### Unexpected Functions of Three Classical Group A Streptococcal Virulence Factors

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Group A Streptococcus (GAS) is a leading human pathogen commonly associated with pharyngitis (“strep throat”) but also capable on occasion of producing severe, invasive diseases including necrotizing fasciitis (“flesh-eating disease”) and toxic shock syndrome, even in previously healthy individuals. GAS systemic disease reflects a large suite of virulence factors that enable the pathogen to avoid eradication by phagocytic defenses of the host immune system. In our studies of the globally-disseminated hyperinvasive GAS M1T1 clone, we have elucidated novel functions of several classical GAS virulence factors in modulating host immunity. The cell-wall anchored M protein, the most abundant and immunodominant antigen on the GAS surface, is known for its fibrinogen-binding and antiphagocytic properties. We find that the hypervariable N-terminal domain can sequester cationic calcium-binding mimetics and histones to promote GAS resistance to killing by neutrophils and neutrophil extracellular traps. M protein also activates the NLRP3 inflammasome triggering rapid pyroptotic cell death. The GAS hyaluronic acid (HA) capsule is upregulated during systemic infection, and mimics a common host glycosaminoglycan to cloak opsonic targets on the bacterial surface. We reveal that GAS uses HA to engage an inhibitory Siglec receptor on the neutrophil cell surface, blunting neutrophil activation and promoting pathogen survival. Finally, the broad-spectrum GAS cysteine protease SpeB degrades host defense molecules such as antimicrobial peptides and immunoglobulins, but is paradoxically inactivated during invasive disease pathogenesis. We describe an caspase- and inflammasome-independent IL-1β signaling pathway to detect bacterial proteases that is critical in host defense against GAS. This pathway may explain an unusually high rate of severe GAS infection in patients receiving IL-1β receptor blocker therapy.

### Streptococcal Just So stories: or, what we can learn from genomics

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It seems increasingly clear that we can sometimes leapfrog the sequence of experiments that were previously necessary to answer a complex research question, simply by sequencing the bacterial genome. Furthermore, the genomic data we acquire allow us to pose entirely new questions that we had not actually ever thought about before. Streptococcal biology is no exception; so-called genomic fishing expeditions helped to identify un-anticipated research questions, such as ‘what is the basis for the link between streptococcal capsule and the lymphatic system?’ Genomic studies have also helped to answer longstanding questions about serotype-specific phenotypes and may yet answer questions about disease and species specificity; including evolution from animal to human pathogen. Evolution of each streptococcal species can be laid bare through genome sequencing, though this requires that strains are accessible, underpinning the importance of curating isolates from the past. Genomic science is also an increasingly important tool within Clinical Infection; confirming or refuting an outbreak is of real importance when trying to prevent the next one. However outbreak studies can provide unique insight as to what streptococci are capable of. Unexpected transmission events occur seemingly routinely in the hospital environment and we need to better-understand the capacity of streptococci to transmit from person to person in such environments.

While the reading and interpretation of genomic data may remain the domain of specialists, it behoves the wider scientific and clinical research community to embrace these findings and identify the most important questions that are yet to be answered.

### Treatment of Group A Strep beyond antibiotics

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Necrotizing fasciitis retains a very high mortality rate despite prompt and adequate antibiotic treatment and surgical debridement. Aiming to improve therapeutic options by in-depth understanding of the pathophysiology of necrotizing fasciitis the role of protein synthesis inhibitors and immunoglobulins will be discussed.

### Immunity after Group A Streptococcal infections: Beyond emm-type specific response

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Immunity after GAS infection is traditionally seen as relying on emm-type specific antibody response. However, the evidences supporting this paradigm are in fact relatively limited. We have analyzed the M protein immune response after GAS skin infection in Fiji. We investigated the human serological response to GAS infection in Fijian schoolchildren, focusing on 3 major emm clusters (E4, E6, and D4). Pre- and post infection sera were assayed by ELISA assay with N-terminal M peptides and bactericidal assays using the infecting-type strain, emm cluster–related strains, and nonrelated strains. While the responses were highly variable, numerous instances of cross-reactive killing were observed. Our study confirms the existence of emm-type specific immunity, but
suggests that this is an incomplete picture and that a combination of “cluster-specific” and “type-specific” responses occur. Our study suggests that cross-reactive immune responses occur following skin infection and raises hope for the development of a broadly protective multivalent vaccine.

