds with the M protein N-terminus. In Alberta, type *emm* 1 or serotype M1 (2, 4) has been predominant in 1993-1999 (8), 2000-2002 (1) and 2004-2011 (6). By understanding the M/*emm* types that are present in a region, correlations between such types and risk factors for invasive disease/invasive disease forms can be established (1-2). For example in Alberta, M1 was mostly correlated with NF and STSS cases in 2000 and 2001 (1).

Presently, there is no licensed vaccine for GAS (9). However, there are several candidates in development. Two of these are the 26-valent M protein-based vaccine (9-10) and the 30-valent M protein-based vaccine (9, 11). The 26-valent vaccine is in the phase II developmental stage, while the 30-valent vaccine is in the phase I developmental

stage (9, 12). Both vaccines consist of four proteins that contain N-terminal peptides of selected M protein serotypes/subtypes, as well as the amino-terminal peptide of streptococcal protective antigen (Spa) from M18 (4, 10-11, 13-14). As the name suggests, the 26-valent vaccine represents 26 different M serotypes/subtypes while the 30-valent vaccine represents 30 different M subtypes. In addition to targeting the GAS M serotypes that they depict, both vaccines seem to be active against a number of non-vaccine M/*emm* types (4, 10-11, 15-16).

Although investigations regarding invasive GAS in Alberta have been conducted, a more thorough analysis of recent years has not been conducted (Table 2.1, 1-2, 6). In addition, the coverage-related implication of utilizing a potentially licensed 26-valent vaccine or 30-valent vaccine in Alberta has not been fully assessed (9-11, 16). The objective of this study was to assess the M/*emm* type of invasive GAS cases in Alberta in 2013- 2016 with respect to future M/*emm* based vaccines.

2.2 Materials and Methods

2.2.1 Selection of invasive GAS cases for analysis

Data files were provided by the Bacterial Typing Unit (ProvLab, Edmonton, Alberta) (2,6). These files contained information regarding GAS cases investigated. The information in the files that became the basis for selection of GAS cases for analysis included collection date, provincial location, specimen source, patient name and *emm* type. In terms of collection date and provincial location, only GAS cases from Alberta in 2013- 2016 were considered. Regarding specimen source, GAS from throat specimens were excluded as this implies pharyngitis, not invasive infection (2, 17-18). All other specimens were inferred to reflect invasive GAS. On the subject of *emm* types, cases without an *emm* type were also excluded (2). Finally, only one of multiple cases associated with the same patient name, same *emm* type and collection date of <1 month apart was considered for analysis. I carried out the prior criteria for 2013, 2015 and 2016 data, while Dr. Gregory Tyrrell implemented the criteria for the 2014 data.

After exclusion, the remaining invasive GAS cases were categorized according to the *emm* type present in each collection year (2). The total number of invasive cases present in each collection month in 2013-2016 was assessed as well (1-2). For GAS *emm* 1, 82 and 101, the number of invasive cases in collection months in 2013-2016 was analyzed. For the 2016 invasive cases, categorization based on gender and age was also conducted.

2.2.2 Theoretical coverage assessments with a 30-valent vaccine and 26-valent vaccine

After the total number of invasive cases associated with each *emm* type in 2013-2016 were determined (2), the theoretical coverage of such cases with a 30-valent vaccine and 26-valent vaccine was assessed using Table 1.2 and Table 2.2 (Table 1.2, Table 2.2, 2, 9-11, 16). Coverage was determined with and without consideration for vaccine cross-reactivity (Table 1.2, Table 2.2, 2, 10-11, 15-16). The percentage of non-coverage was calculated for 2013-2016 by considering the number of invasive cases that could not be covered by either vaccine and the total number of invasive cases in a given year.

2.2.3 Incidence rate calculations

Estimated incidence rate of invasive GAS cases in 2016 for distinct genders and ages were calculated using the formula: number of new cases/estimated mid-year Alberta population for different genders/age ranges X 100 000 (1-2, 7). In this study, new cases were defined to also include similar cases that had ≥1 month separation in terms of their collection dates. Estimated mid-year Alberta populations (adjusted) were obtained from the Alberta Interactive Health Data Application (7, 19-20).

2.3 Results

2.3.1 Invasive GAS emm types in Alberta in 2013- 2016

From 2013-2016, a total of 1 085 invasive GAS cases representing 43 different *emm* types are analyzed (Table 2.3, 1-2). The most predominant *emm* type over the four years is *emm* 1 (204 cases). GAS *emm* 1 is the most common *emm* type seen in 2013-2016 (Table 2.3, Supplementary figure S2.1A-C, 1-2). In addition, it shows seasonality with peaks from December to June in general (Figure 2.2A, 1-2). The second and third most common *emm* types from 2013-2016 are *emm* 82 (71 cases) and *emm* 28 (70 cases) respectively (Table 2.3, 1-2). GAS *emm* 82 invasive cases have risen overall from 14 in 2013 to 29 in 2016, while GAS *emm* 28 has remained consistent over the four years examined. The fourth most predominant *emm* type is *emm* 101 (66 cases). In 2013 and 2014, the number of GAS *emm* 101 invasive cases is two and three respectively. However in 2015 and 2016, this value suddenly rises to 25 and 36 respectively. GAS *emm* 101 shows seasonality in 2015-2016 with peaks in April, July-August and October generally (Figure 2.2C, 1-2). The fifth most common *emm* type in the four years is *emm* 41 (62 cases) (Table 2.3, 1-2). The invasive cases associated with this *emm* type have increased generally from nine in 2013 to 15 in 2016.

Besides the five predominant *emm* types indicated above, other *emm* types of interest in the years examined are *emm* 83, 59, 53 and 74 (Table 2.3, 1-2). GAS *emm* 83 has shown an increase in invasive cases from two in 2013 to 22 in 2016 (Table 2.3, 1-2) and demonstrates no seasonality (Figure 2.2B, 1-2). GAS *emm* 59 invasive cases have risen from five in 2013 to 20 in 2016 (Table 2.3, 1-2). Unlike *emm* 83 and 59, GAS *emm* 53 has decreased in invasive cases from 20 in 2013 to zero in 2016. Finally, 15 GAS *emm* 74 invasive cases are present only in 2016.

2.3.2 Total monthly invasive GAS cases in 2013-2016

From 2013-2016, seasonality of invasive GAS cases in Alberta is becoming more apparent (Figure 2.1, 1-2). In general, invasive GAS cases seem to peak in December-January, April-May and July.

2.3.3 Incidence rates of invasive GAS related to gender and age

Based on the 2016 data, it seems that males have a higher propensity for invasive GAS (incidence rate - 9.9/100 000 population) than females (incidence rate - 6.1/100 000 population) in Alberta (Figure 2.3A, Supplementary Table S2.1, 1-2, 7). In addition, invasive GAS affects 0-10 year olds, 21-50 year olds and 51->80 year olds more than other ages (Figure 2.3B; Supplementary Table S2.1, 1-2, 7). However amongst these age groups, it is those 51->80 years that are most afflicted with invasive GAS. In fact, the incidence rate for >80 year olds is the highest in this age group at 16.2/100 000 population.

2.3.4 Theoretical coverage of a 30-valent vaccine and 26-valent vaccine in Alberta

The potential implementation of a 30-valent vaccine or 26-valent vaccine in Alberta in the possible future to combat invasive GAS could be beneficial (Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, 2, 9-11, 15-16). With either vaccine, whether cross-reactivity is considered or not, at least 65% theoretical coverage of cases is obtained in each year examined. The 30-valent vaccine demonstrates a lower percentage of non-coverage in 2013-2016 than the 26-valent vaccine. Without consideration for cross-reactivity, these percentages are 21.6% (2013), 22.4% (2014), 23.1% (2015) and 28.8% (2016) for the 30-valent vaccine and 25.9% (2013), 28.9% (2014), 29.2% (2015) and 34.7% (2016) for the 26-valent vaccine (Figure 2.4A-B, Supplementary figures S2.1A-D-S2.2A-D, 2, 9-11, 15-16). When cross-reactivity is considered, the percentages are 9.1% (2013), 15.5% (2014), 17.1% (2015) and 17.4% (2016) for the 30-valent vaccine and 22.4% (2013), 25.4% (2014), 26.7% (2015) and 32.6% (2016) for the 26-valent vaccine (Figure 2.4A-B, Supplementary figures S2.3A-D-S2.4A-D, 2, 9-11, 15-16). Based on the prior percentages, it is shown that from 2013- 2016, both vaccines demonstrate progressively higher percentages of non-coverage in Alberta, indicating that their coverage is worsening (Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, 2, 9-11, 15-16).

2.4 Discussion

In this study, a total of 43 distinct *emm* types associated with 1 085 invasive GAS cases are reported from 2013-2016 (Table 2.3, 1-2). The five most predominant of these *emm* types are 1, 82, 28, 101 and 41. GAS M1/*emm* 1 was the most common known M/*emm* type reported in Alberta in 1993-1999 (2, 4, 8), 2000-2002 (1, 2, 4), 2004-2007 and 2010-2011 (2, 6). In addition from 2004-2007, 2009 (2) and 2010-2014 (2,5), *emm* 1 was the most frequent *emm* type reported from GAS isolates and data collected throughout Canada. Therefore, it is not surprising to see *emm* 1 remain as the most common *emm* type in Alberta in this study (Table 2.3, 1-2, 4-5, 8). Seven GAS *emm* 82 invasive cases were first reported in Alberta in 2001 (1-2, 4, 8). In 2007, *emm* 82 invasive cases peaked at 29 before decreasing in 2008-2011 (2, 6). In 2016, *emm* 82 peaked again at 29 invasive cases (Table 2.3, 1-2, 4, 6, 8). This indicates that *emm* 82 has a periodicity associated with its invasive cases. GAS M28/*emm* 28 has been reported in Alberta in 1993-1999 (2, 4, 8), 2000-2002 (1-2, 4) and 2006-2011 (2, 6). Therefore, its appearance in Alberta in 2013-2016 is not surprising (Table 2.3, 1-2, 4, 6, 8). GAS *emm* 101 was not reported in Alberta previously (1-2, 4, 6, 8). However, *emm* 101 has been detected in Canada since the mid to late 2000s (1-2, 4-5, 8, 21- 22). In Alberta, GAS *emm* 101 invasive cases have risen from two in 2013 to 36 in 2016 (Table 2.3, 2). A possible explanation for this increase is that clonal *emm* 101 may have spread from eastern Canada, especially Thunder Bay, to Alberta (Table 2.3, 2, 21, 23). In Thunder Bay, Ontario, incidence rates of invasive GAS reported were higher than those of the province in 2011-2013 (21). During this same period, *emm* 1, 4, 82, 83, 87, 101 and 114 were most commonly associated with invasive disease in Thunder Bay, as well as severe skin and soft tissue infections (1, 21, 24). Amongst these *emm* types, only *emm* 1 and *emm* 4 were the same in terms of being the most common for invasive disease in Thunder Bay and the rest of Ontario (2, 21). In addition, *emm* 4, 82, 83, 87, 101 and 114 were each composed of GAS clonal strains. As *emm* 101 consists of a clonal strain, its possible spread from east to west may account for the increased numbers of this *emm* type from 2013-2016 in Alberta (Table 2.3, 2, 21, 23). However, the question remains as to why Alberta would be vulnerable to this clonal *emm* 101. In Thunder Bay, GAS invasive disease in 2011-2013 was correlated with alcohol abuse, intravenous drug usage, homelessness and infection with hepatitis C virus (21). Therefore, the presence of

the prior risk factors in Alberta may have led to the appearance of *emm* 101 in high numbers (Table 2.3, 2, 21, 23). The indicated risk factors have also been associated with *emm* 59. Canada experienced an invasive GAS epidemic from 2006-2008 that was caused by an *emm* 59 clone (2, 23). This clone disseminated into the United States as well (2, 23, 25- 26). The increase in case number from five in 2013 to 20 in 2016 from *emm* 59 in Alberta may indicate that the *emm* 59 clone has reemerged in the province, because of the prior risk factors for instance (Table 2.3, 2, 21, 23).

GAS *emm* 41 has not been reported in Alberta before this study (Table 2.3, 1-2, 4, 6, 8). However, it has been reported in Canada since 2004 (1-2, 4-5, 8). Thus, the presence of *emm* 41 in Alberta in 2013-2016 makes sense (Table 2.3, 2, 5). The appearance of GAS *emm* 53 in 2013-2015 in Alberta is expected, as this *emm* type has been reported in Alberta in 2006-2007 and 2009-2011 (Table 2.3, 2, 6). However, what is unexpected is that GAS *emm* 53 decreased from a peak of 20 invasive cases in 2013 to zero in 2016. From this information, it is hypothesized that the GAS *emm* 53 in 2013 represents a clonal strain (Table 2.3, 2, 21, 23). The decrease in invasive cases associated with this possible clonal *emm* 53 may be explained by increased herd immunity within Alberta and/or replacement with another clone, such as *emm* 101. This possibly clonal *emm* 53 may be spreading to eastern Canada, as Thunder Bay detected *emm* 53 in 2015. GAS *emm* 74 has not been seen in Alberta prior to 2016 (Table 2.3, 1-2, 4, 6, 8). In Toronto, Ontario, *emm* 74 reemerged in December 2015 (27). The *emm* type was associated with an outbreak at a shelter and was shown to be clonal (2, 27). Thus, the sudden appearance of *emm* 74 in 2016 in Alberta may have to do with the spread of this *emm* 74 clone from Toronto to western Canada (Table 2.3, 1-2, 4, 8, 23). Finally, GAS *emm* 83 has previously been reported in Alberta in 2004-2008 and 2011 (1-2, 6, 8). Therefore, its appearance in 2013-2016 is not surprising (Table 2.3, 1-2, 6, 8).

A seasonal trend is demonstrated for invasive GAS overall in Alberta in 2013-2016 (Figure 2.1, 1-2) as well as for the particular *emm* types, *emm* 1 and 101 (Figure 2.2A-C, 1-2). The choice to conduct a seasonal trend analysis for *emm* 1 is based upon the fact that it is the most common *emm* type reported in 2013-2016 (Table 2.3, Figure 2.2A, 1-2). In contrast, the decision to perform a seasonal trend analysis for *emm* 83 and 101 is based upon the fact that both *emm* types are rising in invasive cases in 2013-2016 (Table

2.3, 1-2, 4). Generally, invasive GAS was predominant in December-January, April-May and July based on the four years examined (Figure 2.1, 1-2). This is similar to what was reported in Alberta from 2000-2002, where the early spring and winter months received the largest number of invasive GAS cases. GAS *emm* 1 showed high prevalence in invasive cases from December-June (Figure 2.2A, 1-2) while *emm* 101 had peaks in cases in April, July-August and October in 2015-2016 (Figure 2.2C, 1-2). For *emm* 1, the seasonality demonstrated based on the 2013- 2016 data is similar to that reported for Canada in 2004-2009 (Figure 2.2A, 1-2). In 2004-2009, *emm* 1 showed the highest case numbers from December to March (2). For *emm* 101, this study may be the first to demonstrate a seasonal pattern associated with this *emm* type in Alberta (Figure 2.2C, 1-2, 5, 8).

According to the 2016 data, the incidence rate of invasive GAS is higher in males than females in Alberta (Figure 2.3A, Supplementary Table S2.1, 1-2, 7). This is not surprising to see, as in two national surveillance studies in Canada, invasive GAS came from males more than females (Figure 2.3A, 1- 2, 5, 7, 28). In addition, the incidence rate of invasive GAS for >80 year olds (16.2/100 000 population) was the highest amongst all age groups considered in Alberta in 2016, while it was the lowest for 11-20 year olds (2.1/100 000 population) (Figure 2.3B, Supplementary Table S2.1, 1-2, 7). Also, the incidence rate for 0-10 year olds was similar to that for 21-30, 31-40 and 41-50 year olds, but lower than that for 51-60, 61-70 and 71-80 year olds. The predominance of invasive GAS in 71-80 and >80 year olds in Alberta is not a new phenomenon, as similar results have been reported previously for the province (Figure 2.3B, 1-2, 7). The same can be said for the similarities in incidence rates between the 0-10 year old group and 21-30, 31-40 and 41-50 year old groups, as well as the lowest incidence rate being reported for 11-20 year olds.

The implementation of a potentially licensed 30-valent vaccine or 26-valent vaccine in Alberta in the possible future is beneficial, as at least 65% theoretical coverage against invasive GAS is obtained in 2013- 2016 (Figure 2.4A-B; Supplementary figures S2.1A-D-S2.4A-D, 2, 4, 9-11, 13, 15-16). The vaccine M serotypes selected for calculating this theoretical coverage value were those that showed any level of killing in bactericidal assays performed by McNeil *et al.*, 2005 and Dale *et al*., 2011 (Table 1.2,

Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, 2, 4, 9-11, 13, 15-16). In contrast, the non-vaccine types chosen for deriving the theoretical coverage were those that demonstrated >40% killing in bactericidal assays conducted by Hu *et al*., 2002, Dale *et al.,* 2011 and Dale *et al.*, 2013 (Table 1.2, Table 2.2, Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, 2, 4, 9-11, 13, 15-16). Therefore, the percentages of non-coverage calculated for 2013- 2016 in Alberta may be underestimations of their true value. This is because 100% killing of GAS M/*emm* types was inferred. In addition, bactericidal assays often used rabbits for assessing percent killing of GAS and did not consider all possible *emm* types for cross-reactivity. Despite these limitations, this study still shows that a 30-valent vaccine or 26-valent vaccine would be of some benefit in Alberta. However, alternatives for or enhancements of these vaccines should be considered as the percentage of non-coverage is worsening over time.

In short, in this study, a more updated and thorough epidemiological assessment of invasive GAS in Alberta has been provided (Table 2.3, Figure 2.1-2.4, 1-2, 4, 8, 29). This assessment showed that aspects, such as the predominance of *emm* 1 and ages associated with invasive GAS, have not changed much in Alberta since the 2000s (Table 2.3, Figure 2.1, Figure 2.3B, 1-2, 4, 8). However, other aspects such as the emergence of a substantial number of cases of *emm* 101 and 74 are fairly new to Alberta (Table 2.3, 1-2, 4, 6, 8).

2.5 References

1. Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. Invasive group A streptococcal disease in Alberta, Canada (2000 to 2002). J Clin Microbiol. 2005 Apr; 43(4):1678–83.
2. Tyrrell GJ, Lovgren M, St. Jean T, Hoang L, Patrick DM, Horsman G, Van Caeseele P, Sieswerda LE, McGeer A, Laurence RA, Bourgault A-M, Low DE. Epidemic of group A *streptococcus* M/emm59 causing invasive disease in Canada. Clin Infect Dis. 2010 Dec 1;51(11):1290–7.
3. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005 Nov; 5(11):685-94.
4. Spellerberg B, Brandt C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci). In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
5. Demczuk WHB, Griffith A, Singh R, Montes K, Sawatsky P, Martin I, Mulvey M. National laboratory surveillance of invasive streptococcal disease in Canada: annual summary 2014 [Internet]. Winnipeg: Public Health Agency of Canada; 2015 Dec [cited 2017 Jul 21]. Available from: http://www.healthycanadians.gc.ca/publications/drugs-products-medicaments-produits/2014-streptococcus/alt/surveillance-streptococca-eng.pdf
6. Government of Alberta [Internet]. Alberta Government. Streptococcal disease – group A, invasive; c2003-2013 [revised 2013 Jun; cited 20 Aug 2017]; [about 17 p]. Available from: https://open.alberta.ca/dataset/b6405d01-6ffd-4b8a-9762-2d6c29772f9c/resource/0fc41de9-1b42-427d-914c-0c687eca0652/download/Guidelines-Streptococcal-A-Invasive-2013.pdf
7. CDC. Principles of epidemiology in public health practice: an introduction to applied epidemiology and biostatistics [Internet]. 3rd ed. Atlanta: CDC; 2006 Oct [updated 2012 May, cited 2017 Jul 21]. Available from: https://www.cdc.gov/ophss/csels/dsepd/ss1978/ SS1978.pdf
8. Tyrrell GJ, Lovgren M, Forwick B, Hoe NP, Musser JM, Talbot JA. M types of group A streptococcal isolates submitted to the National Centre for Streptococcus (Canada)

from 1993 to 1999. J Clin Microbiol. 2002 Dec;40(12):4466–71.

9. Steer AC, Carapetis JR, Dale JB, Fraser JD, Good MF, Guilherme L, Moreland NJ, Mulholland EK, Schodel F, Smeesters PR. Status of research and development of vaccines for *Streptococcus pyogenes*. Vaccine. 2016 Jun 3;34(26): 2953–8.
10. McNeil SA, Halperin SA, Langley JM, Smith B, Warren A, Sharratt GP, Baxendale DM, Reddish MA, Hu MC, Stroop SD, Linden J, Fries LF, Vink PE, Dale JB. Safety and immunogenicity of 26-valent group A *streptococcus* vaccine in healthy adult volunteers. Clin Infect Dis. 2005 Oct 15;41(8):1114–22.
11. Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine. 2011 Oct 26;29(46): 8175–8.
12. Excler JL, Kim JH. Accelerating the development of a group A *streptococcus* vaccine: an urgent public health need. Clin Exp Vaccine Res. 2016 Jul;5(2):101-7.
13. Dale JB, Chiang EY, Liu S, Courtney HS, Hasty DL. New protective antigen of group A streptococci. J Clin Invest. 1999 May 1;103(9):1261–8.
14. CDC *Streptococcus* laboratory: about the *Streptococcus* laboratory [Internet]. Atlanta: CDC; [updated 2016 Aug 17]. Assigning *emm* types and subtypes; [updated/reviewed 2007 Dec 19; cited 2017 Jul 21]; [about 51 lines]. Available from: https://www.cdc.gov/streplab/assigning.html
15. Hu MC, Walls MA, Stroop SD, Reddish MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. Infect Immun. 2002 Apr;70(4):2171–7.
16. Dale JB, Penfound TA, Tamboura B, Sow SO, Nataro JP, Tapia M, Kotloff KL. Potential coverage of a multivalent M protein-based group A streptococcal vaccine. Vaccine. 2013 Mar 15;31(12):1576–81.
17. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the infectious diseases society of America. Clin Infect Dis. 2012 Nov 15;55(10):1–17. Erratum in: Clin Infect Dis. 2014 May;58(10):1496.
18. Seale AC, Davies MR, Anampiu K, Morpeth SC, Nyongesa S, Mwarumba S, Smeesters PR, Efstratiou A, Karugutu R, Mturi N, Willams TN, Scott JAG, Kariuki S,

Dougan G, Berkley JA. Invasive group A *streptococcus* infection among children, rural Kenya. Emerg Infect Dis. 2016 Feb;22(2):224–32.

19. Alberta IHDA: welcome to the interactive health data application [Internet]. Alberta: Government of Alberta;c1983-2017. Alberta IHDA; c1983-2017 [cited 2017 Jul 21]; [about 2 screens]. Available from: http://www.ahw.gov.ab.ca/IHDA_Retrieval /selectSubCategory.do#
20. Alberta IHDA: welcome to the interactive health data application [Internet]. Alberta: Government of Alberta;c1983-2017. Alberta health, surveillance & assessment branch: interactive health data application – adjusted population estimates; 2016 Dec [cited 2017 Jul 21] [about 6 screens]. Available from: http://www.ahw.gov.ab.ca/IHDA_ Retrieval/ShowMetaDataNotesServlet?1942
21. Athey TBT, Teatero S, Sieswerda LE, Gubbay JB, Marchand-Austin A, Li A, Wasserscheid J, Dewar K, McGeer A, Williams D, Fittipaldi N. High incidence of invasive group A *streptococcus* disease caused by strains of uncommon *emm* types in Thunder Bay, Ontario, Canada. J Clin Microbiol. 2016 Jan;54(1):83-92.
22. McDonald RR, Golding GR, Levett PN, Yost CK, Irvine J, Horsman GB, Mulvey MR. Characterization of *Streptococcus pyogenes* and *Staphylococcus aureus* isolates associated with mixed skin and soft tissue infections in northern Saskatchewan. 2008 [cited 2017 Jul 21]: [about 16 slides]. Available from: http://www.narp.ca/pdf/publications/ MCDONALD.pdf
23. Fittipaldi N, Beres SB, Olsen RJ, Kapur V, Shea PR, Watkins ME, Cantu CC, Laucirica DR, Jenkins L, Flores AR, Lovgren M, Ardanuy C, Liñares J, Low DE, Tyrrell GJ, Musser JM. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A *streptococcus*. Am J Pathol. 2012 Apr;180(4):1522–34.
24. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan SL, Montoya JG, Wade JC, Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis. 2014 Jul;59(2):e10-52. Erratum in: Clin Infect Dis. 2014;59:147-50.; Clin Infect Dis. 2015.

25. Fittipaldi N, Tyrrell GJ, Low DE, Martin I, Lin D, Hari KL, Musser JM. Integrated whole-genome sequencing and temporospatial analysis of a continuing group A *streptococcus* epidemic. Emerg Microbes Infect. 2013 Mar;2(3):e13.
26. Engelthaler DM, Valentine M, Bowers J, Pistole J, Driebe EM, Terriquez J, Nienstadt L, Carroll M, Schumacher M, Ormsby ME, Brandy S, Livar E, Yazzie D, Waddell V, Peoples M, Komatsu K, Keim P. Hypervirulent *emm*59 clone in invasive group A *streptococcus* outbreak, southwestern United States. Emerg Infect Dis. 2016 Apr;22(4):734-8.
27. Finkelstein M. Streptococcus, group A – Canada (02): (Ontario) fatal, homeless shelter, emm 74, clonal. ProMED-mail 2017; 5 Apr: 20170406.4952247. <http://www.promedmail.org>. Accessed 21 Jul 2017.
28. Demczuk WHB, Griffith A, Singh R, Martin I, Mulvey M. National laboratory surveillance of invasive streptococcal disease in Canada: annual summary 2013 [Internet]. Winnipeg: Public Health Agency of Canada; 2014 Dec [cited 2017 Jul 21]. Available from: https://www.canada.ca/en/public-health/services/publications/drugs-health-products//laboratory-surveillance-invasive-streptococcal-disease-annual-summary-2013.html
29. Health topics: epidemiology [Internet]. WHO;2017 [cited 2017 Jul 21]. Available from: http://www.who.int/topics/epidemiology/en/
30. CDC *Streptococcus* laboratory: about the *Streptococcus* laboratory [Internet]. Atlanta: CDC; [updated 2016 Aug 17]. New *emm* designations/browse: conversion of st to emm – numerical sort; [updated/reviewed 2014 Apr 2; cited 2017 Jul 21]; [about 24 pages]. Available from: https://www.cdc.gov/streplab/types-emm103-124.html

Table 2.1. Incidence rates of invasive GAS in Alberta from 2007-2016*

Alberta Health iGAS rates.

	Invasive Group A Strep		Severe, Invasive Group A Strep (Necrotizing Fasciitis, Toxic Shock or Death)		All cases of invasive GAS (severe and not severe)	
	Count	Rate per 100,000	Count	Rate per 100,000	Count	Rate per 100,000
2007	214	6.09	42	1.20	256	7.29
2008	173	4.81	48	1.33	221	6.15
2009	160	4.35	39	1.06	199	5.41
2010	148	3.97	30	0.80	178	4.77
2011	184	4.85	36	0.95	220	5.80
2012	183	4.72	61	1.57	244	6.29
2013	220	5.50	47	1.18	267	6.68
2014	216	5.26	44	1.07	260	6.33
2015	220	5.26	68	1.63	288	6.89
2016	273	6.42	54	1.27	327	7.69
2017	78	-	25	-	103	-

*Notes:

a) Table courtesy of Alberta Health

b) iGAS – invasive GAS (1-2, 7)

Table 2.2. GAS non-vaccine M/*emm* types considered for coverage assessments in Alberta

Vaccine type	GAS M/*emm* types*	References
26-valent M protein-based vaccine	M4	4, 10, 15
30-valent M protein-based vaccine	M8 M9 M15 *emm* 25 M30 M33 M36 M40 *emm* 42 M48 M51 M52 *emm* 53 M59 *emm* 60 *emm* 63 *emm* 64 M65 M66 M68 *emm* 69 *emm* 71 *emm* 74 M76 M79 M85 M94 *emm* 95 M97 *emm* 100 M102 M105 M109 M111 M122 *emm* 123 stKNB6 (*emm* 151) stXH1 (*emm* 158) st1389 (*emm* 168)	4, 11, 14, 16, 30

st1731 (*emm* 169)
st2460 (*emm* 180)
st2904 (*emm* 183)
st2911 (*emm* 184)

*Notes:

a) To be selected, GAS M*/emm* types had to demonstrate >40% killing in bactericidal assays (4, 11, 14-15, 16, 30).

b) To be included in the list, *emm* 42, *emm* 53, *emm* 63, *emm* 64, *emm* 71, *emm* 95, *emm* 100 and M122 had to demonstrate one incident of >40% killing (4, 11, 14, 16, 30).

Table 2.3. Documented invasive GAS *emm* types in Alberta from 2013-2016.*

emm type	Number (%) of cases				
	2013	2014	2015	2016	Total
emm 1	44 (19.0%)	51 (22.0%)	47 (16.7%)	62 (18.2%)	204 (18.8%)
emm 82	14 (6.0%)	4 (1.7%)	24 (8.5%)	29 (8.5%)	71 (6.5%)
emm 28	19 (8.2%)	15 (6.5%)	16 (5.7%)	20 (5.9%)	70 (6.5%)
emm 101	2 (0.9%)	3 (1.3%)	25 (8.9%)	36 (10.6%)	66 (6.1%)
emm 41	9 (3.9%)	21 (9.1%)	17 (6.0%)	15 (4.4%)	62 (5.7%)
emm 3	5 (2.2%)	5 (2.2%)	27 (9.6%)	16 (4.7%)	53 (4.9%)
emm 12	10 (4.3%)	11 (4.7%)	12 (4.3%)	18 (5.3%)	51 (4.7%)
emm 89	14 (6.0%)	15 (6.5%)	15 (5.3%)	7 (2.1%)	51 (4.7%)
emm 6	12 (5.2%)	19 (8.2%)	12 (4.3%)	5 (1.5%)	48 (4.4%)
emm 11	16 (6.9%)	7 (3.0%)	9 (3.2%)	11 (3.2%)	43 (4.0%)
emm 59	5 (2.2%)	6 (2.6%)	9 (3.2%)	20 (5.9%)	40 (3.7%)
emm 83	2 (0.9%)	4 (1.7%)	10 (3.6%)	22 (6.5%)	38 (3.5%)
emm 77	18 (7.8%)	6 (2.6%)	6 (2.1%)	6 (1.8%)	36 (3.3%)
emm 4	8 (3.4%)	8 (3.4%)	7 (2.5%)	7 (2.1%)	30 (2.8%)
emm 53	20 (8.6%)	7 (3.0%)	2 (0.7%)	0 (0.0%)	29 (2.7%)
emm 91	8 (3.4%)	7 (3.0%)	6 (2.1%)	5 (1.5%)	26 (2.4%)
emm 22	7 (3.0%)	4 (1.7%)	5 (1.8%)	9 (2.6%)	25 (2.3%)
emm 114	0 (0.0%)	10 (4.3%)	9 (3.2%)	5 (1.5%)	24 (2.2%)
emm 2	4 (1.7%)	10 (4.3%)	2 (0.7%)	4 (1.2%)	20 (1.8%)
emm 87	5 (2.2%)	3 (1.3%)	5 (1.8%)	6 (1.8%)	19 (1.8%)
emm 74	0 (0.0%)	0 (0.0%)	0 (0.0%)	15 (4.4%)	15 (1.4%)
emm 75	1 (0.4%)	2 (0.9%)	4 (1.4%)	3 (0.9%)	10 (0.9%)
emm 81	0 (0.0%)	3 (1.3%)	4 (1.4%)	3 (0.9%)	10 (0.9%)
emm 118	1 (0.4%)	0 (0.0%)	2 (0.7%)	7 (2.1%)	10 (0.9%)
emm 73	1 (0.4%)	2 (0.9%)	0 (0.0%)	2 (0.6%)	5 (0.5%)
emm 9	2 (0.9%)	0 (0.0%)	1 (0.4%)	1 (0.3%)	4 (0.4%)
emm 25	0 (0.0%)	0 (0.0%)	2 (0.7%)	1 (0.3%)	3 (0.3%)
emm 68	0 (0.0%)	1 (0.4%)	1 (0.4%)	1 (0.3%)	3 (0.3%)
emm 80	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	2 (0.2%)
emm 94	0 (0.0%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	2 (0.2%)
emm 102	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.3%)	2 (0.2%)
emm 104	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.3%)	2 (0.2%)
emm 33	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 44	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 58	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 64	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 93	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 115	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)	1 (0.1%)
emm 123	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 170	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 182	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)	1 (0.1%)

emm 218	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
emm 227	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
Total	232	232	281	340	1085

*References from which formatting for table derived – 1,8

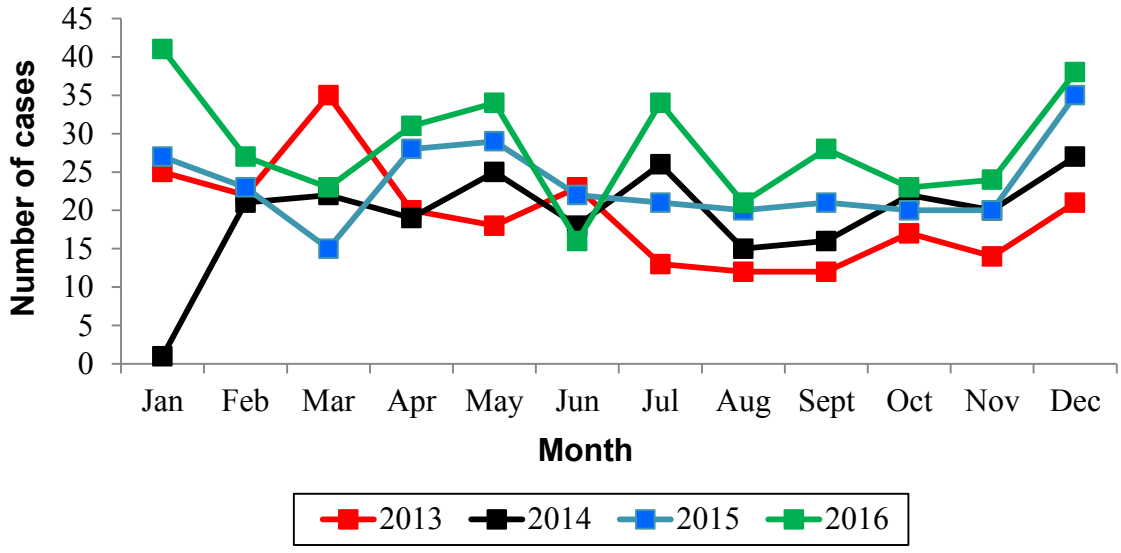
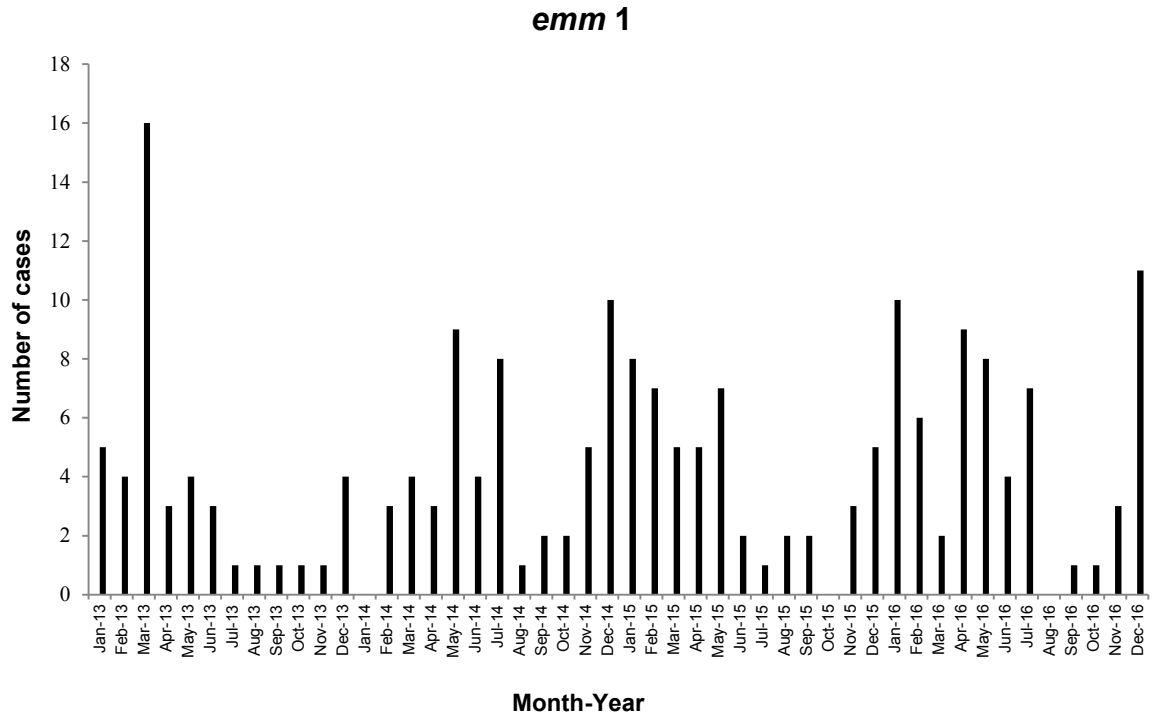
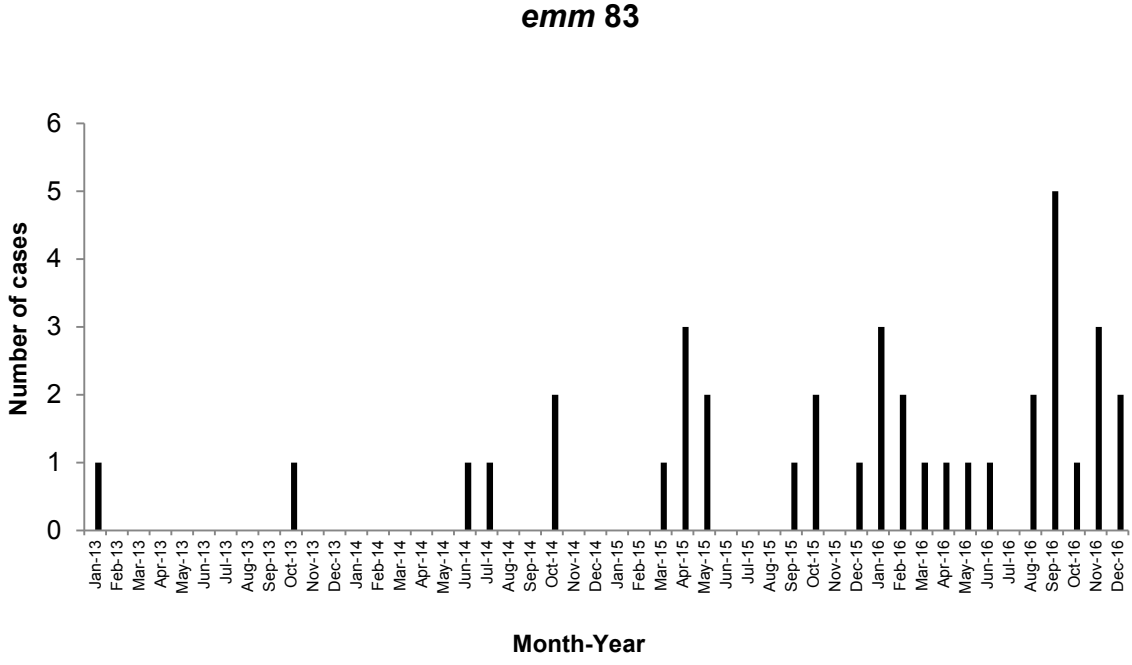


Figure 2.1. Total number of invasive GAS cases reported in each collection month in 2013-2016. Cases in 2013 are indicated by —, in 2014 are depicted by —, in 2015 are represented by — and in 2016 are shown by — (1-2).

A


emm 1
Number of cases
0
2
4
6
8
10
12
14
16
18
Jan-13 Feb-13 Mar-13 Apr-13 May-13 Jun-13 Jul-13 Aug-13 Sep-13 Oct-13 Nov-13 Dec-13 Jan-14 Feb-14 Mar-14 Apr-14 May-14 Jun-14 Jul-14 Aug-14 Sep-14 Oct-14 Nov-14 Dec-14 Jan-15 Feb-15 Mar-15 Apr-15 May-15 Jun-15 Jul-15 Aug-15 Sep-15 Oct-15 Nov-15 Dec-15 Jan-16 Feb-16 Mar-16 Apr-16 May-16 Jun-16 Jul-16 Aug-16 Sep-16 Oct-16 Nov-16 Dec-16
Month-Year

B


emm 83
Number of cases
0
1
2
3
4
5
6
Jan-13 Feb-13 Mar-13 Apr-13 May-13 Jun-13 Jul-13 Aug-13 Sep-13 Oct-13 Nov-13 Dec-13 Jan-14 Feb-14 Mar-14 Apr-14 May-14 Jun-14 Jul-14 Aug-14 Sep-14 Oct-14 Nov-14 Dec-14 Jan-15 Feb-15 Mar-15 Apr-15 May-15 Jun-15 Jul-15 Aug-15 Sep-15 Oct-15 Nov-15 Dec-15 Jan-16 Feb-16 Mar-16 Apr-16 May-16 Jun-16 Jul-16 Aug-16 Sep-16 Oct-16 Nov-16 Dec-16
Month-Year

C

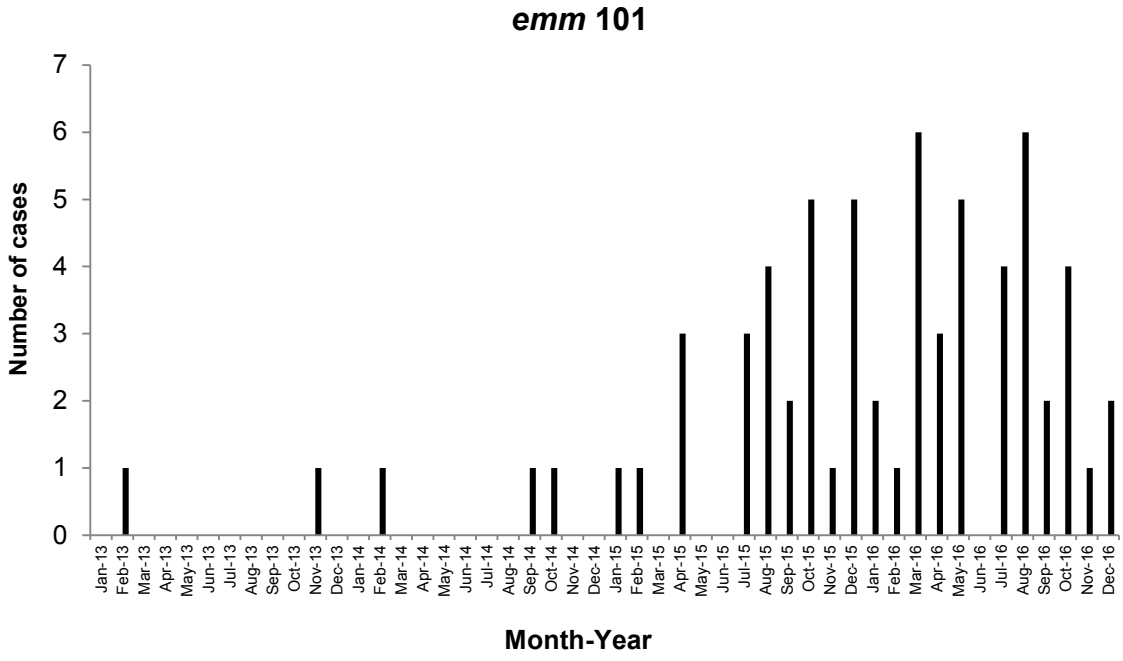
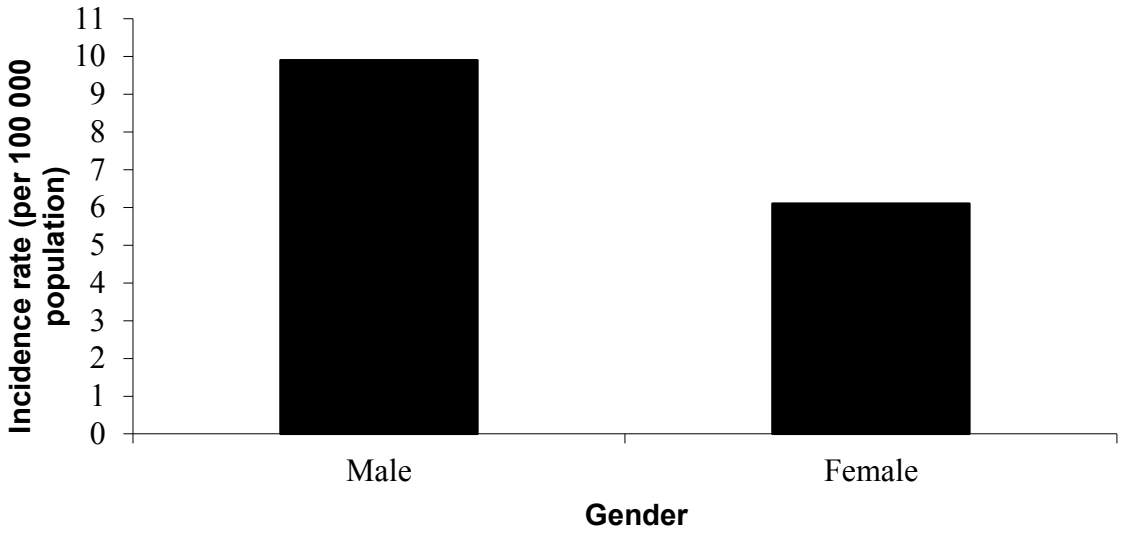


Figure 2.2. Distribution of invasive cases reported for GAS *emm* 1, *emm* 83 and *emm* 101 by month of collection from 2013-2016. A. Distribution of invasive cases from GAS *emm* 1 (2). B. Distribution of invasive cases from GAS *emm* 83. C. Distribution of invasive cases from GAS *emm* 101. Format for graphs is based on that in Tyrrell *et al.,* 2010 (2).

A



B

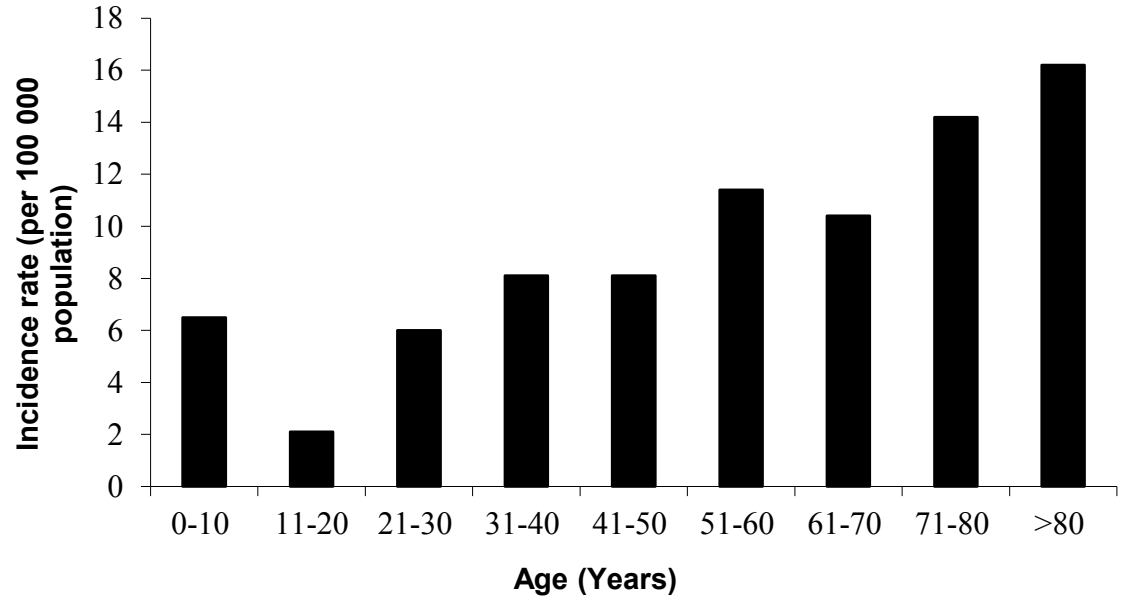
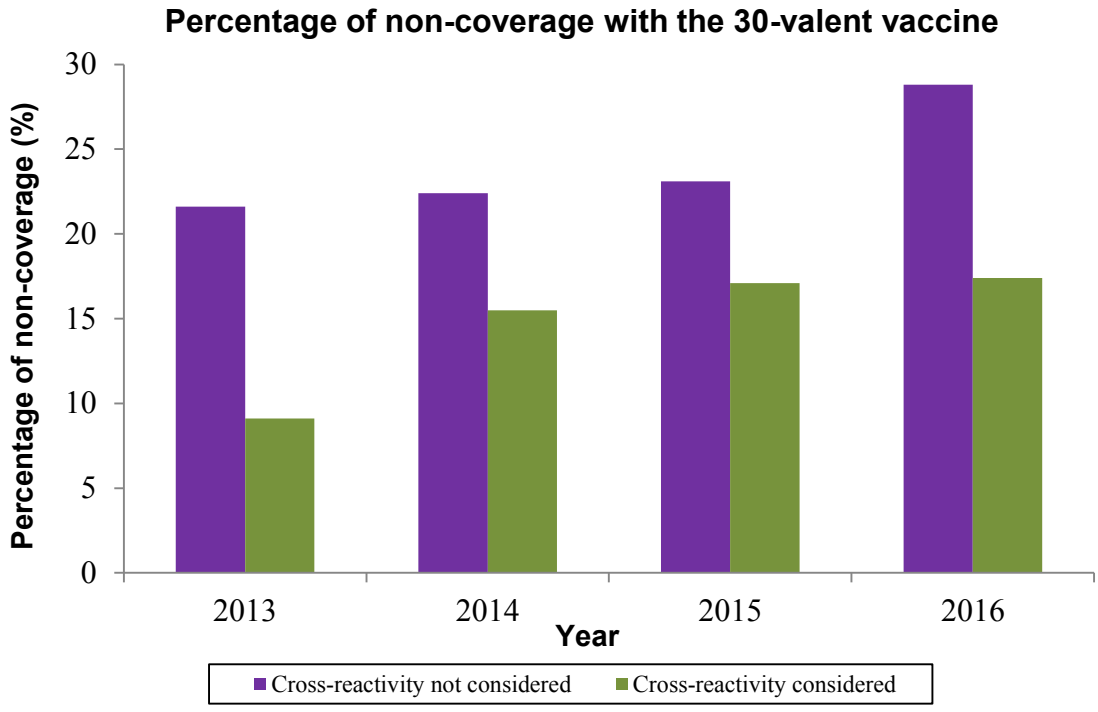


Figure 2.3. Estimates of the incidence rate of invasive GAS based on gender and age. For both gender (A) and age (B) incidence rates, data used to determine such rates are based on reported 2016 cases with confirmed *emm* types (1, 2, 4, 7). In addition, similar cases are considered to be new cases for the purposes of the incidence rate calculations if

they are ≥1 month apart (1-2, 7). The mid-year populations for obtaining the incidence rates were derived from Alberta Interactive Health Data Application (1-2, 7, 19-20). For the numerical incidence rate values for A and B above, refer to Supplementary Table S2.1.