Mechanisms of Blood-Brain Barrier Penetration by Group B Streptococcus

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Bacterial meningitis is a serious infection of the central nervous system and occurs when blood-borne bacteria cross the blood-brain barrier (BBB). Group B Streptococcus (GBS) is the leading cause of neonatal meningitis, however the molecular mechanisms regulating bacterial BBB disruption and penetration are still being elucidated. We found that infection of human brain microvascular endothelial cells (hBMEC) with GBS and other meningeval pathogens resulted in the induction of Snail1, a host transcriptional repressor of tight junction genes. Transcript and protein levels of tight junction components ZO-1, Claudin-5 and Occludin were decreased in hBMEC following GBS infection, which was dependent on Snail1 induction. Additionally we have conducted mass spectrometry analysis of cell wall extracts of GBS deficient in their ability to activate Snail1. Differential protein expression between these samples has created a candidate list of GBS proteins implicated in activating Snail1. Additional proteomic analysis of GBS has been conducted to investigate the contribution of the Diag mutant to Snail1 activation. Taken together our data suggests a novel mechanism of BBB disruption and penetration by GBS and provide a candidate list of the GBS proteins that should be investigated for their contribution to Snail1 activation and BBB disruption.

Population genomics of Group A Streptococci: Application to global vaccine design

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High-throughput genome sequencing has enabled the ability to sequence large numbers of bacterial genomes to unravel the dynamics of bacterial populations at an unprecedented resolution. In its purist form, this approach provides an assessment of the naturally occurring antigenic diversity within a population. We are using this technology to investigate the population structure of the human bacterial pathogen, the Group A Streptococcus (GAS), a leading cause of human morbidity and mortality to which no vaccine exists. One of the major hurdles facing GAS vaccine design is variable antigen carriage and antigenic heterogeneity. In order to advance the progress of the global GAS vaccine, we have analyzed the genome sequences of over 1500 GAS isolates, primarily from regions endemic for streptococcal infection. This GAS genome database comprises of over 140 emm-sequence types, 37 emm-clusters and 402 multi-locus sequence types. We assess antigen carriage of 28 purported GAS vaccine antigens, polymorphism heterogeneity and provide examples of variation in the context of protein structure. The development of genomic databases for vaccine design is equally applicable to future antigenic, epidemiological and pathogenesis studies at a global level.

Global Burden of Rheumatic Heart Disease

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Background:
Rheumatic heart disease remains an important preventable cause of cardiovascular death and disability, particularly in low- and middle-income countries. We estimated global, regional, and national trends in rheumatic heart disease mortality and prevalence as part of the Global Burden of Disease 2015 Study.

Methods:
We systematically reviewed data on fatal and non-fatal rheumatic heart disease from 1990-2015. Two Global Burden of Disease analytic tools, the Cause of Death Ensemble model and DisMod-MR 2.1, were used to produce estimates of mortality rates and prevalence including estimates of uncertainty.

Results:
There were an estimated 319,400 (95% uncertainty interval 297,300 to 337,300) deaths due to rheumatic heart disease in 2015. The global age-standardized death rate due to rheumatic heart disease decreased by 47.8% (95% uncertainty interval 44.7% to 50.9%) from 1990 to 2015, but large differences were observed across regions. In 2015, the highest age-standardized rheumatic heart disease death and prevalence rates were observed in Oceania, South Asia, and Central Sub-Saharan Africa. In 2015, there were an estimated 33.4 million (95% uncertainty interval 29.7 million to 33.4 million) cases of rheumatic heart disease globally and an estimated 10.5 million (95% uncertainty interval 9.6 million to 11.5 million) disability-adjusted life-years due to rheumatic heart disease.

Conclusions:
We estimated global disease prevalence and mortality due to rheumatic heart disease over a 25-year period. The health-related burden of rheumatic heart disease has declined worldwide, but high rates of disease persist in some of the world’s poorest regions.
Despite more than two decades of collaborative research and progress has been made in improving care for people living with Rheumatic Heart Disease (RHD), but not in reducing overall RHD burden. The top 5 causes of overall socio-economic disadvantage, it occurs at among the highest rates in the world in Indigenous Australians living in national focus on “Closing the Gap” of Indigenous disadvantage, but despite considerable investment and worse health outcomes, including shorter life expectancy, than the non-Indigenous population.