A


Percentage of non-coverage with the 30-valent vaccine
Percentage of non-coverage (%)
30
25
20
15
10
5
0
2013
2014
2015
2016
Year
Cross-reactivity not considered
Cross-reactivity considered

B

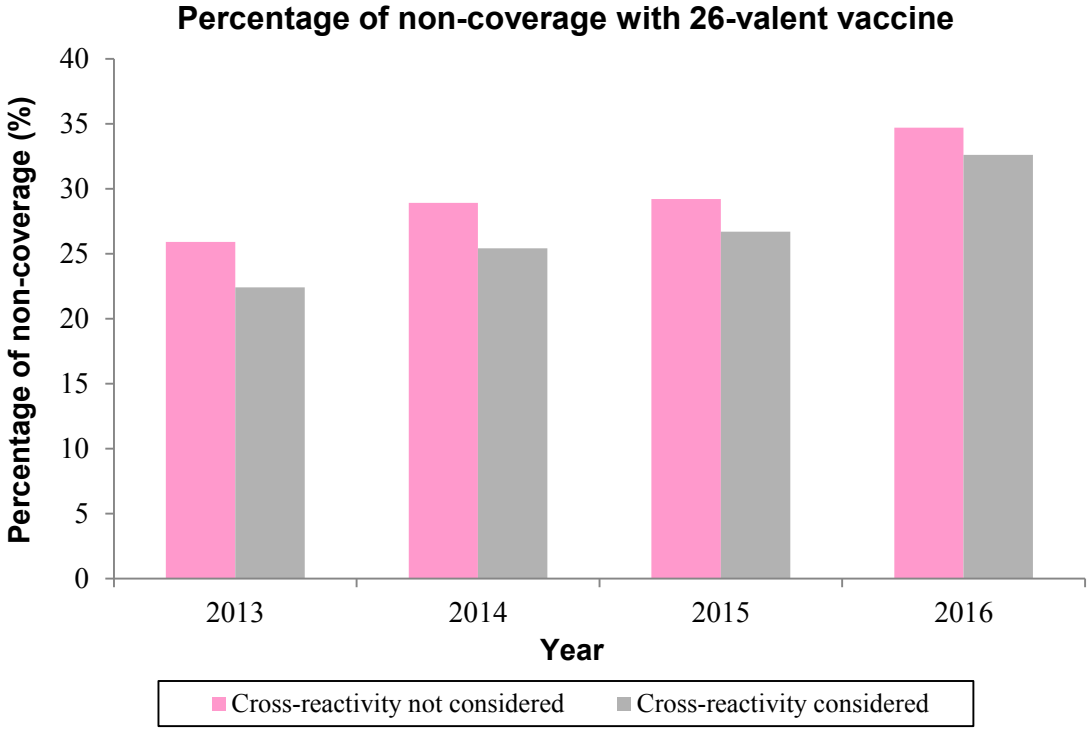

Percentage of non-coverage with 26-valent vaccine
Percentage of non-coverage (%)
40
35
30
25
20
15
10
5
0
2013
2014
2015
2016
Year
Cross-reactivity not considered
Cross-reactivity considered

Figure 2.4. Theoretical percentage of non-coverage in Alberta with the 30-valent vaccine and 26-valent vaccine in 2013-2016. Each invasive GAS *emm* type and its numerical cases in 2013-2016 were assessed as to whether they would be covered by the 30-valent vaccine or 26-valent vaccine using Table 1.2 and Table 2.2 (Table 1.2, Table 2.2, Supplementary figures S2.1-S2.4). The percentage of non-coverage in each year was calculated with and without considering cross-reactivity. A. Percentage of non-coverage with the 30-valent vaccine in 2013-2016. B. Percentage of non-coverage with the 26-valent vaccine in 2013-2016.

Chapter 3: Surface PGK expression on GAS strains

3.1 Introduction

The primary carbon-based energy source for Group A *Streptococcus* (GAS) is glucose (1-2). Glucose can be utilized in the Embden-Meyerhof-Parnas glycolytic pathway of GAS to ultimately produce two $NADH_2$ and two ATP. The enzymes constituting the pathway responsible for the generation of these molecules can be located in the cytoplasm. However, some of these pathway enzymes are also present in non-cytoplasmic locations (1-3). Examples of these include glyceraldehyde-3-phosphate dehydrogenase (GAPDH), enolase and phosphoglycerate kinase (PGK) (1-4).

GAS GAPDH has been discovered in three non-cytoplasmic locations: the cell wall, membrane and outside the bacterium (1-3, 5). Besides its function in glycolysis, GAS GAPDH demonstrates non-glycolytic properties (1-2, 5-8). Examples of these properties include interacting with urokinase plasminogen activator receptor (uPAR)/CD87 on the pharyngeal cell surface, C5a, fibronectin, actin and plasminogen. Similar to GAPDH, GAS enolase and PGK are present outside the bacterium and in the cell wall (2-4). A non-glycolytic property of GAS enolase is its ability to bind plasminogen (1-2, 9). In contrast, neutrophil activation seems to be a non-glycolytic property of GAS PGK (1-2, 10).

There are three *emm* pattern groups associated with GAS (A-C, D and E) (2, 11), which are defined by the chromosomal arrangement of the *emm*, *enn* and *mrp* genes. Generally, GAS *emm* patterns are associated with *emm* types and tissue tropisms, (12); *emm* pattern A-C is correlated with GAS infections of the throat, while *emm* pattern D is associated with superficial skin infections, and pattern E with throat and skin infections.

PGK has been detected on the surface of GAS previously (2-4, 13-16). However, there were only seven GAS M/*emm* types for which testing of surface PGK occurred. In addition, as the microenvironment of GAS seems to influence protein expression and surface attachment, there has been little investigation into the relationship between PGK surface expression and *emm* pattern groups associated with tissue tropisms (4, 12, 14, 17-18). Therefore, the purpose of this investigation was to explore surface PGK expression on a variety of GAS M/*emm* types associated with different *emm* patterns and tissue environments. This data will provide a better understanding of the surface expression of GAS PGK for future studies that further explore its role in virulence (1, 5, 10, 12, 14-18).

3.2 Materials and Methods

3.2.1 Bacteria assessed for surface PGK

The bacteria that were assessed for surface PGK are provided in Table 3.1 (Table 3.1, 2, 4, 13). These bacteria were from the collection available in the Bacterial Typing Unit and Tyrrell research laboratory in the Alberta Provincial Laboratory for Public Health in Edmonton, Alberta (Table 3.1, 2, 4, 13, 19). To determine and quantitate the surface PGK, an enzyme-linked immunosorbent assay (ELISA) method similar to the one conducted by Siddiqua was performed (2, 4, 13). The positive control for the ELISA was a Group B streptococcus (GBS) NCS13 strain, because PGK has already been detected on its surface (Table 3.1, 4, 13, 20). The negative controls were *Escherichia coli* JM101 and THY (3% Bacto™ Todd Hewitt Broth; Becton, Dickinson and Company and 0.2% Bacto™ Yeast Extract; Becton, Dickinson and Company in water) medium only (Table 3.1, 4, 13, 21).

3.2.2 ELISA method for surface PGK determination and quantification

The bacteria in Table 3.1 were inoculated from skim milk stored at -70°C into 1 ml THY medium and incubated at 35°C until the next day (Table 3.1, 2, 4, 13, 15, 22- 23). The resulting suspensions were centrifuged for one minute at 20 800 xg (Eppendorf centrifuge 5417C; Brinkmann Instruments Inc., Westbury, N.Y) before the supernatants were discarded. Then, 1 ml aliquots of 1X tris buffered saline (TBS) are mixed with the pellets before centrifugation for one minute at 20 800 xg occurred again. After the supernatants were removed, the pellets were suspended in 1X TBS. Next, the absorbance at 600 nm (A_{600}) measured using the DU® 730 Life Science UV/Vis Spectrophotometer (Beckman Coulter®) of each bacterial mixture was adjusted to 0.180-0.400 with 1X TBS, if necessary (Table 3.1, 4, 13, 20, 24- 28). The purpose of this was to ensure that differences in surface PGK quantities are not confounded by differences in bacterial concentrations. The negative THY control in 1X TBS acted as the reference sample to which all the test samples were compared. Aliquots of 100 µl of the adjusted mixtures were dispensed into Falcon® 96-flat bottom well polystyrene plates (Corning®) and

incubated at 4°C until the next day for bacterial fixation. After incubation, the remaining liquid in the wells were removed before the wells were washed once with 1X TBS. Then, 150 µl of 3% skim milk (Carnation® fat free instant skim milk powder; Smucker Foods of Canada Co., Markham, ON, Canada) in 1X TBS was added to each well for blocking for approximately one hour at room temperature (Table 3.1, 4, 13, 20, 24-31). Next, the wells were washed with 150 µl of 1X TBS three times. Fifty microliter aliquots of rabbit anti-GBS NCS13 PGK antibodies (research lab stocks) diluted 1 in 300 in 3% skim milk with 1X TBS were then dispensed in the wells. Anti-GBS PGK antibodies should be detectable for surface PGK in GAS, as their amino acid sequences are quite similar (Table 3.1, 4, 13, 30-36). The wells with anti-GBS PGK were incubated at room temperature for approximately one hour (Table 3.1, 4, 13, 20, 24-31, 37). After incubation, the wells were washed before 50 µl of goat anti-rabbit IgG (whole molecule)-alkaline phosphatase (Sigma-Aldrich®) antibodies diluted 1 in 1000 in 3% skim milk with 1X TBS were added. The anti-rabbit IgG-alkaline phosphatase antibodies in the wells were incubated at room temperature for approximately one hour. After the final wash, 100 µl aliquots of alkaline phosphatase yellow (p-nitrophenyl phosphate) liquid substrate system (SIGMA® Life Science) were pipetted into the wells and incubated at room temperature for an estimated 30 minutes (Table 3.1, 4, 13, 20, 24-31, 37-39). Aliquots of 25 µl 3N sodium hydroxide solution were added to the wells before the absorbances at 405 nm (A_{405}) were determined using the Opsys MR™ microplate reader (Dynex Technologies Inc.; Chantilly, VA, USA). The assay was conducted in triplicate and the average A_{405} along with the standard deviation was calculated.

3.3 Results

A total of 21 GAS strains representing 17 different M/*emm* types were tested for the presence of surface PGK using ELISA (Table 3.1, Figure 3.1A-E, 2, 4, 13, 15-16). Out of these 21 strains, four GAS strains demonstrate M/*emm* types that correlated with *emm* pattern A-C (Table 3.1, 2, 12, 15-16, 40). These four GAS strains designated as T1, T3, T5 and T8 show mean A_{405} values of 1.745 ± 0.013, 1.421± 0.120, 1.002 ± 0.090 and 1.451± 0.073 respectively, which are similar to that for the GBS NCS13 positive control (Table 3.1, Figure 3.1A,C, E, 2, 4, 12-13, 16, 40). This indicated that PGK was present on the surface of GAS T1, T3, T5 and T8 in similar quantities to GBS NCS13.

Amongst the 21 GAS strains, eight display M/*emm* types that are related to *emm* pattern D (Table 3.1, 2, 12, 15-16, 40). Four of these eight GAS strains named S8, S12G, S14 and S18 have mean A_{405} values of 1.732 ± 0.100, 1.677 ± 0.054, 1.300 ± 0.256 and 1.745 ± 0.050 respectively, which are similar to GBS NCS13 (Table 3.1, Figure 3.1A,D,E, 2, 4, 12-13, 16, 40). Two of the eight strains known as S20 and S26 have mean A_{405} values of 1.760 ± 0.008 and 1.711 ± 0.020, which exceed that of GBS NCS13 (Table 3.1, Figure 3.1C, 2, 4, 12-13, 16, 40). One of the eight GAS strains known as S12D has a mean A_{405} of 0.898 ± 0.085 that is intermediate between GBS NCS13 (1.290 ± 0.224) and *E. coli* JM101 (0.701 ± 0.054) or THY medium (0.585 ± 0.181), while the last strain called S12J has a mean A_{405} similar to JM101 or THY (Table 3.1, Figure 3.1C, E; 2, 4, 12-13, 16, 40).

Nine of the 21 GAS strains demonstrated M/*emm* types associated with *emm* pattern E (Table 3.1, 2, 12, 15-18, 40). Of these nine, three GAS strains were linked to throat specimens, while the remainder were correlated with body sites containing skin. Between the throat-linked and skin-linked GAS strains, S2 and T4 share the same M/*emm* type of 4, while S7 and T11 show an M/*emm* type of 28. Seven of the nine GAS strains known as T2, T4, T11, S3, S4, S5 and S7 show mean A_{405} values similar to GBS NCS13 (Table 3.1, Figure 3.1A,D,E, 2, 4, 12-13, 16, 40). However, two of the nine strains named S2 and S19 have mean A_{405} values of 1.779 ± 0.006 and 1.682 ± 0.075 respectively, which exceed that of GBS NCS13 (Table 3.1, Figure 3.1B,C, 2, 4, 12-13, 16, 40).

3.4 Discussion

In this study, PGK is shown to be present on the surface of many GAS M/*emm* types (Table 3.1, Figure 3.1A-E, 2, 4, 13, 15-16). These M/*emm* types include M/*emm* 1-4, 5, 9, 11-12, 22, 28, 41, 59, 74, 81-83 and 101. The detection of surface PGK in the M/*emm* types tested here confirms or adds to the list of M/*emm* types previously known to possess surface PGK (Table 3.1, Figure 3.1A-E, 2-4, 13-16). This previous list includes M1, M3, M6, M12, M53, M69 and M89. Therefore, surface PGK is prevalent amongst a variety of GAS M/*emm* types.

Overall, there is no relationship between *emm* pattern and surface PGK expression amongst the GAS tested (Table 3.1, Figure 3.1A-E, 2, 4, 12-13, 15-18, 40). In addition, there is no correlation between surface PGK and throat-linked or skin-linked *emm* pattern E GAS sharing the same M/*emm* type. Surface PGK expression in GAS seems to be strain-specific. This is best demonstrated with S12G and S12J, which are M/*emm* 59 GAS with an *emm* pattern of D (Table 3.1, Figure 3.1C,E, 2, 4, 12-13, 15-16, 40). GAS S12G has an abundant amount of surface PGK, while GAS S12J shows no detectable surface PGK. The mechanism behind this phenomenon is unknown, but may involve other GAS components that influence surface PGK expression (Table 3.1, Figure 3.1C,E, 1, 4, 12-13, 15-16, 20, 31, 40-41). For GAS GAPDH, another glycolytic enzyme, its surface expression in GAS seems to be influenced by the *mga* gene and the M and M-related proteins (1-2, 41). The latter may be involved in GAPDH surface attachment. In contrast, GAS lipoteichoic acid, a cell surface component, was shown to interact with PGK from GBS (20, 31, 42). PGK has been demonstrated on the surface of GBS and its surface expression is influenced by other components, such as the *sag1003* gene.

The presence of surface PGK in *emm* pattern A-C GAS indicates that this protein may be involved in GAS throat-specific infections (Table 3.1, Figure 3.1A,C, 1-2, 4, 7, 12-13, 15-16, 40, 42). Surface GAS GAPDH has been shown to mediate adherence to pharyngeal cells possibly via the uPAR/CD87 pharyngeal cell receptor (1, 5, 7, 43). In addition, surface GAPDH in GAS has been involved in signal transduction within pharyngeal cells (1, 7, 43).

Secreted GAS PGK has been shown to stimulate neutrophil activation (2, 10). Activation of neutrophils seems to contribute to the development of invasive GAS

infections. As PGK is present on the surface of invasive *emm* pattern D and E strains, it may also trigger neutrophil activation from this location (Table 3.1, Figure 3.1A-E, 2, 4, 10, 12-13, 15-16, 40). Besides neutrophil activation, surface PGK in invasive GAS may be involved in binding plasminogen (Table 3.1, Figure 3.1A-E, 1, 5, 7, 10, 20, 31). This has been demonstrated with the other GAS glycolytic enzymes GAPDH and enolase, as well as PGK from GBS. Plasminogen is a host component that can be activated to plasmin, a serine protease (44). Once bound to GAS, the plasmin may degrade the tissue barriers present during infection (2, 44).

In short, this study shows that PGK is present on the surface of many different GAS M/*emm* types (Table 3.1, Figure 3.1A-E, 2-4, 12-13, 15-18, 40). The surface expression of this PGK is not dependent on *emm* pattern or tissue microenvironment, but is more strain-specific. Finally, GAS surface PGK may be involved in the infection process in various ways (described above).

3.5 References

1. Pancholi V, Caparon M. *Streptococcus pyogenes* metabolism. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
2. Fittipaldi N, Beres SB, Olsen RJ, Kapur V, Shea PR, Watkins ME, Cantu CC, Laucirica DR, Jenkins L, Flores AR, Lovgren M, Ardanuy C, Liñares J, Low DE, Tyrrell GJ, Musser JM. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A *streptococcus*. Am J Pathol . 2012 Apr;180(4):1522–34.
3. Cole JN, Ramirez RD, Currie BJ, Cordwell SJ, Djordjevic SP, Walker MJ. Surface analyses and immune reactivities of major cell wall-associated proteins of group A streptococcus. Infect Immun. 2005 May;73(5):3137–46.
4. Siddiqua K. Mutant group B streptococcus surface expressed phosphoglycerate kinase (PGK) with reduced plasminogen binding [master's thesis]. [Edmonton(AB)]: University of Alberta; 2014. 108 p.
5. Boël G, Jin H, Pancholi V. Inhibition of cell surface export of group A streptococcal anchorless surface dehydrogenase affects bacterial adherence and antiphagocytic properties. Infect Immun. 2005 Oct;73(10):6237–48.
6. Pancholi V, Fischetti VA. A major surface protein on group A streptococci is a glyceraldehyde-3-phosphate-dehydrogenase with multiple binding activity. J Exp Med. 1992 Aug 1;176(2):415–26.
7. Jin H, Song YP, Boel G, Kochar J, Pancholi V. Group A streptococcal surface GAPDH, SDH, recognizes uPAR/CD87 as its receptor on the human pharyngeal cell and mediates bacterial adherence to host cells. J Mol Biol. 2005 Jul 1;350(1):27–41.
8. Terao Y, Yamaguchi M, Hamada S, Kawabata S. Multifunctional glyceraldehyde-3-phosphate dehydrogenase of *Streptococcus pyogenes* is essential for evasion from neutrophils. J Biol Chem. 2006 May 19;281(20):14215–23.
9. Derbise A, Song YP, Parikh S, Fischetti VA, Pancholi V. Role of the C-terminal lysine residues of streptococcal surface enolase in Glu- and Lys-plasminogen-binding activities of group A streptococci. Infect Immun. 2004 Jan;72(1):94–105.
10. Uhlmann J, Siemens N, Kai-Larsen Y, Fiedler T, Bergman P, Johansson L, Norrby-

Teglund A. Phosphoglycerate kinase – a novel streptococcal factor involved in neutrophil activation and degranulation. J Infect Dis. 2016 Dec 15; 214(12):1876–83.

11. Bessen DE. Molecular basis of serotyping and the underlying genetic organization of *Streptococcus pyogenes*. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
12. McMillan DJ, Drèze PA, Vu T, Bessen DE, Guglielmini J, Steer AC, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR. Updated model of group A streptococcus M proteins based on a comprehensive worldwide study. Clin Microbiol Infect. 2013 May;19(5):E222-9.
13. Severin A, Nickbarg E, Wooters J, Quazi SA, Matsuka YV, Murphy E, Moutsatsos IK, Zagursky RJ, Olmsted SB. Proteomic analysis and identification of *Streptococcus pyogenes* surface-associated proteins. J Bacteriol. 2007 Mar;189(5):1514–22.
14. Reglinski M, Gierula M, Lynskey NN, Edwards RJ, Sriskandan S. Identification of the *Streptococcus pyogenes* surface antigens recognised by pooled human immunoglobulin. Sci Rep. 2015;5:15825.
15. Spellerberg B, Brandt C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci). In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
16. Tyrrell GJ, Lovgren M, St. Jean T, Hoang L, Patrick DM, Horsman G, Van Caeseele P, Sieswerda LE, McGeer A, Laurence RA, Bourgault A-M, Low DE. Epidemic of group A *streptococcus* M/emm59 causing invasive disease in Canada. Clin Infect Dis. 2010 Dec 1;51(11):1290–7.
17. Eichenbaum Z, Green BD, Scott JR. Iron starvation causes release from the group A streptococcus of the ADP-ribosylating protein called plasmin receptor or surface glyceraldehyde-3-phosphate-dehydrogenase. Infect Immun. 1996 Jun;64(6):1956–60.
18. Dalton TL, Scott JR. CovS inactivates CovR and is required for growth under conditions of general stress in *Streptococcus pyogenes*. J Bacteriol. 2004 Jun;186(12):3928–37.
19. Demczuk WHB, Griffith A, Singh R, Montes K, Sawatsky P, Martin I, Mulvey M.

National laboratory surveillance of invasive streptococcal disease in Canada: annual summary 2014 [Internet]. Winnipeg: Public Health Agency of Canada; 2015 Dec [cited 2017 Jul 21]. Available from: http://www.healthycanadians.gc.ca/publications/drugs-products-medicaments-produits/2014-streptococcus/alt/surveillance-streptococca-eng.pdf

20. Boone TJ. Characterization of phosphoglycerate kinase expressed on the surface of group B streptococcus [PhD's thesis]. [Edmonton (AB)]: University of Alberta; 2012. 249 p.
21. Burnham CA. Interactions of the group B *streptococcus* with epithelial cells: host-cell signal transduction pathways in group B streptococcal invasion and the role of surface-associated group B streptococcal phosphoglycerate kinase in pathogenesis [PhD's thesis]. [Edmonton (AB)]: University of Alberta; 2007. 306 p.
22. Cody WL, Wilson JW, Hendrixson DR, McIver KS, Hagman KE, Ott CM, Nickerson CA, Schurr MJ. Skim milk enhances the preservation of thawed -80°C bacterial stocks. J Microbiol Methods. 2008 Sep;75(1):135–8.
23. The QIAexpressionist™: a handbook for high-level expression and purification of 6xHis-tagged proteins [Internet]. 5th ed. Qiagen; 2003 Jun [cited 2017 Aug 10]. 128 p. Available from: http://kirschner.med.harvard.edu/files/protocols/QIAGEN_ QIAexpressionist_EN.pdf
24. Savic DJ, McShan WM. Long-term survival of *Streptococcus pyogenes* in rich media is pH-dependent. Microbiology. 2012 Jun;158(Pt 6):1428–36.
25. Saab-Rincón G, Juárez VR, Osuna J, Sánchez F, Soberón X. Different strategies to recover the activity of monomeric triosephosphate isomerase by directed evolution. Protein Eng. 2001 Mar 1;14(3):149–55.
26. Gobinet J, Auzou G, Nicolas JC, Sultan C, Jalaguier S. Characterization of the interaction between androgen receptor and a new transcriptional inhibitor, SHP. Biochemistry. 2001 Dec 18;40(50):15369–77.
27. London Biohackspace [Internet]. London: London Biohackspace; c2017. London Biohackspace: OD600 spectrophotometer; c2017 [cited 2017 Aug 10];[about 13 screens]. Available from: https://biohackspace.org/projects/od600-spectrophotometer/
28. VWR: we enable science [Internet]. VWR. Falcon® 96-well cell culture plates,

Corning®; [cited 2017 Aug 10];[about 2 screens]. Available from: https://ca.vwr.com/store/product/en/4678980/falcon-96-well-cell-culture-plates-corning#

29. Aviva systems biology [Internet]. San Diego: Aviva systems biology corporation; c2010-2017. Enzyme-linked immunosorbent assay (ELISA) protocol; c2010-2017 [cited 2017 Aug 10];[about 2 screens]. Available from: http://www.avivasysbio.com/protocols-procedures/elisa
30. Sompayrac L. How the immune system works. 3rd ed. Malden (MA): Blackwell Publishing; 2008.
31. Smyth DJ, Shera J, Bauer MJ, Cameron A, McNeilly CL, Sriprakash KS, McMillan DJ. Conjugative transfer of ICESde3396 between three β-hemolytic streptococcal species. BMC Res Notes. 2014 Aug 12;7: 521.
32. Alhhazmi A. Phosphoglycerate kinase NCS13 [unpublished work]. 2015.[a]
33. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 2003 Jul 1;31(13):3784–8.
34. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipan DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997 Sep 1;25(17):3389-402.
35. Altschul SF, Wootton JC, Gertz EM, Agarwala R, Morgulis A, Schäffer AA, Yu YK. Protein database searches using compositionally adjusted substitution matrices. FEBS J. 2005 Oct;272(20):5101-9.
36. Hazes B. Section: distant homology detection. Edmonton: University of Alberta;2016.
37. Sigma-Aldrich® is now Millipore Sigma [Internet]. Sigma-Aldrich Co. LLC.; c2017. Product information: anti-rabbit IgG (whole molecule)-alkaline phosphatase produced in goat, affinity isolated antibody; [cited 2017 Aug 10];[about 2 p.]. Available from: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/6/a3687dat.pdf
38. Sigma-Aldrich® is now Millipore Sigma [Internet]. Sigma-Aldrich Co. LLC.; c2017. Product information: alkaline phosphatase yellow liquid substrate for ELISA; [cited 2017 Aug 10]; [about 1 p.]. Available from: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/5/p7998dat.pdf

39. Opsys MR^{TM} microplate reader: user's guide [Internet]. Chantilly (VA): Dynex Technologies Inc.; c1999 [revised 1999 Feb 1; cited 2017 Aug 10]. Available from: http://www.medteh.info/_fr/31/Opsys_MR.pdf
40. Sanderson-Smith M, De Oliveira DM, Guglielmini J, McMillan DJ, Vu T, Holien JK, Henningham A, Steer AC, Bessen DE, Dale JB, Curtis N, Beall BW, Walker MJ, Parker MW, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR, M Protein Study Group. A systematic and functional classification of *Streptococcus pyogenes* that serves as a new tool for molecular typing and vaccine development. J Infect Dis. 2014 Oct 15; 210(8):1325–38.
41. D'Costa SS, Romer TG, Boyle MD. Analysis of expression of a cytosolic enzyme on the surface of *Streptococcus pyogenes*. Biochem Biophys Res Commun. 2000 Nov 30;278(3):826-32.
42. Ryan PA, Juncosa B. Group A streptococcal adherence. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
43. Pancholi V, Fischetti VA. Regulation of the phosphorylation of human pharyngeal cell proteins by group A streptococcal surface dehydrogenase: signal transduction between streptococci and pharyngeal cells. J Exp Med. 1997 Nov 17;186(10):1633–43.
44. Winram SB, Lottenberg R. The plasmin-binding protein Plr of group A streptococci is identified as glyceraldehyde-3-phosphate dehydrogenase. Microbiology. 1996 Aug;142(Pt 8): 2311–20.
45. Tyrrell GJ, Kennedy A, Shokoples SE, Sherburne RK. Binding and invasion of HeLa and MRC-5 cells by *Streptococcus agalactiae*. Microbiology. 2002 Dec;148 (Pt 12):3921–31.
46. Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. Invasive group A streptococcal disease in Alberta, Canada (2000 to 2002). J Clin Microbiol. 2005 Apr; 43(4):1678–83.
47. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the infectious diseases society of America. Clin Infect Dis. 2012 Nov 15;55(10):1–17. Erratum in: Clin Infect Dis. 2014 May;58(10):1496.

[a]Permission has been granted by Alhhazmi A to use the information in this thesis.

Table 3.1. Bacteria investigated for surface PGK using ELISA*

Designation in study	Accession number/strain	M/*emm* type	*emm* pattern	Provincial location	Source
E. coli JM101	*E. coli* JM101	N/A	N/A	N/A	Research lab stocks
GBS NCS13 CL278	GBS NCS13 CL278	N/A	N/A	N/A	Soft tissue wound
GAS T1	M13SR000181	1	A-C	NR	Throat
GAS T2	M09SR000723	2	E	NR	Throat
GAS T3	M09SR001064	3	A-C	NR	Throat
GAS T4	M10SR000214	4	E	NR	Throat
GAS T5	M05SR000130	5	A-C	NR	Throat
GAS T8	M09SR000204	12	A-C	NR	Throat
GAS T11	M09SR000203	28	E	NR	Throat
GAS S2	SS-15-0000755	4	E	British Columbia	Elbow, Left
GAS S3	M11SR000028	9	E	Alberta	Tissue Nail
GAS S4	SS-15-0000843	11	E	Alberta	Hand, Left
GAS S5	SS-15-0000617	22	E	Alberta	Shoulder
GAS S7	SS-15-0001073	28	E	Alberta	Hip, Left
GAS S8	SS-15-0000761	41	D	Alberta	Arm, Right
GAS S12D	SS-16-0000720	59	D	Alberta	Thigh
GAS S12G	M12SR000437	59	D	Alberta	Arm right
GAS S12J	M08SR000359 or MGAS15252	59	D	Manitoba	Tissue rt thigh
GAS S14	SS-16-0000762	74	D	Alberta	Axilla
GAS S18	SS-14-0000728	81	D	Alberta	Knee, Left
GAS S19	SS-15-0001107	82	E	Alberta	Hand
GAS S20	SS-15-0001077	83	D	Alberta	Finger
GAS S26	SS-15-0000913	101	D	Alberta	Thigh

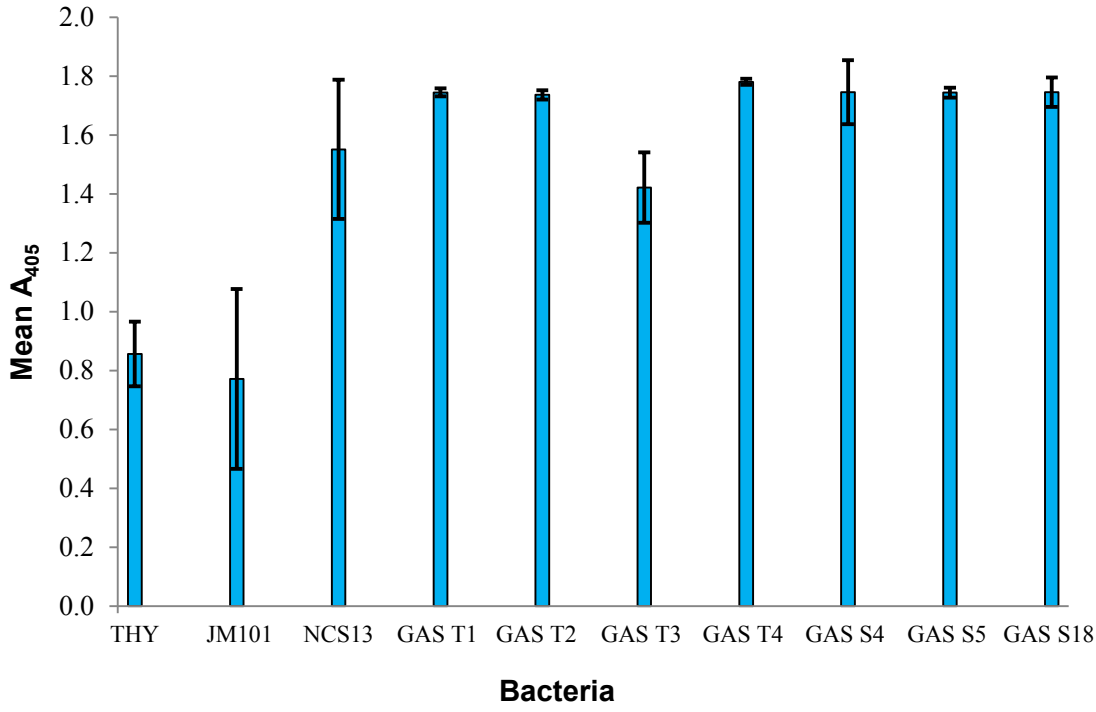
*Notes:

a) References – 2-4, 12, 15, 18, 21, 40, 45

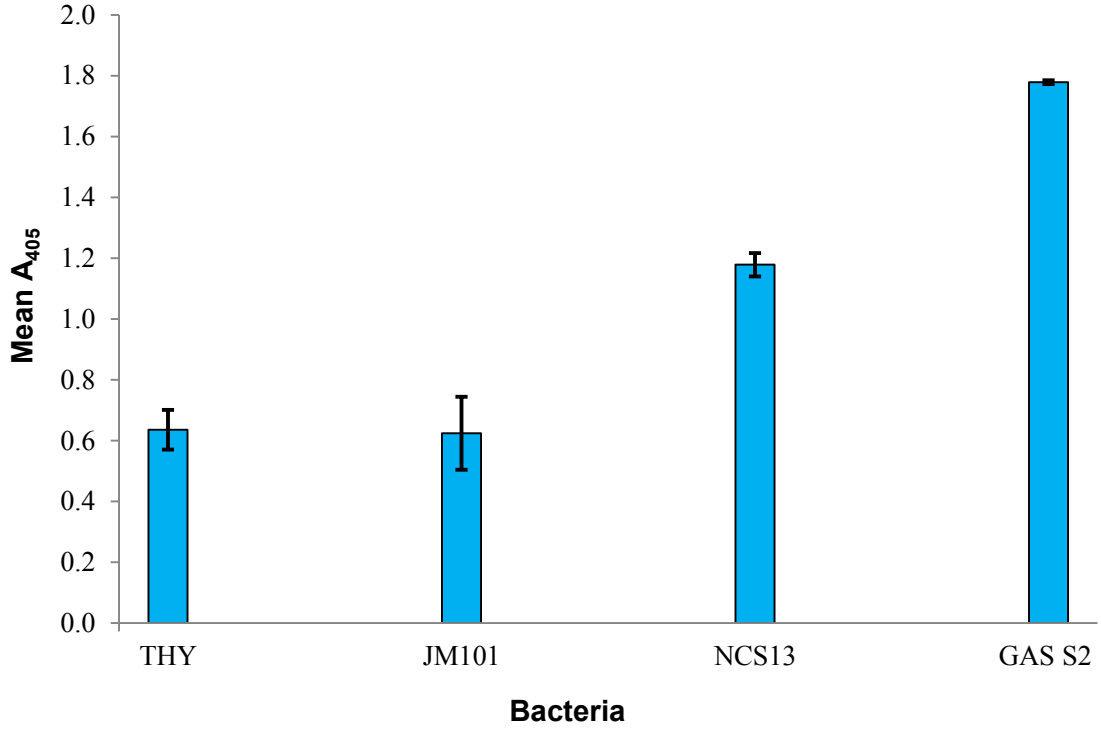
b) Abbreviations – GBS (Group B streptococcus) (4), N/A (not applicable) and NR (not reported)

c) The non-throat GAS are invasive strains (2, 46-47)

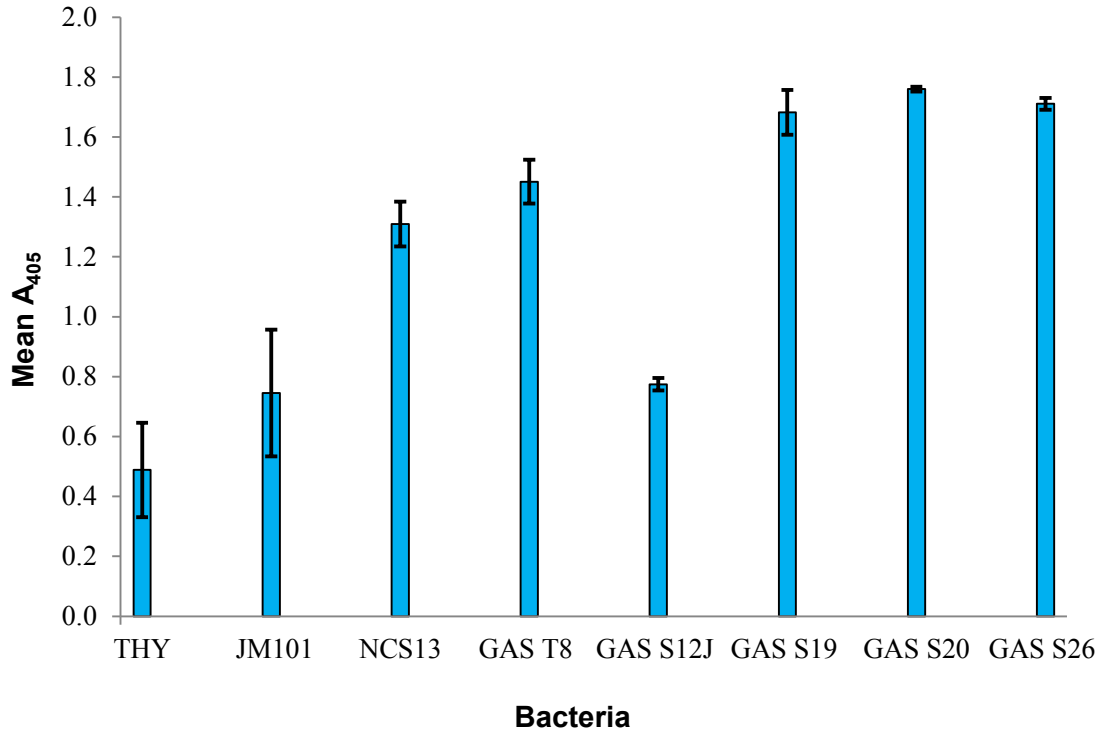
A


2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Mean A_{405}
THY
JM101
NCS13
GAS T1
GAS T2
GAS T3
GAS T4
GAS S4
GAS S5
GAS S18
Bacteria

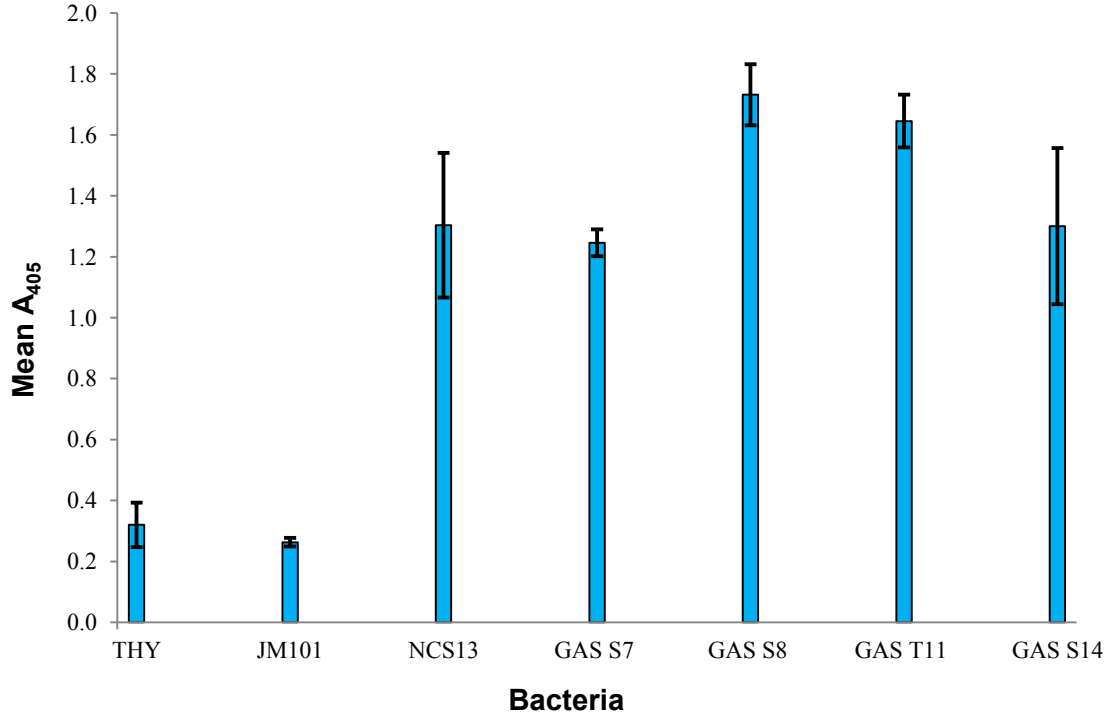
B


2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Mean A_{405}
THY
JM101
NCS13
GAS S2
Bacteria

C


2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Mean A_{405}
THY
JM101
NCS13
GAS T8
GAS S12J
GAS S19
GAS S20
GAS S26
Bacteria

D


2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Mean A_{405}
THY
JM101
NCS13
GAS S7
GAS S8
GAS T11
GAS S14
Bacteria

E

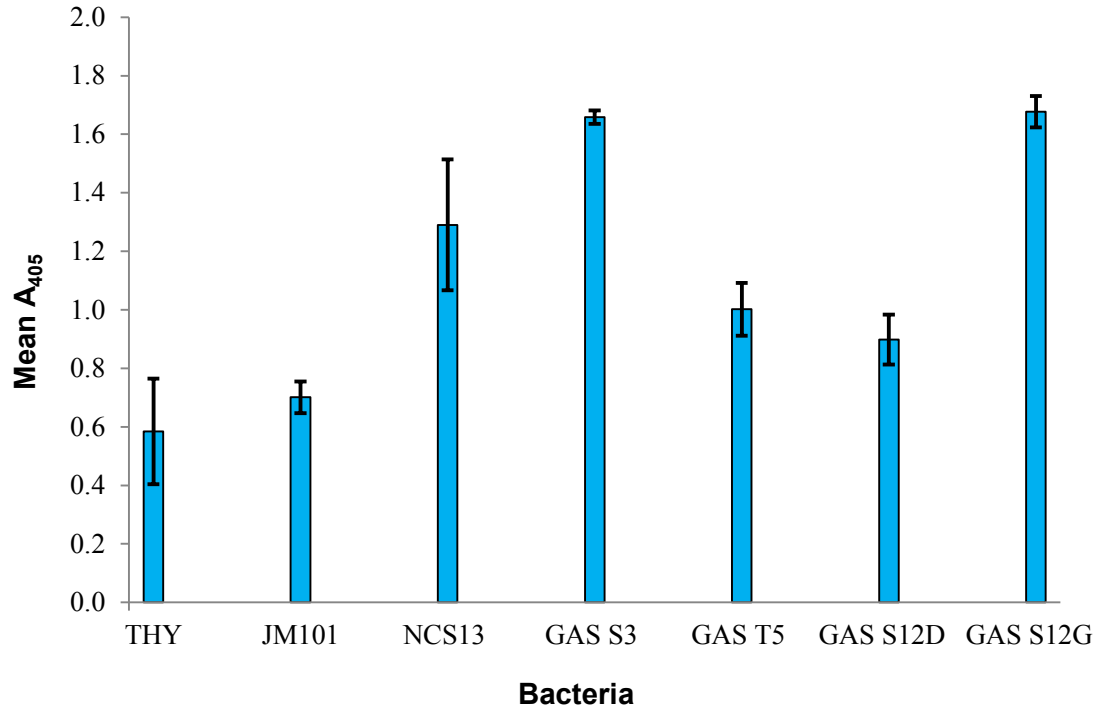


Figure 3.1A-E. Determination and quantification of surface PGK on different GAS strains. An ELISA method (further described in section 3.2.2) is used to measure surface PGK on the GAS strains (further described in Table 3.1) indicated above (Table 3.1, 4, 13, 21). The positive control for this method is GBS NCS13, as it has already demonstrated PGK on its surface. In contrast, the negative controls are *E. coli* JM101 and THY medium. The mean A_{405} is generated from triplicate results. For figures 3.1A and 3.1E, the graphs are adjusted after one outlier A_{405} result each from *E. coli* JM101 are removed. Abbreviations – THY (Todd Hewitt and yeast extract) (13) and A_{405} (absorbance at 405 nm) (4).

Chapter 4: Discussion and Future Studies

4.1 Discussion and future studies

In this thesis, two areas are explored (Chapter 2-3). The first investigates the recent epidemiology of invasive group A streptococci (GAS) in Alberta (Chapter 2, 1-4). Invasive GAS is studied for the years 2013-2016 in Alberta. The results show that many epidemiological features of invasive GAS in Alberta have similar consistency years later (Table 2.3, Figure 2.1, Figure 2.2A, Figure 2.3B, 1-7). This includes the predominance of invasive GAS *emm* 1 in the province. In addition, invasive GAS cases continue to peak in the early spring and winter months and incidence rates are highest in the very elderly. More interesting epidemiology for invasive GAS in the recent years examined in Alberta is the appearance and increase in *emm* 101 associated cases (Table 2.3, 1-4, 6). GAS *emm* 101 has not been reported in Alberta in previous studies (1-3, 5, 6). The increase in *emm* 101 GAS cases in Alberta may be the result of the spread from east to west of clonal *emm* 101 reported in Thunder Bay, Ontario (Table 2.3, 1-3, 5-6, 8-9). In order to confirm this, whole genome SNP analysis of recent Alberta *emm* 101 GAS strains and comparison with those in Thunder Bay needs to be conducted. In addition, complete genome sequencing of Alberta *emm* 101 invasive GAS strains should be completed, in order to identify virulence-enhancing genetic changes that may explain the rise in cases. In the Thunder Bay study, risk factors such as alcohol abuse, homelessness, intravenous drug use and infection with hepatitis C virus are associated with invasive GAS in the period where *emm* 101 is predominant (2, 9). Similar risk factors should be assessed for invasive *emm* 101 GAS in Alberta, in order to determine host susceptibilities for acquiring such bacteria (Table 2.3, 2-3, 8-9).

Other surprising epidemiological features seen in Alberta in 2013- 2016 are the gradual decrease in invasive GAS *emm* 53 cases and the appearance of *emm* 74 (Table 2.3, 1-3, 6). There were 20 invasive cases of GAS *emm* 53 reported in Alberta in 2013, which gradually decreased to zero in 2016 (Table 2.3, 2). In 2015, Thunder Bay has reported the appearance of invasive GAS *emm* 53 (9). It can be hypothesized that *emm* 53 may have spread from western to eastern Canada (Table 2.3, 3, 9-10). In contrast, fourteen invasive GAS *emm* 74 cases have appeared in Alberta in 2016, but not in 2013- 2015 (Table 2.3, 2). In December 2015, clonal GAS *emm* 74 was reported in Toronto, Ontario and linked to a shelter outbreak (2, 11). Together, this implies that *emm* 74 may have spread from

eastern to western Canada (Table 2.3, 2-3, 11). In order to confirm the clonal nature of *emm* 53 that justifies the hypothesis regarding its spread from western to eastern Canada, whole genome SNP analysis and comparison of Alberta and Thunder Bay GAS *emm* 53 should at least be conducted (Table 2.3, 2-3, 8-10). Similarly for *emm* 74, whole genome SNP analysis of Alberta strains and comparison with the clonal ones in Toronto should be accomplished (Table 2.3, 2-3, 8-9, 11). In addition to whole genome SNP analysis, both Alberta *emm* 53 and 74 strains should have their genomes sequenced completely and risk factors for their acquisition assessed, in order to respectively identify any bacterial virulence-enhancing genetic changes and host susceptibilities (Table 2.3, 2-3, 8-9).

GAS *emm* 53, 74 and 101 are associated with *emm* pattern D (Table 1.1, Table 2.3, 2-3, 12-13). *Emm* pattern D GAS have a tropism for skin (8, 12). Therefore, invasive *emm* 53, 74 and 101 GAS in Alberta may have an association with more skin-linked invasive diseases (Table 1.1, Table 2.3, 1-3, 12-16). To confirm this, invasive diseases correlated with the prior *emm* types should be explored. Another research direction with *emm* 53, 74 and 101 that should be investigated is their correlation with different ages and genders of individuals with invasive GAS (Table 2.3, 1-2, 5). Through this study, it has been established that males have a higher incidence rate of invasive GAS than females (Figure 2.3A, Supplementary Table S2.1, 1-2, 7) and only *emm* 101 demonstrates seasonality with peaks in April, July-August and October (Table 2.3, Figure 2.2C, 2).

GAS *emm* 59 invasive cases have risen in Alberta from five in 2013 to 20 in 2016 (Table 2.3, 2). These *emm* 59 cases may represent the reemergence of a clonal *emm* 59 that caused an epidemic in Canada from 2006-2008 (Table 2.3, 2, 8). To confirm this, once again whole genome SNP analysis and comparison of GAS strains from these two time periods should be conducted (Table 2.3, 2, 8-9). In addition, complete genome sequencing and host risk factor determination should be performed, in order to respectively identify any bacterial genetic changes or host susceptibilities that may have led to the reappearance of *emm* 59 in Alberta.

Presently, there is no licensed vaccine for GAS (17). However, there are candidates in development. Two of these candidates are the 26-valent M protein-based vaccine and the 30-valent M protein-based vaccine (17-19). Although either candidate could develop into a vaccine for licensure, the impact of such a vaccine in Alberta has not

been assessed in previous studies (1-2, 5-6, 17-20). This study shows that assuming 100% killing, minimum 65% of invasive GAS cases should be covered with either vaccine over the years examined (Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, 2-3, 17-23). However, the theoretical non-coverage of invasive cases is gradually worsening from 2013 to 2016 with either candidate, whether cross-reactivity is considered or not. This worsening progression seems to be less for the 30-valent vaccine than for the 26-valent vaccine, making it the better choice for use in Alberta theoretically. In order to enhance the coverage of the 30-valent vaccine, another antigen could be added to the formulation. This candidate antigen may be GAS phosphoglycerate kinase (PGK) (Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, 2-3, 17, 19-20, 22-25).

GAS PGK is a glycolytic enzyme shown to be reactive with pooled human immunoglobulin or sera (Chapter 3, 2-3, 24, 26-28). It has been found on the surface of a few M types, but the extent of its coverage is not known. Therefore, the second aspect of this project was to determine surface PGK expression on a variety of GAS M/*emm* types. Amongst the 21 GAS strains tested for surface PGK, 19 definitely possess the protein (Table 3.1, Figure 3.1A-E, 2-3, 8, 25, 28). These 19 GAS represent 17 different M/*emm* types. Of these 17 M/*emm* types, 14 have not previously been shown to possess surface PGK (Table 3.1, Figure 3.1A-E, 2-3, 8, 24-25, 27-28). Thus combined with previous data, a total of 21 GAS M/*emm* types demonstrate surface PGK so far. However, not all GAS strains/isolates with surface PGK demonstrate reactivity with human sera or immunoglobulin (8, 24-25, 27). In the Reglinski *et al.* study for example, 18 of 20 GAS isolates show pooled human immunoglobulin-reactive PGK (8, 25, 27). In addition, although human immunoglobulin/sera are reactive with surface PGK, its bactericidal effect against GAS has not been demonstrated (8, 19, 23-25, 27). Therefore, in order to assess whether GAS PGK would be a good candidate antigen to augment the coverage of the 30-valent vaccine, more GAS strains of especially different M/*emm* types need to be tested for surface PGK using enzyme-linked immunosorbent assay (ELISA) (Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, Table 3.1, Figure 3.1A-E, 2-3, 8, 17, 19-20, 22-25, 27-28). In addition, the reactivity of surface PGK to pooled human immunoglobulin/sera in current and future M/*emm* types tested need to be determined using Western blot analysis of cell wall extracts. Finally, injection of GAS PGK in rabbits

and the use of the corresponding serum in *in vitro* opsonophagocytic killing assays involving human blood will determine bactericidal activity.