Australia is a country blessed with a high standard of living, long life expectancy and an excellent health care system. Yet Australia’s Indigenous population experiences social and economic disadvantage and has much worse health outcomes, including shorter life expectancy, than the non-Indigenous population. This has led to a national focus on “Closing the Gap” of Indigenous disadvantage, but despite considerable investment and attention, little progress is being made. Rheumatic heart disease is an exemplar disease of “the Gap”. Borne of socio-economic disadvantage, it occurs at among the highest rates in the world in Indigenous Australians living in regional and remote areas, and is the leading cause of differential cardiovascular disease burden, and among the top 5 causes of overall differential burden, between Indigenous and non-Indigenous Australians. Considerable progress has been made in improving care for people living with RHD, but not in reducing overall RHD burden, despite more than two decades of collaborative research and RHD control programs. Recognising that a more
comprehensive approach is needed, researchers, service providers and community stakeholders are partnering in END RHD – an alliance of research, service and community organisations taking a three-pillared approach (research, evidence based policy recommendations (the “Endgame Strategy”) and advocacy) in the hope of eliminating RHD as a public health issue of importance in Australia.

Where are we at with treatment and control of group A streptococcal impetigo? 
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The global burden of impetigo at any one time is in excess of 160 million children, with impetigo featuring in the top 50 diseases in global burden studies. In tropical contexts, impetigo is predominantly driven by Group A streptococcus (GAS). Recent advances in treatment of impetigo include the use of short course oral cotrimoxazole and treatment of scabies with ivermectin to reduce the community burden of both scabies and impetigo. Translating this evidence into clinical practice through guidelines and clinical trials is ongoing in Australia and internationally.

The role of Streptococcus pyogenes and other beta-hemolytic streptococci in erysipelas and cellulitis. 
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Cellulitis is a diffuse bacterial skin infection with an incidence rate of around 200/100 000/year. Erysipelas is the superficial variant characterized by sharply demarcated erythema. Streptococcus pyogenes (GAS) and other β-hemolytic streptococci are the main causes of erysipelas. However, in deeper forms of cellulitis, the etiology has been more unclear. Previous studies using culture of biopsies have suggested a major role for Staphylococcus aureus, but recent molecular studies and clinical trials indicate that S. aureus found in cellulitis might often represent colonization.

We have combined serology, culture, and penicillin response data in prospective studies comprising 230 adult patients hospitalized with erysipelas or deeper cellulitis. Evidence of a streptococcal origin was found in more than 80% of the cases, including the majority of patients without typical signs of erysipelas and those with facial location, purulence, or comorbidities such as diabetes mellitus, β-hemolytic group C or G streptococci (GCS/GGS) were more commonly isolated than GAS. GCS/GGS infections were primarily located in the lower extremities and were associated with a rise in anti-streptolysin-O just as often as GAS. Most cases with S. aureus cultured from swabs had serological evidence of streptococcal infection. Together, the findings suggest that cellulitis essentially is a streptococcal disease.

Cardiovascular disease and overweight were associated with delayed treatment response, illustrating the importance of host factors in the course of streptococcal disease. We also frequently observed inflammation that increased during treatment and inflammation that persisted after treatment, reflecting the ambiguous relationship between infection and inflammation and possibly variable pathogen toxicity.

Cellulitis displays the importance and variability of host and pathogen factors in streptococcal disease.

The Novel Group A Streptococcus antigen SpnA combined with Bead-based Immunoassay Technology improves Streptococcal Serology for the Diagnosis of Acute Rheumatic Fever
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Streptococcal serological tests provide evidence of prior infection by Group A Streptococcus (GAS). These tests are crucial to the diagnosis of the post-infectious immune sequelae acute rheumatic fever (ARF) and glomerulonephritis since these syndromes develop several weeks after a GAS infection. Current tests measure anti-streptolysin-O (ASO) and anti-DNaseB (ADB) antibodies. Though widely used, these tests have limitations including a slow rate of decay in antibody response that can increase the risk of false positives in settings where GAS is endemic, and incompatible methodology, requiring the two assays to be run in parallel. In this study, the utility of a novel GAS antigen, SpnA, was assessed in a multiplex bead-based assay that also incorporated streptolysin-O and DNaseB. Recombinant streptolysin-O, DNaseB and SpnA were conjugated to polystyrene beads for measurement of serum antibody binding in a Cytometric Bead Array. Multiplex assay were run on sera samples collected from participants in three groups: ARF; ethnically matched healthy children; and healthy adults.

The ability of the antigens to detect a previous GAS exposure for ARF diagnosis was assessed using the 80th centile of the healthy children group as a cut-off (upper limit of normal). Using these experimentally determined cut-offs SpnA had the highest sensitivity at 88% compared to 75% for ASO and 56% for DNaseB. In conclusion, SpnA has favourable immunokinetics for streptococcal serology, and the combination of SpnA with ASO and ADB in a multiplex assay should improve the efficiency and accuracy of streptococcal serology.

Feasibility and safety of mass co-administration of azithromycin and ivermectin to control trachoma and scabies: the AIM study
Background/Aims: Unlike acute rheumatic fever (ARF), there is a lack of comprehensive national surveillance in New Zealand (NZ) for Rheumatic Heart Disease (RHD). The epidemiology, morbidity, mortality and costs of RHD are incompletely understood. We aimed to describe hospitalisations for RHD among patients aged 5–45 years admitted to Auckland City Hospital in 2012. We also sought to identify patients presenting with established RHD as compared to ARF.

Methods: Retrospective study of patients aged 5–45 years admitted to ADHB in 2012 with RHD. Cases identified via ICD codes for RHD (I05–I09). A detailed review of clinical, echocardiographic and laboratory records was performed.

Results: 144 hospitalisations in 104 individuals. Median age was 15 yrs. 54% were female. 96% were Pacific or Māori. 81% were NZ residents; 19% were from Pacific Islands nations. Common admission themes were for operative surgery (74/144, 51%), congestive cardiac failure (45/144, 31%), arrhythmia (33/144, 23%), pre-operative investigations (23/144, 16%), and complications of pregnancy (10/144, 6.7%). There were 4 thromboembolic events, 3 episodes of endocarditis and 1 RHD-related death. 12/104 (12%) patients hospitalised in 2012 presented with newly diagnosed RHD without prior history of ARF and 42/104 (40%) of the patients in the study had no prior documented history of ARF.
Conclusions: This study highlights the complications and substantial morbidity among young people with RHD in New Zealand. 40% of those studied had no prior diagnosis of ARF, highlighting the importance of early detection of RHD within New Zealand's ARF/RHD control programme.

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**Not a coiled coil: structural characterization of the hypervariable region of M3 and its role in collagen binding and biofilm**

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The multiple and often serotype-specific functions of M proteins are generally poorly understood in molecular terms, arguably due to a lack of structural information. All available evidence supports the idea of M proteins adopting an elongated dimeric, parallel coiled-coil conformation. This makes M proteins difficult targets for conventional structural biology. We combined EPR and NMR spectroscopy to map the hypervariable region (HVR) in full length M3 protein in solution and derive a structural model. M3-HVR was found to form a well-defined folded structure that deviates from coiled coil topology. This fold presents collagen-binding motifs associated with rheumatic fever in a structural context that depends on the dimeric state of the protein and is required for binding activity. Using triple-helical peptide libraries we identified M3-binding regions within the triple-helical domain of collagen II. The M protein:collagen interaction was found to mediate biofilm formation of M3 clinical isolates.

Our study reveals a surprising structural complexity of the M3 protein with implications for its function in rheumatic fever and biofilms. It also suggests that M proteins may be structurally more diverse than assumed.

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**Band 3 Protein Activation by Group A Streptococcus Streptolysin S Initiates Erythrocyte Hemolysis**

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Group A Streptococcus (GAS) is one of the most significant global pathogens, causing many common symptoms such as pharyngitis, cellulitis, and impetigo. It is also responsible for more severe diseases such as rheumatic fever, necrotizing fasciitis, and toxic shock syndrome. A landmark feature of GAS is its ability to produce a powerful toxin known as Streptolysin S (SLS). It has been widely held that SLS acts as a major contributing factor during invasive Group A Streptococcus infection through rapid membrane-based destruction of cells and tissues during the infection process. However, recent studies by our laboratory and others suggest that SLS may also play a more complex role in disease at physiologically relevant levels, including its ability to precisely target and inactivate host proteins. SLS has been identified to belong to a conserved family of small, ribosomally produced bacteriocin-like peptides that are structurally distinguished by the posttranslational installation of heterocycles on specific amino acid residues. Many of these related bacteriocins have defined cellular targets and have not been shown to function as general lytic agents of cellular membranes. Indeed, our recent work has shown that the mechanism by which SLS exerts its beta-hemolytic activity is via targeted disruption of the Band 3 anion transporter.