The GAS M/*emm* types tested for surface PGK in this study are associated with A-C, D or E *emm* patterns (Table 3.1, Figure 3.1A-E, 2-3, 8, 12-13, 25-26, 28-33). However, no relationship between surface PGK expression and *emm* pattern is seen for the GAS investigated. Furthermore, between *emm* pattern E GAS sharing the same M/*emm* type but differing in their specimen source, no correlation between throat or skin-linked specimens and expression of surface PGK is detected. Surface PGK expression in GAS seems to be strain dependent. For example, GAS S12J (M08SR000359) and S12G (M12SR000437) are *emm* 59 strains with an *emm* pattern of D. While GAS S12G possesses surface PGK, GAS S12J has no detectable surface PGK. To understand why this is the case, it may be useful to sequence and compare the genomes of both the S12J and S12G strains, in order to determine the genetic changes that result in these differences in surface PGK expression. Once identified, more specific mutagenesis experiments can be conducted to determine the specific gene(s) involved in surface GAS PGK expression and the effect on other GAS properties that occur when these gene(s) are mutated.

The GAS glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and enolase can be surface-localized and bind to plasminogen (8, 26, 33-34). In Group B streptococcus (GBS), a bacterium related to GAS, PGK is also surface expressed and binds plasminogen (29-30). Therefore, surface GAS PGK may be able to also complex with plasminogen (26, 29-30, 33-34). To confirm this, invasive GAS S12G and S12J should be exposed to plasminogen in an ELISA (Table 3.1, Figure 3.1C, E, 23, 26, 28-30, 33-36). This will not only determine whether surface PGK binds plasminogen, but also the relative contribution of PGK to the overall GAS plasminogen-binding ability.

Seven GAS throat strains of *emm* pattern A-C or E are shown in the present study to possess surface PGK (Table 3.1, Figure 3.1A,C-E, 2-3, 8, 12-13, 25, 28). In addition, GAPDH can aid in GAS adherence to pharyngeal cells (33-34, 37). Therefore, it is possible that surface PGK in GAS also helps in mediating adherence to pharyngeal cells (Table 3.1, Figure 3.1A,C-E, 2-3, 8, 12-13, 25-26, 28, 33-34, 37). To test this hypothesis, inhibition of adherence to a human pharyngeal cell line should be measured in the presence of purified GAS PGK and the throat GAS strains.

One major concern regarding the use of GAS PGK in the 30-valent vaccine is the development of autoimmunity (Figure 2.4A-B, Supplementary figures S2.1A-D-S2.4A-D, 2-3, 17, 19-20, 22-26, 29-30). PGK is present in both humans and GAS, making it possible for cross-reactivity to develop. In order to avert this issue, it will be necessary to investigate GAS PGK-specific amino acid regions for their ability to generate bactericidal antibodies.

4.2 References

1. Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. Invasive group A streptococcal disease in Alberta, Canada (2000 to 2002). J Clin Microbiol. 2005 Apr; 43(4):1678–83.
2. Tyrrell GJ, Lovgren M, St. Jean T, Hoang L, Patrick DM, Horsman G, Van Caeseele P, Sieswerda LE, McGeer A, Laurence RA, Bourgault A-M, Low DE. Epidemic of group A *streptococcus* M/emm59 causing invasive disease in Canada. Clin Infect Dis. 2010 Dec 1;51(11):1290–7.
3. Spellerberg B, Brandt C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci). In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
4. Health topics: epidemiology [Internet]. WHO;2017 [cited 2017 Jul 21]. Available from: http://www.who.int/topics/epidemiology/en/
5. Tyrrell GJ, Lovgren M, Forwick B, Hoe NP, Musser JM, Talbot JA. M types of group A streptococcal isolates submitted to the National Centre for Streptococcus (Canada) from 1993 to 1999. J Clin Microbiol. 2002 Dec;40(12):4466–71.
6. Government of Alberta [Internet]. Alberta Government. Streptococcal disease – group A, invasive; c2003-2013 [revised 2013 Jun; cited 20 Aug 2017]; [about 17 p]. Available from: https://open.alberta.ca/dataset/b6405d01-6ffd-4b8a-9762-2d6c29772f9c/resource/0fc41de9-1b42-427d-914c-0c687eca0652/download/Guidelines-Streptococcal-A-Invasive-2013.pdf
7. CDC. Principles of epidemiology in public health practice: an introduction to applied epidemiology and biostatistics [Internet]. 3rd ed. Atlanta: CDC; 2006 Oct [updated 2012 May, cited 2017 Jul 21]. Available from: https://www.cdc.gov/ophss/csels/dsepd/ss1978/ SS1978.pdf
8. Fittipaldi N, Beres SB, Olsen RJ, Kapur V, Shea PR, Watkins ME, Cantu CC, Laucirica DR, Jenkins L, Flores AR, Lovgren M, Ardanuy C, Liñares J, Low DE, Tyrrell GJ, Musser JM. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A *streptococcus*. Am J Pathol. 2012 Apr;180(4):1522–34.
9. Athey TBT, Teatero S, Sieswerda LE, Gubbay JB, Marchand-Austin A, Li A,

Wasserscheid J, Dewar K, McGeer A, Williams D, Fittipaldi N. High incidence of invasive group A *streptococcus* disease caused by strains of uncommon *emm* types in Thunder Bay, Ontario, Canada. J Clin Microbiol. 2016 Jan;54(1):83-92.

10. Demczuk WHB, Griffith A, Singh R, Martin I, Mulvey M. National laboratory surveillance of invasive streptococcal disease in Canada: annual summary 2013 [Internet]. Winnipeg: Public Health Agency of Canada; 2014 Dec [cited 2017 Jul 21]. Available from: https://www.canada.ca/en/public-health/services/publications/drugs-health-products//laboratory-surveillance-invasive-streptococcal-disease-annual-summary-2013.html
11. Finkelstein M. Streptococcus, group A – Canada (02): (Ontario) fatal, homeless shelter, emm 74, clonal. ProMED-mail 2017; 5 Apr: 20170406.4952247. <http://www.promedmail.org>. Accessed 21 Jul 2017.
12. McMillan DJ, Drèze PA, Vu T, Bessen DE, Guglielmini J, Steer AC, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR. Updated model of group A streptococcus M proteins based on a comprehensive worldwide study. Clin Microbiol Infect. 2013 May;19(5):E222-9.
13. Sanderson-Smith M, De Oliveira DM, Guglielmini J, McMillan DJ, Vu T, Holien JK, Henningham A, Steer AC, Bessen DE, Dale JB, Curtis N, Beall BW, Walker MJ, Parker MW, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR, M Protein Study Group. A systematic and functional classification of *Streptococcus pyogenes* that serves as a new tool for molecular typing and vaccine development. J Infect Dis. 2014 Oct 15; 210(8):1325–38.
14. Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, Wade JC, Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin Infect Dis. 2005 Nov 15;41(10):1373–406. Erratum in: Clin Infect Dis. 2005 Dec 15;41(12):1830.; Clin Infect Dis. 2006 Apr 15;42(8): 1219.
15. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan SL, Montoya JG, Wade JC, Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis.

2014 Jul;59(2):e10-52. Erratum in: Clin Infect Dis. 2014;59:147-50.; Clin Infect Dis. 2015.

16. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the infectious diseases society of America. Clin Infect Dis. 2012 Nov 15;55(10):1–17. Erratum in: Clin Infect Dis. 2014 May;58(10):1496.
17. Steer AC, Carapetis JR, Dale JB, Fraser JD, Good MF, Guilherme L, Moreland NJ, Mulholland EK, Schodel F, Smeesters PR. Status of research and development of vaccines for *Streptococcus pyogenes*. Vaccine. 2016 Jun 3;34(26): 2953–8.
18. McNeil SA, Halperin SA, Langley JM, Smith B, Warren A, Sharratt GP, Baxendale DM, Reddish MA, Hu MC, Stroop SD, Linden J, Fries LF, Vink PE, Dale JB. Safety and immunogenicity of 26-valent group A *streptococcus* vaccine in healthy adult volunteers. Clin Infect Dis. 2005 Oct 15;41(8):1114–22.
19. Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine. 2011 Oct 26;29(46): 8175–8.
20. Dale JB, Penfound TA, Tamboura B, Sow SO, Nataro JP, Tapia M, Kotloff KL. Potential coverage of a multivalent M protein-based group A streptococcal vaccine. Vaccine. 2013 Mar 15;31(12):1576–81.
21. Hu MC, Walls MA, Stroop SD, Reddish MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. Infect Immun. 2002 Apr;70(4):2171–7.
22. Dale JB, Chiang EY, Liu S, Courtney HS, Hasty DL. New protective antigen of group A streptococci. J Clin Invest. 1999 May 1;103(9):1261–8.
23. Sompayrac L. How the immune system works. 3rd ed. Malden (MA): Blackwell Publishing; 2008.
24. Cole JN, Ramirez RD, Currie BJ, Cordwell SJ, Djordjevic SP, Walker MJ. Surface analyses and immune reactivities of major cell wall-associated proteins of group A streptococcus. Infect Immun. 2005 May;73(5):3137–46.

25. Siddiqua K. Mutant group B streptococcus surface expressed phosphoglycerate kinase (PGK) with reduced plasminogen binding [master's thesis]. [Edmonton(AB)]: University of Alberta; 2014. 108 p.
26. Pancholi V, Caparon M. *Streptococcus pyogenes* metabolism. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
27. Reglinski M, Gierula M, Lynskey NN, Edwards RJ, Sriskandan S. Identification of the *Streptococcus pyogenes* surface antigens recognised by pooled human immunoglobulin. Sci Rep. 2015;5:15825.
28. Severin A, Nickbarg E, Wooters J, Quazi SA, Matsuka YV, Murphy E, Moutsatsos IK, Zagursky RJ, Olmsted SB. Proteomic analysis and identification of *Streptococcus pyogenes* surface-associated proteins. J Bacteriol. 2007 Mar;189(5):1514–22.
29. Boone TJ. Characterization of phosphoglycerate kinase expressed on the surface of group B streptococcus [PhD's thesis]. [Edmonton (AB)]: University of Alberta; 2012. 249 p.
30. Smyth DJ, Shera J, Bauer MJ, Cameron A, McNeilly CL, Sriprakash KS, McMillan DJ. Conjugative transfer of ICESde3396 between three β-hemolytic streptococcal species. BMC Res Notes. 2014 Aug 12;7: 521.
31. Eichenbaum Z, Green BD, Scott JR. Iron starvation causes release from the group A streptococcus of the ADP-ribosylating protein called plasmin receptor or surface glyceraldehyde-3-phosphate-dehydrogenase. Infect Immun. 1996 Jun;64(6):1956–60.
32. Dalton TL, Scott JR. CovS inactivates CovR and is required for growth under conditions of general stress in *Streptococcus pyogenes*. J Bacteriol. 2004 Jun;186(12):3928–37.
33. Jin H, Agarwal S, Agarwal S, Pancholi V. Surface export of GAPDH/SDH, a glycolytic enzyme, is essential for *Streptococcus pyogenes* virulence. MBio. 2011 May 31;2(3):e00068-11.
34. Boël G, Jin H, Pancholi V. Inhibition of cell surface export of group A streptococcal anchorless surface dehydrogenase affects bacterial adherence and antiphagocytic properties. Infect Immun. 2005 Oct;73(10):6237–48.
35. Winram SB, Lottenberg R. The plasmin-binding protein Plr of group A streptococci is identified as glyceraldehyde-3-phosphate dehydrogenase. Microbiology. 1996 Aug;142(Pt 8): 2311–20.

36. Agarwal S, Kulshreshtha P, Bambah Mukku D, Bhatnagar R. α-Enolase binds to human plasminogen on the surface of *Bacillus anthracis*. Biochim Biophys Acta. 2008 Jul-Aug;1784(7-8):986.94.
37. Jin H, Song YP, Boel G, Kochar J, Pancholi V. Group A streptococcal surface GAPDH, SDH, recognizes uPAR/CD87 as its receptor on the human pharyngeal cell and mediates bacterial adherence to host cells. J Mol Biol. 2005 Jul 1; 350(1):27–41.

Bibliography

Agarwal S, Kulshreshtha P, Bambah Mukku D, Bhatnagar R. α-Enolase binds to human plasminogen on the surface of *Bacillus anthracis*. Biochim Biophys Acta. 2008 Jul-Aug;1784(7-8):986.94.

Agrahari G, Liang Z, Mayfield JA, Balsara RD, Ploplis VA, Castellino FJ. Complement-mediated opsonization of invasive group A *Streptococcus pyogenes* strain AP53 is regulated by the bacterial two-component cluster of virulence responder/sensor (CovRS) system. J Biol Chem. 2013 Sep 20;288(38):27494-504.

Alberta IHDA: welcome to the interactive health data application [Internet]. Alberta: Government of Alberta;c1983-2017. Alberta health, surveillance & assessment branch: interactive health data application – adjusted population estimates; 2016 Dec [cited 2017 Jul 21] [about 6 screens]. Available from: http://www.ahw.gov.ab.ca/IHDA_ Retrieval/ShowMetaDataNotesServlet?1942

Alberta IHDA: welcome to the interactive health data application [Internet]. Alberta: Government of Alberta;c1983-2017. Alberta IHDA; c1983-2017 [cited 2017 Jul 21]; [about 2 screens]. Available from: http://www.ahw.gov.ab.ca/IHDA_Retrieval /selectSubCategory.do#

Alhhazmi A. Phosphoglycerate kinase NCS13 [unpublished work]. 2015.[a]

Allen UD, Moore DL. Invasive group A streptococcal disease: Management and chemoprophylaxis. Paediatr Child Health. 2010;15(5):295-302.

Allmon A, Deane K, Martin KL. Common skin rashes in children. Am Fam Physician. 2015 Aug 1;92(3):211-6.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipan DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997 Sep 1;25(17):3389-402.

Altschul SF, Wootton JC, Gertz EM, Agarwala R, Morgulis A, Schäffer AA, Yu YK. Protein database searches using compositionally adjusted substitution matrices. FEBS J. 2005 Oct;272(20):5101-9.

André I, Persson J, Blom AM, Nilsson H, Drakenberg T, Lindahl G, Linse S. Streptococcal M protein: structural studies of the hypervariable region, free and bound to human C4BP. Biochemistry. 2006 Apr 11;45(14):4559-68.

Antikainen J, Kuparinen V, Lähteenmäki K, Korhonen TK. pH-dependent asssociation of enolase and glyceraldehyde-3-phosphate dehydrogenase of *Lactobacillus crispatus* with the cell wall and lipoteichoic acids. J Bacteriol. 2007 Jun;189(12):4539-43.

Armstrong C. Practice guidelines: AHA guidelines on prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. Am Fam Physician. 2010 Feb 1; 81(3):346-59.

Athey TBT, Teatero S, Sieswerda LE, Gubbay JB, Marchand-Austin A, Li A, Wasserscheid J, Dewar K, McGeer A, Williams D, Fittipaldi N. High incidence of invasive group A *streptococcus* disease caused by strains of uncommon *emm* types in Thunder Bay, Ontario, Canada. J Clin Microbiol. 2016 Jan; 54(1):83-92.

Aviva systems biology [Internet]. San Diego: Aviva systems biology corporation; c2010-2017. Enzyme-linked immunosorbent assay (ELISA) protocol; c2010-2017 [cited 2017 Aug 10];[about 2 screens]. Available from: http://www.avivasysbio.com/protocols-procedures/elisa

Barnett TC, Liebl D, Seymour LM, Gillen CM, Lim JY, LaRock CN, Davies MR, Schulz BL, Nizet V, Teasdale RD, Walker MJ. The globally disseminated M1T1 clone of group A streptococcus evades autophagy for intracellular replication. Cell Host Microbe. 2013 Dec 11;14(6):675–82.

Batsford SR, Mezzano S, Mihatsch M, Schiltz E, Rodríguez-Iturbe B. Is the nephritogenic antigen in post-streptococcal glomerulonephritis pyrogenic exotoxin B (SPEB) or GAPDH? Kidney Int. 2005 Sep;68(3):1120-9.

Batzloff MR, Hayman WA, Davies MR, Zeng M, Pruksakorn S, Brandt ER, Good MF. Protection against group A streptococcus by immunization with J8-diphtheria toxoid: contribution of J8- and diphtheria toxoid-specific antibodies to protection. J Infect Dis. 2003 May 15;187(10):1598-608.

Baytunca MB, Donuk T, Erermiş S. [Evaluation of a Neuropsychiatric Disorder: From PANDAS to PANS and CANS]. Turk Psikiyatri Derg. 2016;27(2). Turkish.

Beall B, Gherardi G, Lovgren M, Facklam RR, Forwick BA, Tyrrell GJ. *Emm* and *sof* gene sequence variation in relation to serological typing of opacity-factor-positive group A streptococci. Microbiology. 2000 May;146(Pt 5):1195-209.

Ben Zakour NL, Davies MR, You Y, Chen JH, Forde BM, Stanton-Cook M, Yang R, Cui Y, Barnett TC, Venturini C, Ong CL, Tse H, Dougan G, Zhang J, Yuen KY, Beatson SA, Walker MJ. Transfer of scarlet fever-associated elements into the group A streptococcus M1T1 clone. Sci Rep. 2015 Nov 2;5:15877.

Bergmann S, Rohde M, Hammerschmidt S. Glyceraldehyde-3-phosphate dehydrogenase of Streptococcus pneumoniae is a surface-displayed plasminogen-binding protein. Infect Immun. 2004 Apr;72(4):2416-9.

Bessen DE. Molecular basis of serotyping and the underlying genetic organization of *Streptococcus pyogenes*. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.

Bessen DE, McGregor KF, Whatmore AM. Relationships between *emm* and multilocus sequence types within a global collection of *Streptococcus pyogenes*. BMC Microbiol. 2008 Apr 11;8:59.

Bläckberg A, Trell K, Rasmussen M. Erysipelas, a large retrospective study of aetiology and clinical presentation. BMC Infect Dis. 2015 Sep 30; 15:402.

Boël G, Jin H, Pancholi V. Inhibition of cell surface export of group A streptococcal anchorless surface dehydrogenase affects bacterial adherence and antiphagocytic properties. Infect Immun. 2005 Oct;73(10):6237–48.

Bono-Neri F. Acute rheumatic fever: global persistence of a preventable disease. J Pediatr Health Care. 2017 May-Jun;31(3):275-84.

Boone TJ. Characterization of phosphoglycerate kinase expressed on the surface of group B streptococcus [PhD's thesis]. [Edmonton (AB)]: University of Alberta; 2012. 249 p.

Bowen AC, Mahé A, Hay RJ, Andrews RM, Steer AC, Tong SY, Carapetis JR. The global epidemiology of impetigo: a systematic review of the population prevalence of impetigo and pyoderma. PLoS One. 2015 Aug 28; 10(8):e0136789.

Burnham CA. Interactions of the group B *streptococcus* with epithelial cells: host-cell signal transduction pathways in group B streptococcal invasion and the role of surface-associated group B streptococcal phosphoglycerate kinase in pathogenesis [PhD's thesis]. [Edmonton (AB)]: University of Alberta; 2007. 306 p.

Burnham CA, Shokoples SE, Tyrrell GJ. Phosphoglycerate kinase inhibits epithelial cell invasion by group B streptococci. Microb Pathog. 2005 May-Jun;38(5-6):189-200.
Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005 Nov; 5(11):685-94.
CDC. Principles of epidemiology in public health practice: an introduction to applied epidemiology and biostatistics [Internet]. 3rd ed. Atlanta: CDC; 2006 Oct [updated 2012 May, cited 2017 Jul 21]. Available from: https://www.cdc.gov/ophss/csels/dsepd/ss1978/SS1978.pdf
CDC *Streptococcus* laboratory: about the *Streptococcus* laboratory [Internet]. Atlanta: CDC; [updated 2016 Aug 17]. Assigning *emm* types and subtypes; [updated/reviewed 2007 Dec 19; cited 2017 Jul 21]; [about 51 lines]. Available from: https://www.cdc.gov/streplab/assigning.html
CDC *Streptococcus* laboratory: about the *Streptococcus* laboratory [Internet]. Atlanta: CDC; [updated 2016 Aug 17]. New *emm* designations/browse: conversion of st to emm – numerical sort; [updated/reviewed 2014 Apr 2; cited 2017 Jul 21]; [about 24 pages]. Available from: https://www.cdc.gov/streplab/types-emm103-124.html
Chalker V, Jironkin A, Coelho J, Al-Shahib A, Platt S, Kapatai G, Daniel R, Dhami C, Laranjeira M, Chambers T, Guy R, Lamagni T, Harrison T, Chand M, Johnson AP, Underwood A, Scarlet Fever Incident Management Team. Genome analysis following a national increase in scarlet fever in England 2014. BMC Genomics. 2017 Mar 10;18(1):224.
Chhatwal GS. Anchorless adhesins and invasins of Gram-positive bacteria: a new class of virulence factors. Trends Microbiol. 2002 May;10(5):205-8.
Cleary PP, Matsuka YV, Huynh T, Lam H, Olmsted SB. Immunization with C5a peptidase from either group A or B streptococci enhances clearance of group A streptococci from intranasally infected mice. Vaccine. 2004 Oct 22;22(31-32):4332-41.
Cody WL, Wilson JW, Hendrixson DR, McIver KS, Hagman KE, Ott CM, Nickerson CA, Schurr MJ. Skim milk enhances the preservation of thawed -80°C bacterial stocks. J Microbiol Methods. 2008 Sep;75(1):135–8.
Cole C, Gazewood J. Diagnosis and treatment of impetigo. Am Fam Physician. 2007 Mar 15;75(6):859–64.

Cole JN, Barnett TC, Nizet V, Walker MJ. Molecular insight into invasive group A streptococcal disease. Nat Rev Microbiol. 2011 Sep 16;9(10):724-36.

Cole JN, Pence MA, von Köckritz-Blickwede M, Hollands A, Gallo RL, Walker MJ, Nizet V. M protein and hyaluronic acid capsule are essential for *in vivo* selection of *covRS* mutations characteristic of invasive serotype M1T1 group A *streptococcus*. MBio. 2010 Sep-Oct;1(4): e00191-10.

Cole JN, Ramirez RD, Currie BJ, Cordwell SJ, Djordjevic SP, Walker MJ. Surface analyses and immune reactivities of major cell wall-associated proteins of group A streptococcus. Infect Immun. 2005 May;73(5):3137–46.

Cork AJ, Ericsson DJ, Law RH, Casey LW, Valkov E, Bertozzi C, Stamp A, Jovcevski B, Aquilina JA, Whisstock JC, Walker MJ, Kobe B. Stability of the octameric structure affects plasminogen-binding capacity of streptococcal enolase. PLoS One. 2015 Mar 25;10(3):e0121764.

Courtney HS, Hasty DL, Dale JB. Serum opacity factor (SOF) of *Streptococcus pyogenes* evokes antibodies that opsonize homologous and heterologous SOF-positive serotypes of group A streptococci. Infect Immun. 2003 Sep;71(9):5097-103.

Courtney HS, Li Y. Non-immune binding of human IgG to M-related proteins confers resistance to phagocytosis of group A streptococci in blood. PLoS One. 2013 Oct 25;8(10):e78719.

Courtney HS, Li Y, Twal WO, Argraves WS. Serum opacity factor is a streptococcal receptor for the extracellular matrix protein fibulin-1. J Biol Chem. 2009 May 8;284(19):12966-71.

Dale JB, Chiang EY, Liu S, Courtney HS, Hasty DL. New protective antigen of group A streptococci. J Clin Invest. 1999 May 1;103(9):1261–8.

Dale JB, Niedermeyer SE, Agbaosi T, Hysmith ND, Penfound TA, Hogn CM, Pullen M, Bright MI, Murrell DS, Shenep LE, Courtney HS. Protective immunogenicity of group A streptococcal M-related proteins. Clin Vaccine Immunol. 2015 Mar;22(3):344-50.

Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine. 2011 Oct 26;29(46): 8175–8.

Dale JB, Penfound TA, Tamboura B, Sow SO, Nataro JP, Tapia M, Kotloff KL. Potential coverage of a multivalent M protein-based group A streptococcal vaccine. Vaccine. 2013 Mar 15;31(12):1576–81.

Dalton TL, Scott JR. CovS inactivates CovR and is required for growth under conditions of general stress in *Streptococcus pyogenes*. J Bacteriol. 2004 Jun;186(12):3928–37.

Darenberg J, Henriques-Normark B, Lepp T, Tegmark-Wisell K, Tegnell A, Widgren K. Increased incidence of invasive group A streptococcal infections in Sweden, January 2012-February 2013. Euro Surveill. 2013 Apr 4;18(14):20443.

D'Costa SS, Romer TG, Boyle MD. Analysis of expression of a cytosolic enzyme on the surface of *Streptococcus pyogenes*. Biochem Biophys Res Commun. 2000 Nov 30;278(3):826-32.

De Amicis KM, Freschi de Barros S, Alencar RE, Postól E, Martins Cde O, Arcuri HA, Goulart C, Kalil J, Guilherme L. Analysis of the coverage capacity of the StreptInCor candidate vaccine against *Streptococcus pyogenes*. Vaccine. 2014 Jul 7;32(32):4104-10.

Demczuk WHB, Griffith A, Singh R, Martin I, Mulvey M. National laboratory surveillance of invasive streptococcal disease in Canada: annual summary 2013 [Internet]. Winnipeg: Public Health Agency of Canada; 2014 Dec [cited 2017 Jul 21]. Available from: https://www.canada.ca/en/public-health/services/publications/drugs-health-products//laboratory-surveillance-invasive-streptococcal-disease-annual-summary-2013.html

Demczuk WHB, Griffith A, Singh R, Montes K, Sawatsky P, Martin I, Mulvey M. National laboratory surveillance of invasive streptococcal disease in Canada: annual summary 2014 [Internet]. Winnipeg: Public Health Agency of Canada; 2015 Dec [cited 2017 Jul 21]. Available from: http://www.healthycanadians.gc.ca/publications/drugs-products-medicaments-produits/2014-streptococcus/alt/surveillance-streptococca-eng.pdf

Derbise A, Song YP, Parikh S, Fischetti VA, Pancholi V. Role of the C-terminal lysine residues of streptococcal surface enolase in Glu- and Lys-plasminogen-binding activities of group A streptococci. Infect Immun. 2004 Jan;72(1):94–105.

Eichenbaum Z, Green BD, Scott JR. Iron starvation causes release from the group A streptococcus of the ADP-ribosylating protein called plasmin receptor or surface glyceraldehyde-3-phosphate-dehydrogenase. Infect Immun. 1996 Jun;64(6):1956–60.

Engelthaler DM, Valentine M, Bowers J, Pistole J, Driebe EM, Terriquez J, Nienstadt L, Carroll M, Schumacher M, Ormsby ME, Brandy S, Livar E, Yazzie D, Waddell V, Peoples M, Komatsu K, Keim P. Hypervirulent *emm*59 clone in invasive group A *streptococcus* outbreak, southwestern United States. Emerg Infect Dis. 2016 Apr;22(4):734-8.

Enright MC, Spratt BG, Kalia A, Cross JH, Bessen DE. Multilocus sequence typing of *Streptococcus pyogenes* and the relationships between *emm* type and clone. Infect Immun. 2001 Apr;69(4):2416-27.

Excler JL, Kim JH. Accelerating the development of a group A *streptococcus* vaccine: an urgent public health need. Clin Exp Vaccine Res. 2016 Jul;5(2):101-7.

Facklam RF, Martin DR, Lovgren M, Johnson DR, Efstratiou A, Thompson TA, Gowan S, Kriz P, Tyrrell GJ, Kaplan E, Beall B. Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: *emm*103 to *emm*124. Clin Infect Dis. 2002 Jan 1;34(1):28-38.

Falugi F, Zingretti C, Pinto V, Mariani M, Amodeo L, Manetti AG, Capo S, Musser JM, Orefici G, Margarit I, Telford JL, Grandi G, Mora M. Sequence variation in group A *streptococcus* pili and association of pilus backbone types with Lancefield T serotypes. J Infect Dis. 2008 Dec 15;198(12):1834-41.

Finkelstein M. Streptococcus, group A – Canada (02): (Ontario) fatal, homeless shelter, emm 74, clonal. ProMED-mail 2017; 5 Apr: 20170406.4952247. <http://www.promedmail.org>. Accessed 21 Jul 2017.

Fischetti VA. M protein and other surface proteins on streptococci. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.

Fittipaldi N, Beres SB, Olsen RJ, Kapur V, Shea PR, Watkins ME, Cantu CC, Laucirica DR, Jenkins L, Flores AR, Lovgren M, Ardanuy C, Liñares J, Low DE, Tyrrell GJ, Musser JM. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A *streptococcus*. Am J Pathol. 2012 Apr;180(4):1522–34.

Fittipaldi N, Tyrrell GJ, Low DE, Martin I, Lin D, Hari KL, Musser JM. Integrated whole-genome sequencing and temporospatial analysis of a continuing group A

streptococcus epidemic. Emerg Microbes Infect. 2013 Mar;2(3):e13.
Fontán PA, Pancholi V, Nociari MM, Fischetti VA. Antibodies to streptococcal surface enolase react with human alpha-enolase: implications in poststreptococcal sequelae. J Infect Dis. 2000 Dec;182(6):1712-21.
Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 2003 Jul 1;31(13):3784–8.
Gewitz MH, Baltimore RS, Tani LY, Sable CA, Shulman ST, Carapetis J, Remenyi B, Taubert KA, Bolger AF, Beerman L, Mayosi BM, Beaton A, Pandian NG, Kaplan EL, American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young. Revision of the Jones criteria for the diagnosis of acute rheumatic fever in the era of Dopper echocardiography: a scientific statement from the American Heart Association. Circulation. 2015 May 19;131(20):1806-18.
Gidaris D, Zafeiriou D, Mavridis P, Gombakis N. Scarlet fever and hepatitis: a case report. Hippokratia. 2008 Jul-Sep;12(3):186-7.
Gillen CM, Courtney HS, Schulze K, Rohde M, Wilson MR, Timmer AM, Guzman CA, Nizet V, Chhatwal GS, Walker MJ. Opacity factor activity and epithelial cell binding by the serum opacity factor protein of *Streptococcus pyogenes* are functionally discrete. J Biol Chem. 2008 Mar 7;283(10):6359-66.
Gobinet J, Auzou G, Nicolas JC, Sultan C, Jalaguier S. Characterization of the interaction between androgen receptor and a new transcriptional inhibitor, SHP. Biochemistry. 2001 Dec 18;40(50):15369–77.
Government of Alberta [Internet]. Alberta Government. Streptococcal disease – group A, invasive; c2003-2013 [revised 2013 Jun; cited 20 Aug 2017]; [about 17 p]. Available from: https://open.alberta.ca/dataset/b6405d01-6ffd-4b8a-9762-2d6c29772f9c/resource/0fc41de9-1b42-427d-914c-0c687eca0652/download/Guidelines-Streptococcal-A-Invasive-2013.pdf
Guarner J, Sumner J, Paddock CD, Shieh WJ, Greer PW, Reagan S, Fischer M, Van Beneden CA, Zaki SR. Diagnosis of invasive group A streptococcal infections by using immunohistochemical and molecular assays. Am J Clin Pathol. 2006 Jul;126(1):148–55.

Hartman-Adams H, Banvard C, Juckett G. Impetigo: diagnosis and treatment. Am Fam Physician. 2014 Aug 15;90(4):229–35.
Hazes B. Section: distant homology detection. Edmonton: University of Alberta;2016.
Health topics: epidemiology [Internet]. WHO;2017 [cited 2017 Jul 21]. Available from: http://www.who.int/topics/epidemiology/en/
Henningham A, Barnett TC, Maamary PG, Walker MJ. Pathogenesis of group A streptococcal infections. Discov Med. 2012 May; 13(72): 329-42.
Hu MC, Walls MA, Stroop SD, Reddish MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. Infect Immun. 2002 Apr;70(4):2171–7.
Hulbah MJ, Tyrrell GJ. Identification of a gene in group B streptococcus involved in the surface expression of GBS-phosphoglycerate kinase. Poster session presented at: Woman and Children's Health Research Institute Research Day;2015 Oct 28; Edmonton,AB.
Hytönen J, Haataja S, Gerlach D, Podbielski A, Finne J. The SpeB virulence factor of *Streptococcus pyogenes*, a multifunctional secreted and cell surface molecule with strepadhesin, laminin-binding and cysteine protease activity. Mol Microbiol. 2001 Jan;39(2):512-9.
Inghammar M, Rasmussen M, Linder A. Recurrent erysipelas - risk factors and clinical presentation. BMC Infect Dis. 2014 May 18;14:270.
Jin H, Agarwal S, Agarwal S, Pancholi V. Surface export of GAPDH/SDH, a glycolytic enzyme, is essential for *Streptococcus pyogenes* virulence. MBio. 2011 May 31;2(3):e00068-11.
Jin H, Song YP, Boel G, Kochar J, Pancholi V. Group A streptococcal surface GAPDH, SDH, recognizes uPAR/CD87 as its receptor on the human pharyngeal cell and mediates bacterial adherence to host cells. J Mol Biol. 2005 Jul 1; 350(1):27–41.
Kang SO, Caparon MG, Cho KH. Virulence gene regulation by CvfA, a putative RNase: the CvfA-enolase complex in *Streptococcus pyogenes* links nutritional stress, growth-phase control, and virulence gene expression. Infect Immun. 2010 Jun;78(6):2754-67.
Kemble SK, Westbrook A, Lynfield R, Bogard A, Koktavy N, Gall K, Lappi V, DeVries AS, Kaplan E, Smith KE. Foodborne outbreak of group A streptococcus pharyngitis associated with a high school dance team banquet - Minnesota, 2012. Clin Infect Dis. 2013 Sep;57(5):648–54.

Khil J, Im M, Heath A, Ringdahl U, Mundada L, Carey Engleberg N, Fay WP. Plasminogen enhances virulence of group A streptococci by streptokinase-dependent and streptokinase—ndependent mechanisms. J Infect Dis. 2003 Aug 15;188(4):497-505.

Kratovac Z, Manoharan A, Luo F, Lizano S, Bessen DE. Population genetics and linkage analysis of loci within the FCT region of *Streptococcus pyogenes*. J Bacteriol. 2007 Feb;189(4):1299-310.

Lancefield RC. The antigenic complex of Streptococcus haemolyticus: I. demonstration of a type-specific substance in extracts of Streptococcus haemolyticus. J Exp Med. 1928 Jan 1;47(1):91-103.

Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. J Exp Med. 1933 Mar 31;57(4):571-95.

Lancefield RC, Dole VP. The properties of T antigens extracted from group A hemolytic streptococci. J Exp Med. 1946 Oct 31;84(5):449-71.

Lenz LL, Mohammadi S, Geissler A, Portnoy DA. SecA2-dependent secretion of autolytic enzymes promotes *Listeria monocytogenes* pathogenesis. Proc Natl Acad Sci USA. 2003 Oct 14;100(21):12432-7.

Li Y, Courtney HS. Promotion of phagocytosis of *Streptococcus pyogenes* in human blood by a fibrinogen-binding peptide. Microbes Infect. 2011 Apr;13(4):413-8.

Liang Z, Zhang Y, Agrahari G, Chandrahas V, Glinton K, Donahue DL, Balsara RD, Ploplis VA, Castellino FJ. A natural inactivating mutation in the CovS component of the CovRS regulatory operon in a pattern D *Streptococcal pyogenes* strain influences virulence-associated genes. J Biol Chem. 2013 Mar 1;288(9):6561-73.

Linke C, Caradoc-Davies TT, Young PG, Proft T, Baker EN. The laminin-binding protein Lbp from *Streptococcus pyogenes* is a zinc receptor. J Bacteriol. 2009 Sep;191(18):5814-23.

Linke-Winnebeck C, Paterson NG, Young PG, Middleditch MJ, Greenwood DR, Witte G, Baker EN. Structural model for covalent adhesion of the *Streptococcus pyogenes* pilus through a thioester bond. J Biol Chem. 2014 Jan 3;289(1):177-89.

Liu S, Su X, Pan P, Zhang L, Hu Y, Tan H, Wu D, Liu B, Li H, Li H, Li Y, Dai M, Li Y, Hu C, Tsung A. Neutrophil extracellular traps are indirectly triggered by lipopolysaccharide and contribute to acute lung inury. Sci Rep. 2016 Nov 16;6:37252.

Logsdon LK, Håkansson AP, Cortés G, Wessels MR. Streptolysin O inhibits clathrin-dependent internalization of group A *streptococcus*. MBio. 2011 Feb 15;2(1):e00332-10.
London Biohackspace [Internet]. London: London Biohackspace; c2017. London Biohackspace: OD600 spectrophotometer; c2017 [cited 2017 Aug 10];[about 13 screens]. Available from: https://biohackspace.org/projects/od600-spectrophotometer/
Madureira P, Baptista M, Vieira M, Magalhães V, Camelo A, Oliveira L, Ribeiro A, Tavares D, Trieu-Cuot P, Vilanova M, Ferreira P. *Streptococcus agalactiae* GAPDH is a virulence-associated immunomodulatory protein. J Immunol. 2007 Feb 1;178(3):1379-87.
Mahara G, Wan C, Yang K, Chen S, Guo J, Gao Q, Wang W, Wang Q, Guo X. The association between environmental factors and scarlet fever incidence in Beijing region: using GIS and spacial regression models. Int J Environ Res Public Health. 2016 Nov;13(11):1083.
Malmström J, Karlsson C, Nordenfelt P, Ossola R, Weisser H, Quandt A, Hansson K, Aebersold R, Malmström L, Björck L. *Streptococcus pyogenes* in human plasma: *adaptive mechanisms analyzed by mass spectrometry-based proteomics*. J Biol Chem. 2012 Jan 6;287(2):1415-25.
Martins CO, Demarchi L, Ferreira FM, Pomerantzeff PM, Brandao C, Sampaio RO, Spina GS, Kalil J, Cunha-Neto E, Guilherme L. Rheumatic heart disease and myxomatous degeneration: differences and similarities of valve damage resulting from autoimmune reactions and matrix disorganization. PLoS One. 2017 Jan 25;12(1):e0170191.
McDonald RR, Golding GR, Levett PN, Yost CK, Irvine J, Horsman GB, Mulvey MR. Characterization of *Streptococcus pyogenes* and *Staphylococcus aureus* isolates associated with mixed skin and soft tissue infections in northern Saskatchewan. 2008 [cited 2017 Jul 21]: [about 16 slides]. Available from: http://www.narp.ca/pdf/ publications/ MCDONALD.pdf
McGregor KF, Spratt BG, Kalia A, Bennett A, Bilek N, Beall B, Bessen DE. Multilocus sequence typing of *Streptococcus pyogenes* representing most known emm types and distinctions among subpopulation genetic structures. J Bacteriol. 2004 Jul;186(13):4285-94.
McMillan DJ, Drèze PA, Vu T, Bessen DE, Guglielmini J, Steer AC, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR. Updated model of group A streptococcus M

proteins based on a comprehensive worldwide study. Clin Microbiol Infect. 2013 May;19(5):E222-9.

McNeil SA, Halperin SA, Langley JM, Smith B, Warren A, Sharratt GP, Baxendale DM, Reddish MA, Hu MC, Stroop SD, Linden J, Fries LF, Vink PE, Dale JB. Safety and immunogenicity of 26-valent group A *streptococcus* vaccine in healthy adult volunteers. Clin Infect Dis. 2005 Oct 15;41(8):1114–22.

Megged O, Yinnon AM, Raveh D, Rudensky B, Schlesinger Y. Group A streptococcus bacteraemia: comparison of adults and children in a single medical centre. Clin Microbiol Infect. 2006 Feb;12(2):156-62.

Miyoshi-Akiyama T, Takamatsu D, Koyanagi M, Zhao J, Imanishi K, Uchiyama T. Cytocidal effect of *Streptococcus pyogenes* on mouse neutrophils in vivo and the critical role of streptolysin S. J Infect Dis. 2005 Jul 1;192(1):107-16.

Molloy EM, Cotter PD, Hill C, Mitchell DA, Ross RP. Streptolysin S-like virulence factors: the continuing sagA. Nat Rev Microbiol. 2011 Aug 8;9(9):670-81.

Mora M, Bensi G, Capo S, Falugi F, Zingaretti C, Manetti AG, Maggi T, Taddei AR, Grandi G, Telford JL. Group A streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. Proc Natl Acad Sci USA. 2005 Oct 25;102(43):15641-6.

Nelson GE, Pondo T, Toews KA, Farley MM, Lindegren ML, Lynfield R, Aragon D, Zansky SM, Watt JP, Cieslak PR, Angeles K, Harrison LH, Petit S, Beall B, Van Beneden CA. Epidemiology of invasive group A streptococcal infections in the United States, 2005-2012. Clin Infect Dis. 2016 Aug 15;63(4):478-86.

Oliveira L, Madureira P, Andrade EB, Bouaboud A, Morello E, Ferreira P, Poyart C, Trieu-Cuot P, Dramsi S. Group B streptococcus GAPDH is released upon cell lysis, associates with bacterial surface, and induces apoptosis in murine macrophages. PLoS One. 2012;7(1):e29963.

Olsen RJ, Raghuram A, Cantu C, Hartman MH, Jimenez FE, Lee S, Jgo A, Rice KA, Saddington D, Spillman H, Valson C, Flores AR, Beres SB, Long W, Nasser W, Musser JM. The majority of 9,729 group A streptococcus strains causing disease secrete SpeB cysteine protease: pathogenesis implications. Infect Immun. 2015 Dec;83(12):4750-8.

Opsys MR™ microplate reader: user's guide [Internet]. Chantilly (VA): Dynex Technologies Inc.; c1999 [revised 1999 Feb 1; cited 2017 Aug 10]. Available from: http://www.medteh.info/_fr/31/Opsys_MR.pdf

Pancholi V, Caparon M. *Streptococcus pyogenes* metabolism. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.

Pancholi V, Fischetti VA. A major surface protein on group A streptococci is a glyceraldehyde-3-phosphate-dehydrogenase with multiple binding activity. J Exp Med. 1992 Aug 1;176(2):415–26.

Pancholi V, Fischetti VA. Glyceraldehyde-3-phosphate dehydrogenase on the surface of group A streptococci is also an ADP-ribosylating enzyme. Proc Natl Acad Sci USA. 1993 Sept 1;90(17):8154-8.

Pancholi V, Fischetti VA. Regulation of the phosphorylation of human pharyngeal cell proteins by group A streptococcal surface dehydrogenase: signal transduction between streptococci and pharyngeal cells. J Exp Med. 1997 Nov 17;186(10):1633–43.

Pancholi V, Fischetti VA. α-Enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. J Biol Chem. 1998 Jun 5;273(23):14503-15.

Pandey M, Langshaw E, Hartas J, Lam A, Batzloff MR, Good MF. A synthetic M protein peptide syngergizes with a CXC chemokine protease to induce vaccine-mediated protection against virulence streptococcal pyoderma and bacteremia. J Immunol. 2015 Jun 15;194(12):5915-25.

Parvez Y, Thomas SV. Acute cholecystitis in a child with scarlet fever: a rare association. S Afr J Child Health. 2016;10(3):188-9.

Phillips GN Jr, Flicker PF, Cohen C, Manjula BN, Fischetti VA. Streptococcal M protein: alpha-helical coiled-coil structure and arrangement on the cell surface. Proc Natl Acad Sci USA. 1981 Aug;78(8):4689-93.

Reglinski M, Gierula M, Lynskey NN, Edwards RJ, Sriskandan S. Identification of the *Streptococcus pyogenes* surface antigens recognised by pooled human immunoglobulin. Sci Rep. 2015;5:15825.

Russell EA, Tran L, Baker RA, Bennetts JS, Brown A, Reid CM, Tam R, Walsh WF, Maguire GP. A review of valve surgery for rheumatic heart disease in Australia. BMC Cardiovasc Disord. 2014 Oct 2; 14:134.

Ryan PA, Juncosa B. Group A streptococcal adherence. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.

Saab-Rincón G, Juárez VR, Osuna J, Sánchez F, Soberón X. Different strategies to recover the activity of monomeric triosephosphate isomerase by directed evolution. Protein Eng. 2001 Mar 1;14(3):149–55.

Sanderson-Smith M, De Oliveira DM, Guglielmini J, McMillan DJ, Vu T, Holien JK, Henningham A, Steer AC, Bessen DE, Dale JB, Curtis N, Beall BW, Walker MJ, Parker MW, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR, M Protein Study Group. A systematic and functional classification of *Streptococcus pyogenes* that serves as a new tool for molecular typing and vaccine development. J Infect Dis. 2014 Oct 15; 210(8):1325–38.

Savic DJ, McShan WM. Long-term survival of *Streptococcus pyogenes* in rich media is pH-dependent. Microbiology. 2012 Jun;158(Pt 6):1428–36.

Seale AC, Davies MR, Anampiu K, Morpeth SC, Nyongesa S, Mwarumba S, Smeesters PR, Efstratiou A, Karugutu R, Mturi N, Willams TN, Scott JAG, Kariuki S, Dougan G, Berkley JA. Invasive group A *streptococcus* infection among children, rural Kenya. Emerg Infect Dis. 2016 Feb;22(2):224–32.

Seidler KA, Seidler NW. Role of extracellular GAPDH in *Streptococcus pyogenes* virulence. Mo Med. May-June 2013;110(3):236-40.

Seifert KN, McArthur WP, Bleiweis AS, Brady LJ. Characterization of group B streptococcal glyceraldehyde-3-phosphate dehydrogenase: surface localization, enzymatic activity, and protein-protein interactions. Can J Microbiol. 2003 May;49(5):350-6.

Severin A, Nickbarg E, Wooters J, Quazi SA, Matsuka YV, Murphy E, Moutsatsos IK, Zagursky RJ, Olmsted SB. Proteomic analysis and identification of *Streptococcus pyogenes* surface-associated proteins. J Bacteriol. 2007 Mar;189(5):1514–22.

Shet A, Kaplan EL, Johnson DR, Cleary PP. Immune response to group A streptococcal C5a peptidase in children: implications for vaccine development. J Infect Dis. 2003 Sept 15;188(6):809-17.
Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the infectious diseases society of America. Clin Infect Dis. 2012 Nov 15;55(10):1–17. Erratum in: Clin Infect Dis. 2014 May;58(10):1496.
Shulman ST, Stollerman G, Beall B, Dale JB, Tanz RR. Temporal changes in streptococcal M protein types and the near-disappearance of acute rheumatic fever in the United States. Clin Infect Dis. 2006 Feb 15;42(4):441-7.
Shulman ST, Tanz RR, Dale JB, Beall B, Kabat W, Kabat K, Cederlund E, Patel D, Rippe J, Li Z, Sakota V, North American Streptococcal Pharyngitis Surveillance Group. Seven-year surveillance of North American pediatric group A streptococcal pharyngitis isolates. Clin Infect Dis. 2009 Jul 1;49(1):78–84.
Siddiqua K. Mutant group B streptococcus surface expressed phosphoglycerate kinase (PGK) with reduced plasminogen binding [master's thesis]. [Edmonton(AB)]: University of Alberta; 2014. 108 p.
Sigma-Aldrich® is now Millipore Sigma [Internet]. Sigma-Aldrich Co. LLC.; c2017. Product information: alkaline phosphatase yellow liquid substrate for ELISA; [cited 2017 Aug 10]; [about 1 p.]. Available from: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/5/p7998dat.pdf
Sigma-Aldrich® is now Millipore Sigma [Internet]. Sigma-Aldrich Co. LLC.; c2017. Product information: anti-rabbit IgG (whole molecule)-alkaline phosphatase produced in goat, affinity isolated antibody; [cited 2017 Aug 10];[about 2 p.]. Available from: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/6/a3687dat.pdf
Smyth DJ, Shera J, Bauer MJ, Cameron A, McNeilly CL, Sriprakash KS, McMillan DJ. Conjugative transfer of ICESde3396 between three β-hemolytic streptococcal species. BMC Res Notes. 2014 Aug 12;7: 521.
Sompayrac L. How the immune system works. 3rd ed. Malden (MA): Blackwell Publishing; 2008.

Spellerberg B, Brandt C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci). In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.

Steemson JD, Moreland NJ, Williamson D, Morgan J, Carter PE, Proft T. Survey of the bp/tee genes from clinical group A streptococcus isolates in New Zealand - implications for vaccine development. J Med Microbiol. 2014 Dec;63(Pt 12):1670–8.

Steer AC, Carapetis JR, Dale JB, Fraser JD, Good MF, Guilherme L, Moreland NJ, Mulholland EK, Schodel F, Smeesters PR. Status of research and development of vaccines for *Streptococcus pyogenes*. Vaccine. 2016 Jun 3;34(26): 2953–8.

Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan SL, Montoya JG, Wade JC, Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis. 2014 Jul;59(2):e10-52. Erratum in: Clin Infect Dis. 2014;59:147-50.; Clin Infect Dis. 2015.

Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, Wade JC, Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin Infect Dis. 2005 Nov 15;41(10):1373–406. Erratum in: Clin Infect Dis. 2005 Dec 15;41(12):1830.; Clin Infect Dis. 2006 Apr 15;42(8): 1219.

Sumby P, Barbian KD, Gardner DJ, Whitney AR, Welty DM, Long RD, Bailey JR, Parnell MJ, Hoe NP, Adams GG, Deleo FR, Musser JM. Extracellular deoxyribonuclease made by group A streptococcus assists pathogenesis by enhancing evasion of the innate immune response. Proc Natl Acad Sci USA. 2005 Feb 1;102(5):1679-84.

Sumitomo T, Nakata M, Higashino M, Jin Y, Terao Y, Fujinaga Y, Kawabata S. Streptolysin S contributes to group A streptococcal translocation across an epithelial barrier. J Biol Chem. 2011 Jan 28;286(4):2750-61.

Tan J, Smith CH, Goldman RD. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. Can Fam Physician. 2012 Sep;58(9):957-9.

Terao Y, Kawabata S, Kunitomo E, Nakagawa I, Hamada S. Novel laminin-binding protein of *Streptococcus pyogenes*, Lbp, is involved in adhesion to epithelial cells. Infect Immun. 2002 Feb;70(2):993-7.

Terao Y, Yamaguchi M, Hamada S, Kawabata S. Multifunctional glyceraldehyde-3-phosphate dehydrogenase of *Streptococcus pyogenes* is essential for evasion from neutrophils. J Biol Chem. 2006 May 19;281(20):14215-23.

Terrasse R, Amoroso A, Vernet T, Di Guilmi AM. *Streptococcus pneumoniae* GAPDH is released by cell lysis and interacts with peptidoglycan. PLoS One. 2015 Apr 30; 10(4):e0125377.

The QIAexpressionist™: a handbook for high-level expression and purification of 6xHis-tagged proteins [Internet]. 5th ed. Qiagen; 2003 Jun [cited 2017 Aug 10]. 128 p. Available from: http://kirschner.med.harvard.edu/files/protocols/QIAGEN_ QIAexpressionist_EN.pdf

Tsai JC, Loh JM, Clow F, Lorenz N, Proft T. The group A streptococcus serotype M2 pilus plays a role in host cell adhesion and immune evasion. Mol Microbiol. 2017 Jan;103(2):282-98.

Tyrrell GJ, Kennedy A, Shokoples SE, Sherburne RK. Binding and invasion of HeLa and MRC-5 cells by *Streptococcus agalactiae*. Microbiology. 2002 Dec;148 (Pt 12):3921–31.

Tyrrell GJ, Lovgren M, Forwick B, Hoe NP, Musser JM, Talbot JA. M types of group A streptococcal isolates submitted to the National Centre for Streptococcus (Canada) from 1993 to 1999. J Clin Microbiol. 2002 Dec;40(12):4466–71.

Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. Invasive group A streptococcal disease in Alberta, Canada (2000 to 2002). J Clin Microbiol. 2005 Apr; 43(4):1678–83.

Tyrrell GJ, Lovgren M, St. Jean T, Hoang L, Patrick DM, Horsman G, Van Caeseele P, Sieswerda LE, McGeer A, Laurence RA, Bourgault A-M, Low DE. Epidemic of group A *streptococcus* M/emm59 causing invasive disease in Canada. Clin Infect Dis. 2010 Dec 1;51(11):1290–7.

Uchiyama S, Döhrmann S, Timmer AM, Dixit N, Ghochani M, Bhandari T, Timmer JC, Sprague K, Bubeck-Wardenburg J, Sinom SI, Nizet V. Streptolysin O rapidly impairs neutrophil oxidative burst and antibacterial responses to group A streptococcus. Front Immunol. 2015 Nov 16;6:581.

Uhlmann J, Siemens N, Kai-Larsen Y, Fiedler T, Bergman P, Johansson L, Norrby-Teglund A. Phosphoglycerate kinase – a novel streptococcal factor involved in neutrophil activation and degranulation. J Infect Dis. 2016 Dec 15; 214(12):1876–83.
VanDeVoorde RG 3rd. Acute poststreptococcal glomerulonephritis: the most common acute glomerulonephritis. Pediatr Rev. 2015 Jan; 36(1): 3-12; quiz 13.
van Sorge NM, Cole JN, Kuipers K, Henningham A, Aziz RK, Kasirer-Friede A, Lin L, Berends ETM, Davies MR, Dougan G, Zhang F, Dahesh S, Shaw L, Gin J, Cunningham M, Merriman JA, Hütter J, Lepenies B, Rooijakkers SHM, Malley R, Walker MJ, Shattil SJ, Schlievert PM, Choudhury B, Nizet V. The classical Lancefield antigen of group A *streptococcus* is a virulence determinant with implications for vaccine design. Cell Host Microbe. 2014 Jun 11;15(6):729-40.
Vázquez-Zamorano ZE, González-Lóopez MA, Romero-Espejel ME, Azuara-Liceaga El, López-Casamichana M, Olivares-Trejo Jde J. *Streptococcus pneumoniae* secretes a glyceraldehyde-3-phosphate dehydrogenase, which binds haemoglobin and haem. Biometals. 2014 Aug;27(4):683-93.
VWR: we enable science [Internet]. VWR. Falcon® 96-well cell culture plates, Corning®; [cited 2017 Aug 10];[about 2 screens]. Available from: https://ca.vwr.com/store/product/en/4678980/falcon-96-well-cell-culture-plates-corning#
Wessels MR. Pharyngitis and scarlet fever. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes*: basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.
Winram SB, Lottenberg R. The plasmin-binding protein Plr of group A streptococci is identified as glyceraldehyde-3-phosphate dehydrogenase. Microbiology. 1996 Aug;142(Pt 8): 2311–20.
Young PG, Moreland NJ, Loh JM, Bell A, Atatoa Carr P, Proft T, Baker EN. Structural conservation, variability, and immunogenicity of the T6 backbone pilin of serotype M6 *Streptococcus pyogenes*. Infect Immun. 2014 Jul;82 (7):2949–57.
Zhu L, Olsen RJ, Musser JM. Opacification domain of serum opacity factor inhibits beta-hemolysis and contributes to virulence of *Streptococcus pyogenes.* mSphere. 2017 Mar-Apr;2(2):e00147-17.

[a]Permission has been granted by Alhhazmi A to use the information in this thesis.

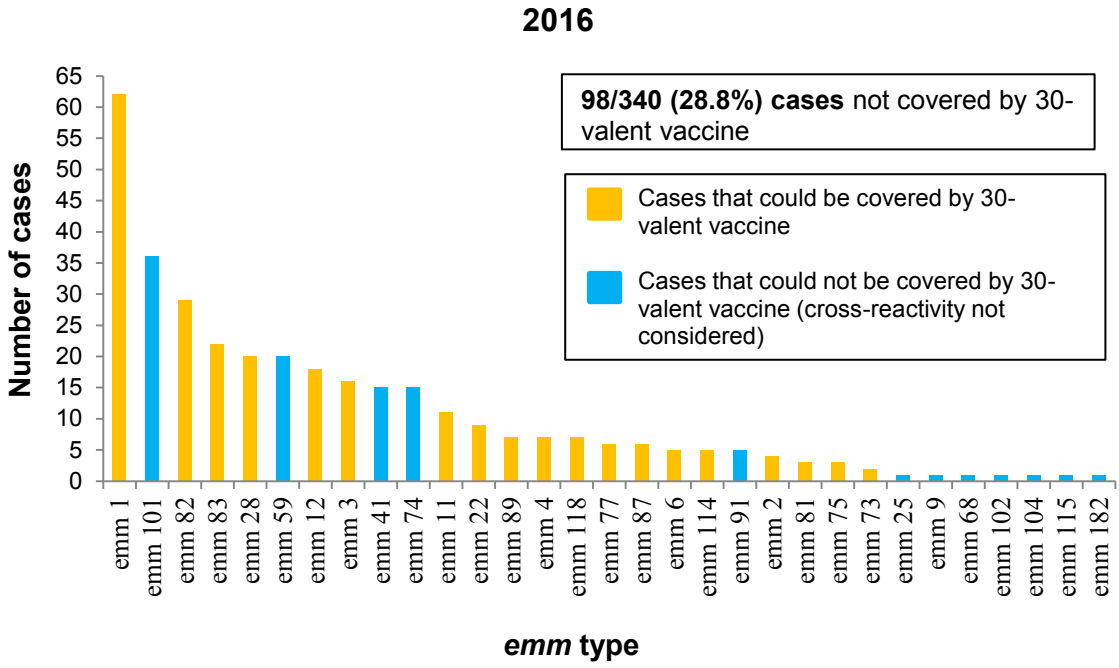
Appendix

Supplementary Table S2.1. Estimated incidence rates of invasive GAS in Alberta in 2016 according to gender and age.*

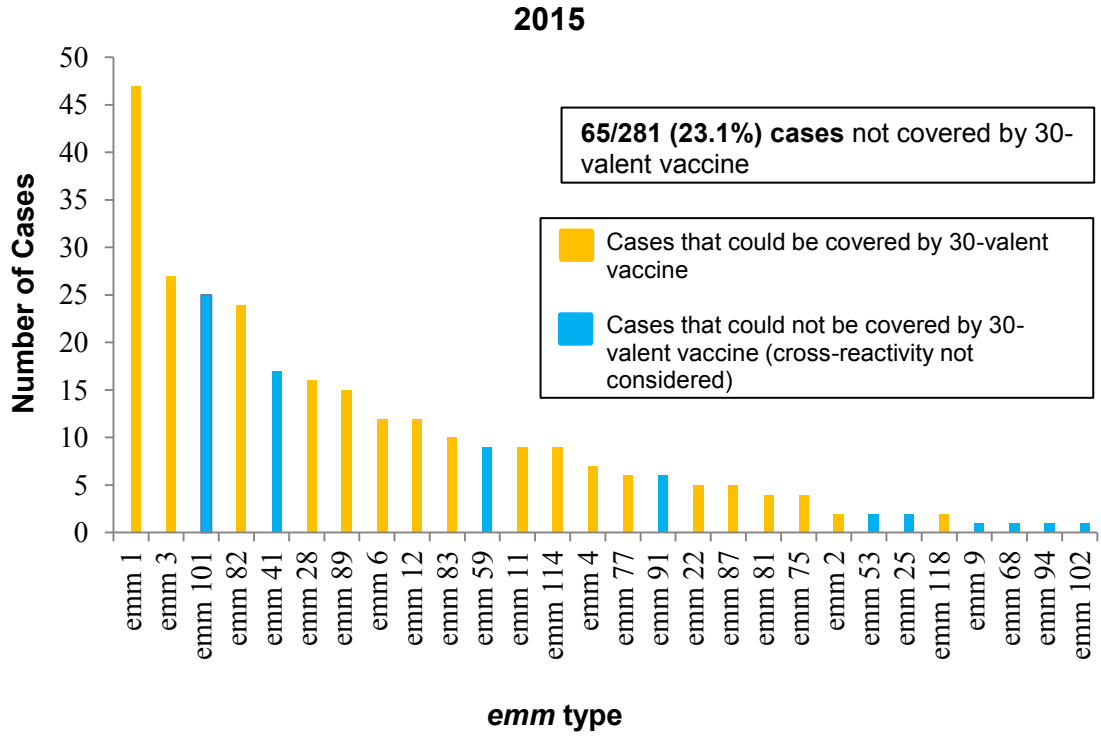
Category	Categorical Components	Incidence rate (/100 000 population)
Gender	Male	9.9
	Female	6.1
Age	0-10 years	6.5
	11-20 years	2.1
	21-30 years	6.0
	31-40 years	8.1
	41-50 years	8.1
	51-60 years	11.4
	61-70 years	10.4
	71-80 years	14.2
	>80 years	16.2

*For a graphical representation of the incidence rates, refer to Figure 2.3.

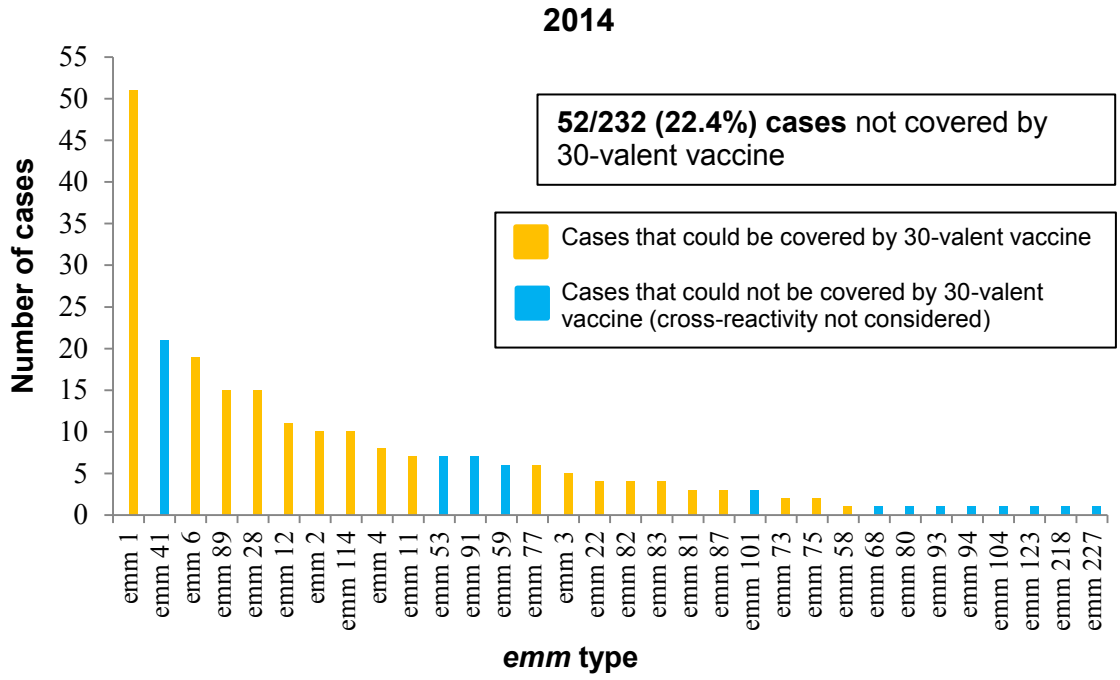
A


2016
Number of cases
65
60
55
50
45
40
35
30
25
20
15
10
5
0
98/340 (28.8%) cases not covered by 30-valent vaccine
Cases that could be covered by 30-valent vaccine
Cases that could not be covered by 30-valent vaccine (cross-reactivity not considered)
emm 1
emm 101
emm 82
emm 83
emm 28
emm 59
emm 12
emm 3
emm 41
emm 74
emm 11
emm 22
emm 89
emm 4
emm 118
emm 77
emm 87
emm 6
emm 114
emm 91
emm 2
emm 81
emm 75
emm 73
emm 25
emm 9
emm 68
emm 102
emm 104
emm 115
emm 182
emm type

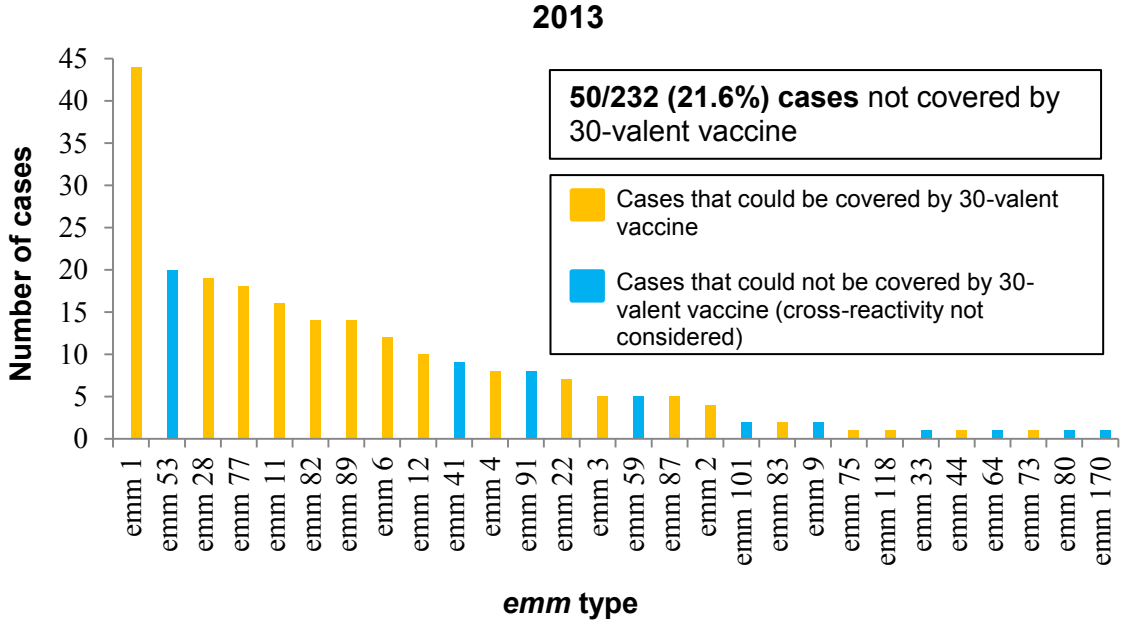
B


2015
Number of Cases
50
45
40
35
30
25
20
15
10
5
0
65/281 (23.1%) cases not covered by 30-valent vaccine
Cases that could be covered by 30-valent vaccine
Cases that could not be covered by 30-valent vaccine (cross-reactivity not considered)
emm 1
emm 3
emm 101
emm 82
emm 41
emm 28
emm 89
emm 6
emm 12
emm 83
emm 59
emm 11
emm 114
emm 4
emm 77
emm 91
emm 22
emm 87
emm 81
emm 75
emm 2
emm 53
emm 25
emm 118
emm 9
emm 68
emm 94
emm 102
emm type

C



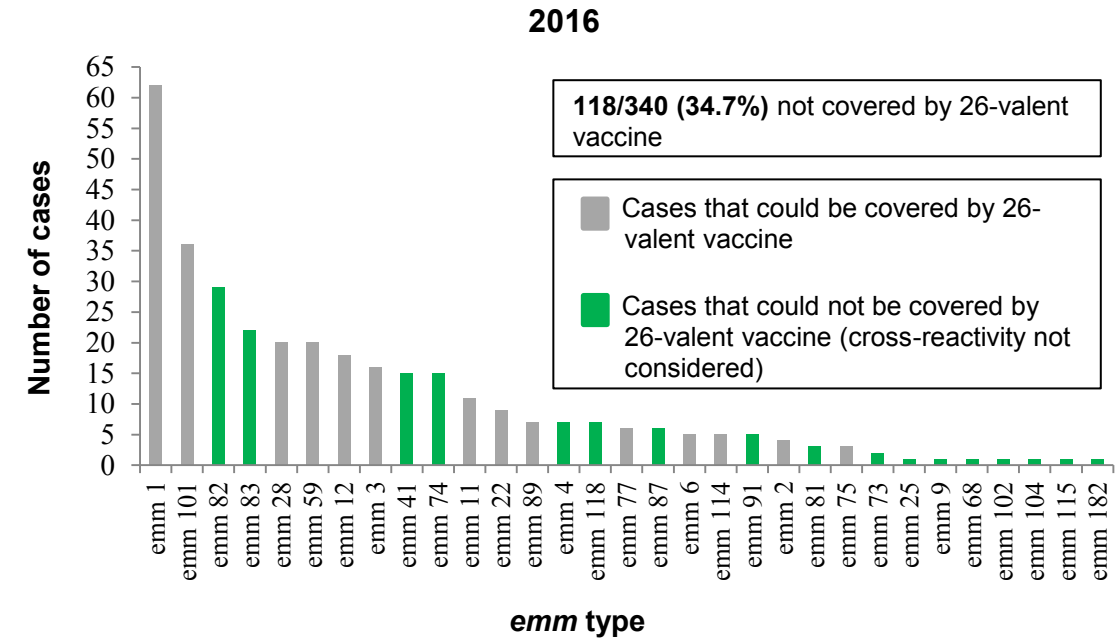
D



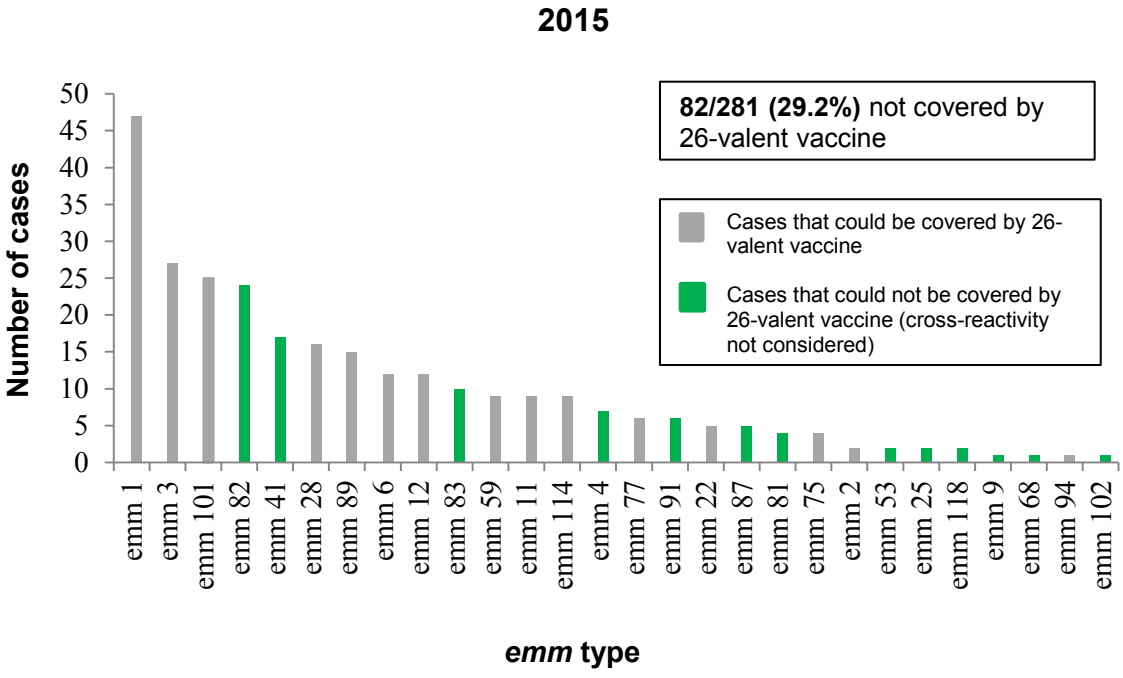
Supplementary Figure S2.1. Coverage of invasive GAS cases with the 30-valent vaccine in Alberta without accounting for vaccine cross-reactivity with non-vaccine *emm* types. Theoretical vaccine coverage of cases for 2016 (A), 2015 (B), 2014 (C) and

2013 (D) was assessed (2, 4, 8, 11, 16) using Table 1.2. The theoretical percentage of non-coverage for invasive GAS cases (8, 11, 16) in 2013 (D), 2014 (C), 2015 (B) and 2016 (A) is 21.6%, 22.4%, 23.1% and 28.8% respectively (bolded above).

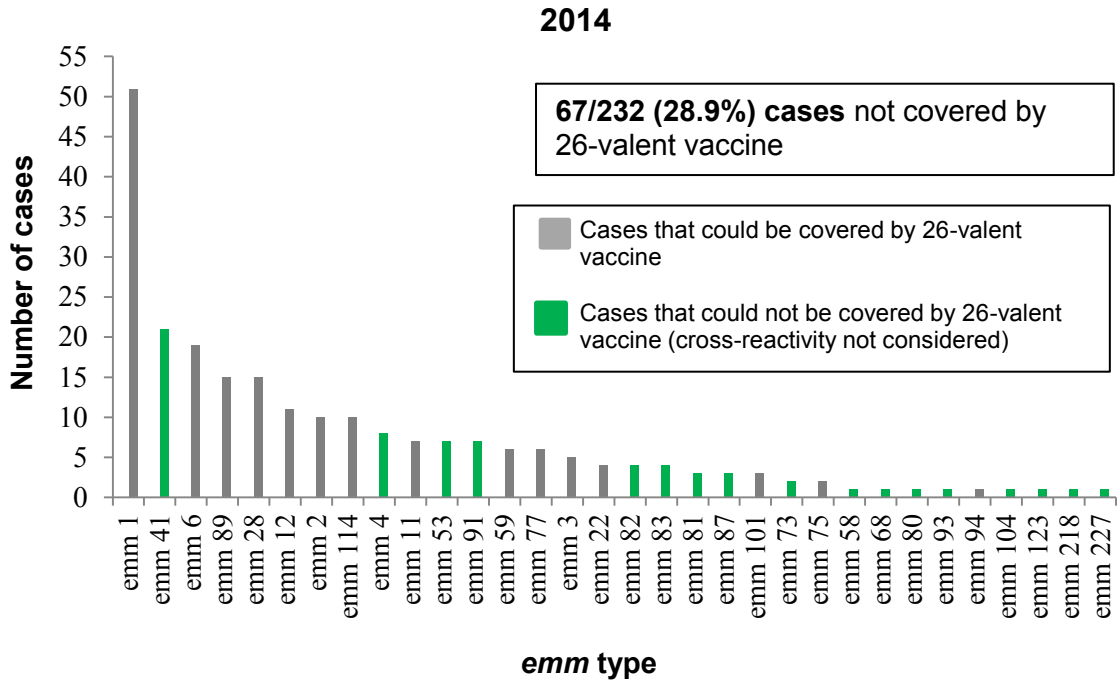
A


2016
Number of cases
65
60
55
50
45
40
35
30
25
20
15
10
5
0
118/340 (34.7%) not covered by 26-valent vaccine
Cases that could be covered by 26-valent vaccine
Cases that could not be covered by 26-valent vaccine (cross-reactivity not considered)
emm 1
emm 101
emm 82
emm 83
emm 28
emm 59
emm 12
emm 3
emm 41
emm 74
emm 11
emm 22
emm 89
emm 4
emm 118
emm 77
emm 87
emm 6
emm 114
emm 91
emm 2
emm 81
emm 75
emm 73
emm 25
emm 9
emm 68
emm 102
emm 104
emm 115
emm 182
emm type

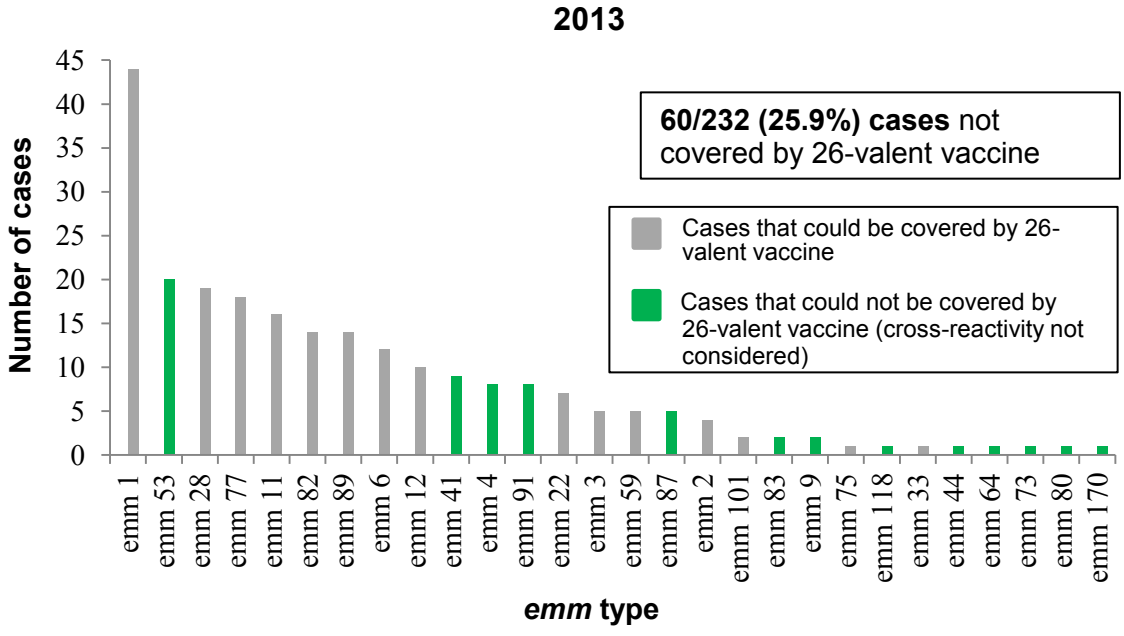
B


2015
Number of cases
50
45
40
35
30
25
20
15
10
5
0
82/281 (29.2%) not covered by 26-valent vaccine
Cases that could be covered by 26-valent vaccine
Cases that could not be covered by 26-valent vaccine (cross-reactivity not considered)
emm 1
emm 3
emm 101
emm 82
emm 41
emm 28
emm 89
emm 6
emm 12
emm 83
emm 59
emm 11
emm 114
emm 4
emm 77
emm 91
emm 22
emm 87
emm 81
emm 75
emm 2
emm 53
emm 25
emm 118
emm 9
emm 68
emm 94
emm 102
emm type

C



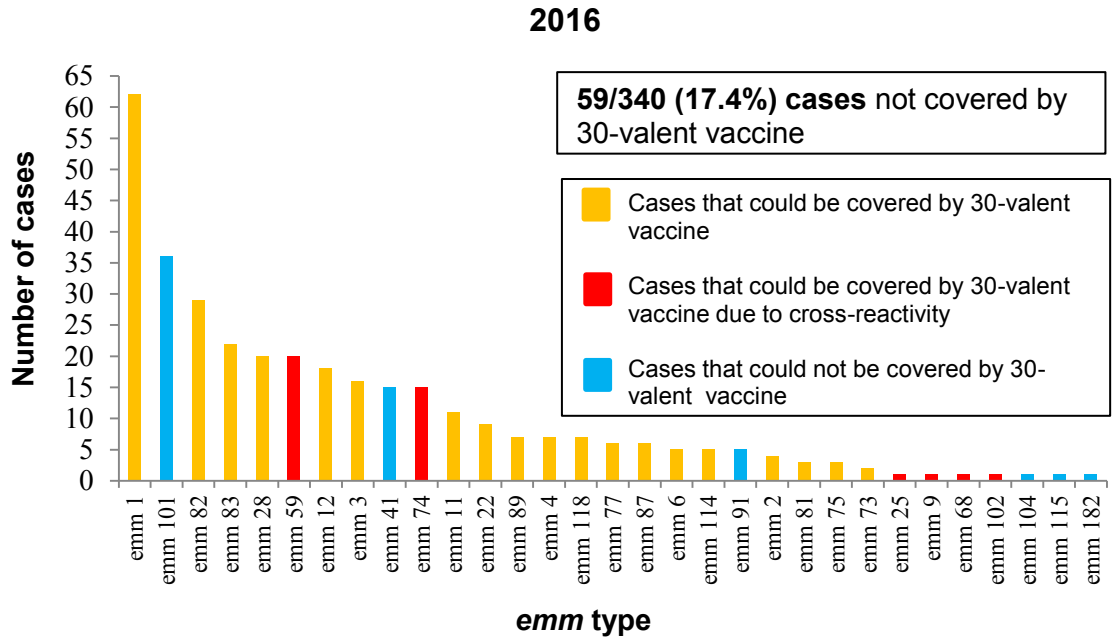
D



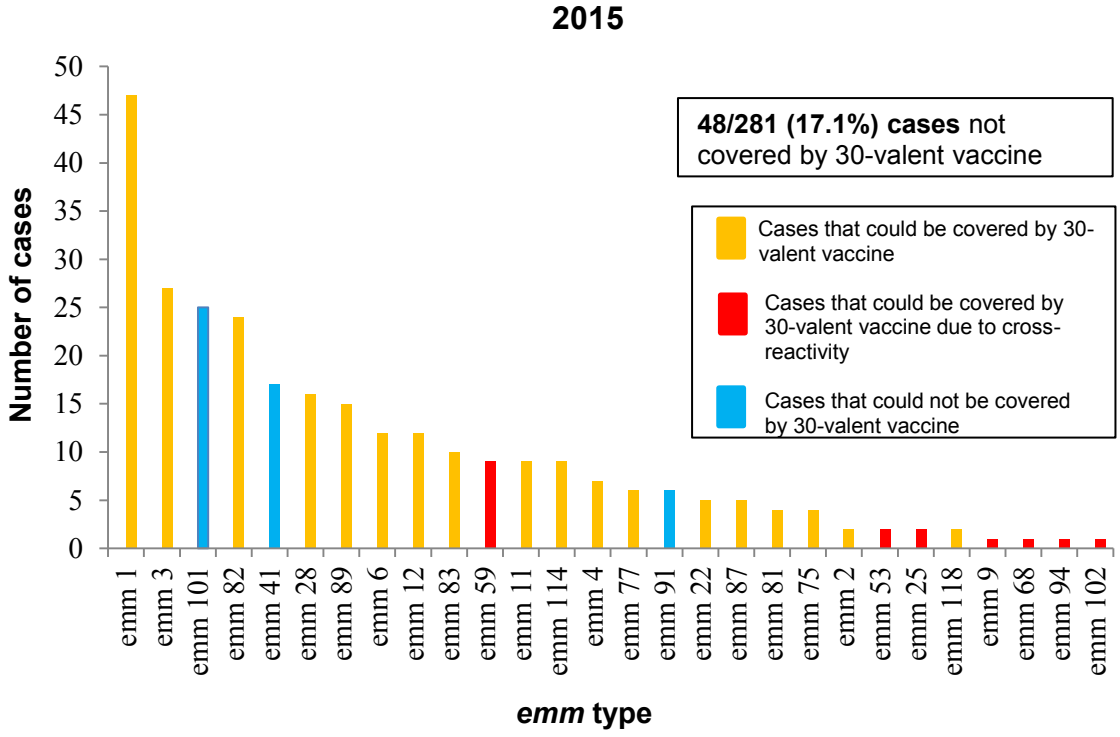
Supplementary Figure S2.2. Coverage of invasive GAS cases with the 26-valent vaccine in Alberta without accounting for vaccine cross-reactivity with non-vaccine

***emm* types.** Theoretical vaccine coverage of cases for 2016 (A), 2015 (B), 2014 (C) and 2013 (D) was assessed (2, 4, 8, 10-11, 15-16) using Table 1.2. The theoretical percentage of non-coverage for invasive GAS cases (8, 10-11, 15-16) in 2013 (D), 2014 (C), 2015 (B) and 2016 (A) is 25.9%, 28.9%, 29.2% and 34.7% respectively (bolded above).

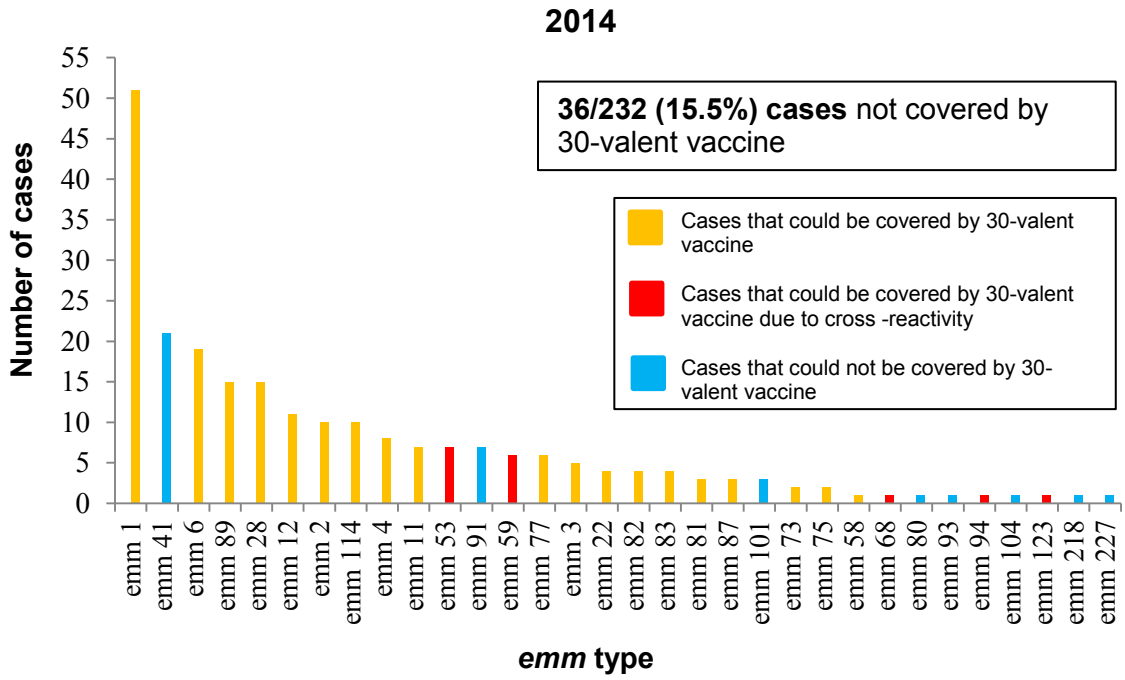
A


2016
59/340 (17.4%) cases not covered by 30-valent vaccine
Cases that could be covered by 30-valent vaccine
Cases that could be covered by 30-valent vaccine due to cross-reactivity
Cases that could not be covered by 30-valent vaccine
Number of cases
0
5
10
15
20
25
30
35
40
45
50
55
60
65
emm 1
emm 101
emm 82
emm 83
emm 28
emm 59
emm 12
emm 3
emm 41
emm 74
emm 11
emm 22
emm 89
emm 4
emm 118
emm 77
emm 87
emm 6
emm 114
emm 91
emm 2
emm 81
emm 75
emm 73
emm 25
emm 9
emm 68
emm 102
emm 104
emm 115
emm 182
emm type

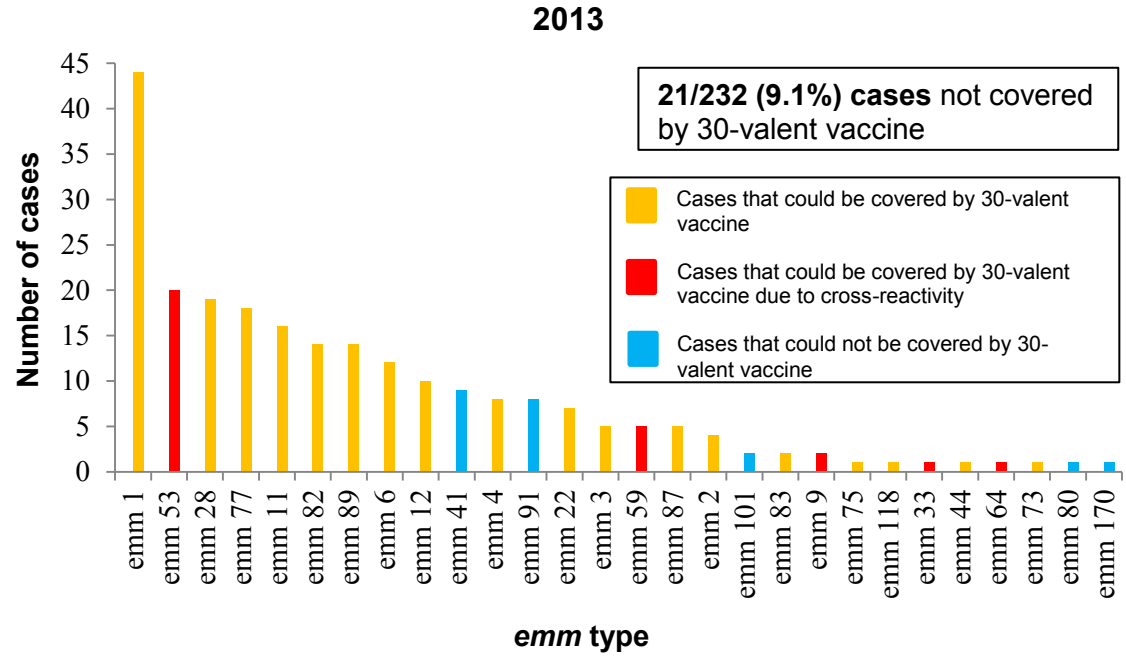
B


2015
48/281 (17.1%) cases not covered by 30-valent vaccine
Cases that could be covered by 30-valent vaccine
Cases that could be covered by 30-valent vaccine due to cross-reactivity
Cases that could not be covered by 30-valent vaccine
Number of cases
0
5
10
15
20
25
30
35
40
45
50
emm 1
emm 3
emm 101
emm 82
emm 41
emm 28
emm 89
emm 6
emm 12
emm 83
emm 59
emm 11
emm 114
emm 4
emm 77
emm 91
emm 22
emm 87
emm 81
emm 75
emm 2
emm 53
emm 25
emm 118
emm 9
emm 68
emm 94
emm 102
emm type

C



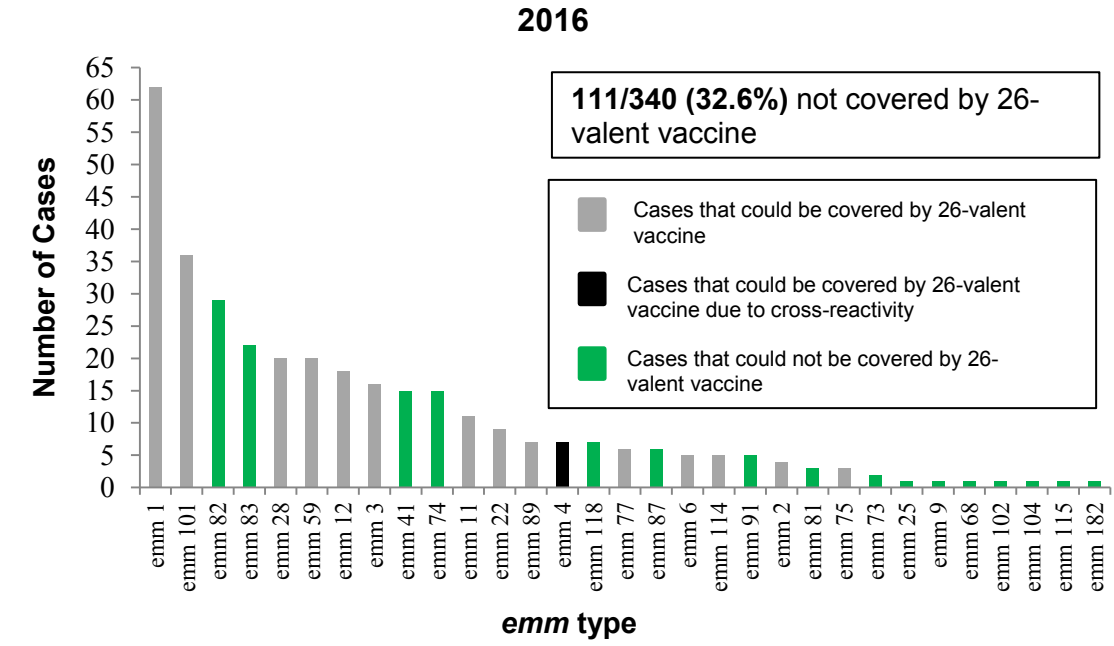
D



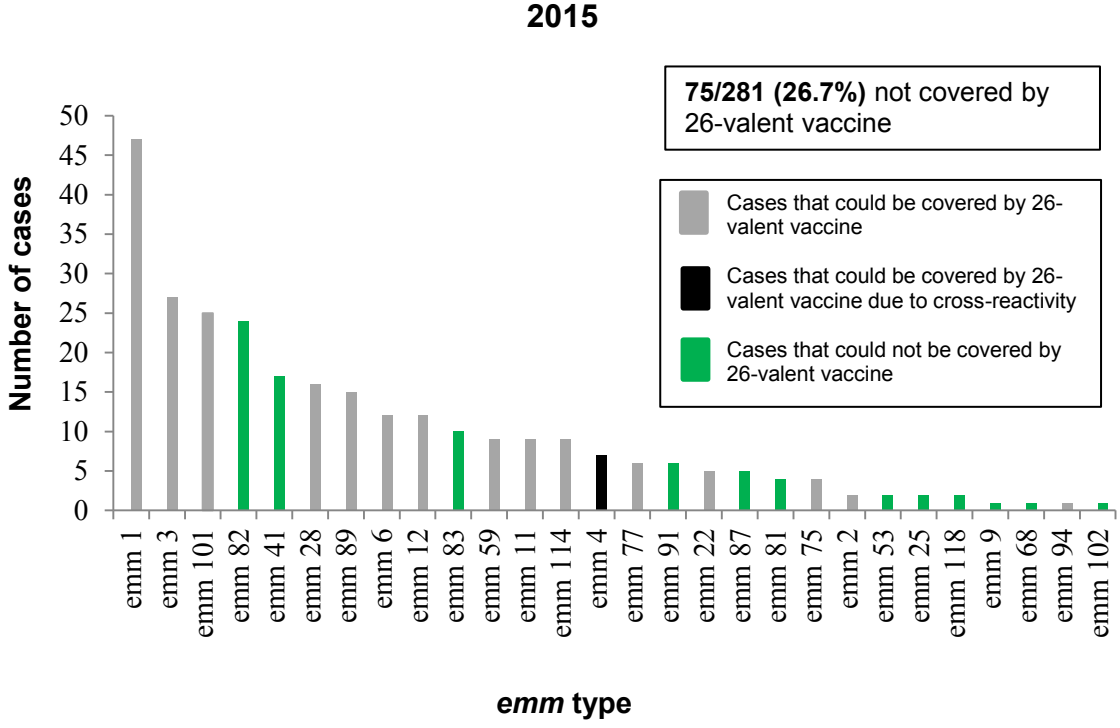
Supplementary Figure S2.3. Coverage of invasive GAS cases with the 30-valent vaccine in Alberta accounting for vaccine cross-reactivity with non-vaccine *emm* types. Theoretical vaccine coverage of cases for 2016 (A), 2015 (B), 2014 (C) and 2013

(D) was assessed (2, 4, 8, 11, 16) using Table 1.2 and Table 2.2. The theoretical percentage of non-coverage for invasive GAS cases (8, 11, 16) in 2013 (D), 2014 (C), 2015 (B) and 2016 (A) is 9.1%, 15.5%, 17.1% and 17.4% respectively (bolded above).

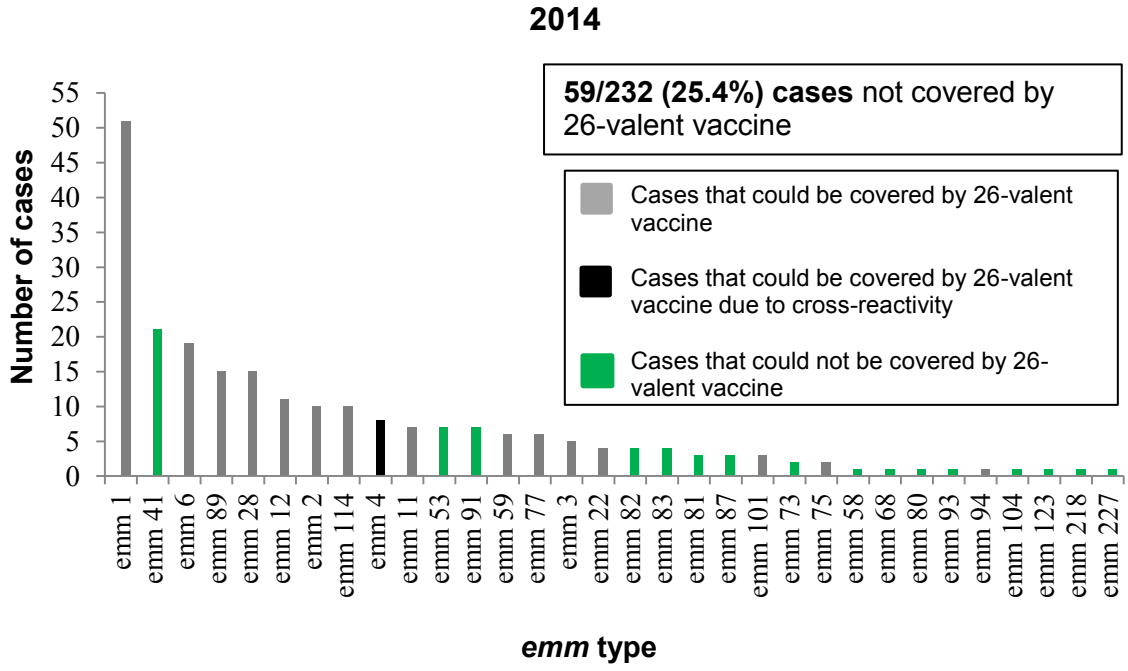
A


2016
111/340 (32.6%) not covered by 26-valent vaccine
Cases that could be covered by 26-valent vaccine
Cases that could be covered by 26-valent vaccine due to cross-reactivity
Cases that could not be covered by 26-valent vaccine
Number of Cases
0
5
10
15
20
25
30
35
40
45
50
55
60
65
emm 1
emm 101
emm 82
emm 83
emm 28
emm 59
emm 12
emm 3
emm 41
emm 74
emm 11
emm 22
emm 89
emm 4
emm 118
emm 77
emm 87
emm 6
emm 114
emm 91
emm 2
emm 81
emm 75
emm 73
emm 25
emm 9
emm 68
emm 102
emm 104
emm 115
emm 182
emm type

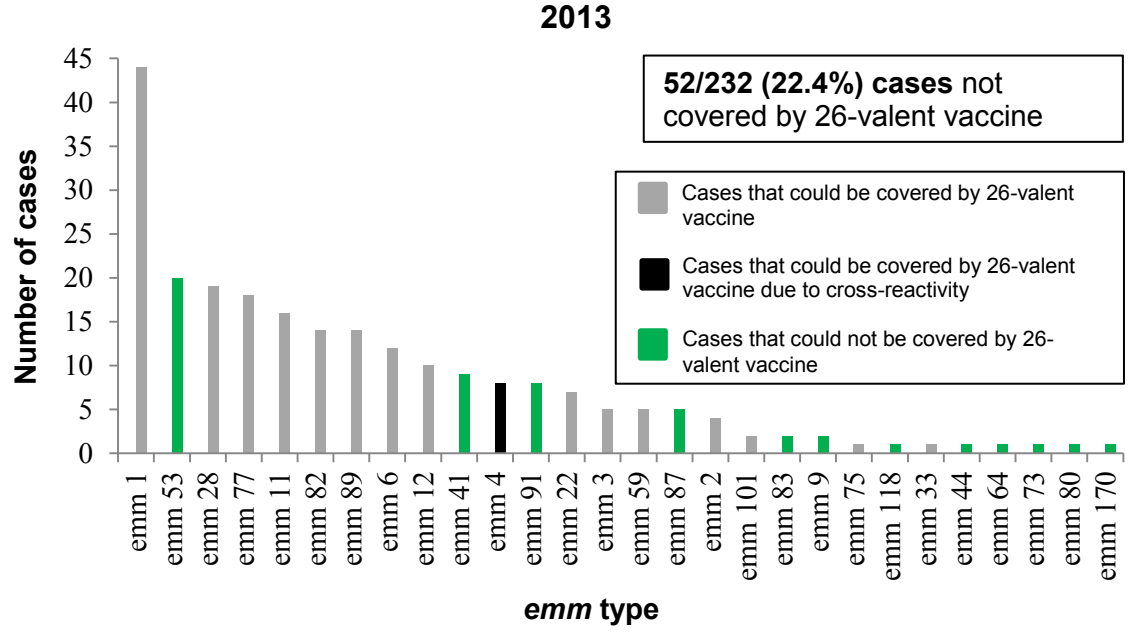
B


2015
75/281 (26.7%) not covered by 26-valent vaccine
Cases that could be covered by 26-valent vaccine
Cases that could be covered by 26-valent vaccine due to cross-reactivity
Cases that could not be covered by 26-valent vaccine
Number of cases
0
5
10
15
20
25
30
35
40
45
50
emm 1
emm 3
emm 101
emm 82
emm 41
emm 28
emm 89
emm 6
emm 12
emm 83
emm 59
emm 11
emm 114
emm 4
emm 77
emm 91
emm 22
emm 87
emm 81
emm 75
emm 2
emm 53
emm 25
emm 118
emm 9
emm 68
emm 94
emm 102
emm type

C



D



Supplementary Figure S2.4. Coverage of invasive GAS cases with the 26-valent vaccine in Alberta accounting for vaccine cross-reactivity with non-vaccine *emm* types. Theoretical vaccine coverage of cases for 2016 (A), 2015 (B), 2014 (C) and 2013

(D) was assessed (2, 4, 8, 10-11, 15-16) using Table 1.2 and Table 2.2. The theoretical percentage of non-coverage for invasive GAS cases (8, 10-11, 15-16) in 2013 (D), 2014 (C), 2015 (B) and 2016 (A) is 22.4%, 25.4%, 26.7% and 32.6% respectively (bolded above).