We show for the first time, using high-resolution live cell imaging, that SLS induces a dramatic osmotic change in red blood cells, leading to cell lysis. This osmotic change was characterized by the rapid influx of $\text{Cl}^-$ ions into the red blood cells through SLS-mediated disruption of the major erythrocyte anion exchange protein, band 3. Chemical inhibition of band 3 function significantly reduced the hemolytic activity of streptolysin S, and reduced pathology in an in vivo skin model of GAS infection.

Findings from our study will have important implications for developing targeted therapeutics against Group A Streptococcus as well as revealing novel strategies to treat and prevent Streptococcal disease.

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**Innovative Strategies for Antivirulence Therapeutics to Neutralize Streptolysin O-mediated Cytotoxicity**

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Group A Streptococcus (GAS) expresses a multitude of virulence factors with diverse modes of action that act to overcome the host's innate immune defense mechanisms. The pore-forming toxin (PFT) streptolysin O (SLO), a well-characterized virulence factor produced by nearly all GAS clinical isolates, is necessary and sufficient to promote epithelial injury and increased pathogenicity in animal models of invasive GAS infection. In the present work, we describe two companion approaches toward novel therapeutics that block SLO-mediated toxicities. The first is through the pharmacological presentation of red blood cell (RBC)-derived biomimetic nanoparticles ("nanosponges"), which can sequester SLO and block the ability of GAS to damage host cells, preserving innate immune function and increasing bacterial clearance in vitro and in vivo.

Nanosponge administration protected human neutrophils, macrophages and keratinocytes against SLO-mediated cytotoxicity, and increased GAS killing by the respective phagocytic cells. In a murine model of GAS necrotizing skin infection, local nanosponge administration was associated with decreased lesion size and reduced bacterial colony-forming unit recovery. This simple “decoy capture” platform represents a novel strategy that could prove a powerful
adjunctive therapy in severe GAS infections. Our second approach employs a CRISPR-based mutagenized haploid cell genetic screen to identify novel host factors involved in mediating SLO toxicity. Through this method, we have discovered gene candidates involved in cholesterol metabolism whose reduced expression promotes resistance to SLO cytotoxicity. Individual “hits” have been validated by CRISPR knockdown, and their viability as targets for drug intervention extended to models of SLO-mediated cytopathology and GAS infection.

### PmtA is a PerR-regulated ferrous iron efflux system of group A Streptococcus

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Streptococcus pyogenes (group A Streptococcus; GAS) is an obligate human pathogen responsible for a broad spectrum of human disease. GAS has a requirement for metal homeostasis within the human host and as such, tightly modulates metal uptake and efflux during infection. Metal acquisition systems are required to combat metal sequestration by the host, while metal efflux systems are essential to protect against metal overload poisoning. Here, we investigated the function of PmtA (PerR-regulated metal transporter A), a P1B,4 type ATPase efflux pump, in the invasive GAS M1T1 strain 5448. We reveal that PmtA functions as a ferrous iron [Fe(II)] efflux system. In the presence of high Fe(II) concentrations, the 5448ΔpmtA deletion mutant exhibited diminished growth and accumulated 5-fold higher intracellular Fe(II) compared to the wild-type and complemented mutant. The 5448ΔpmtA deletion mutant also showed enhanced susceptibility to killing by the Fe-dependent antibiotic, streptonigrin, as well as increased sensitivity to hydrogen peroxide and superoxide. We suggest that the PerR-mediated control of Fe(II) efflux by PmtA is important for bacterial defense against oxidative stress. PmtA represents an exemplar for a Fe(II) efflux system in a host-adapted Gram-positive bacterial pathogen. The interplay of Fe(II) and other metals within this system will be discussed.

### Complement independent activity of C5a peptidase contributes to Streptococcal pathogenesis and impairs the host innate immune response

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C5a peptidase (ScpA) is widely conserved amongst the streptococcal species, and plays a critical role in pathogenesis within the host. The only function of ScpA reported to date is the cleavage and inactivation of the complement anaphylatoxin C5a. Using complement deficient mice we established a novel complement-independent role for ScpA in promoting systemic infection, a phenotype we further characterised using a combination of in vitro assays and in vivo murine models. Using Group A streptococcus as a model species, we generated isogenic ScpA knock-out strains in serotypes M1 and M89. ScpA promoted bacterial resistance to clearance early during infection in a murine soft-tissue infection model in both wildtype mice and those lacking complement component C5. Unexpectedly ScpA also promoted systemic dissemination via the bloodstream in mice lacking both C3 and C5. We went on to characterise ScpA as an adhesin, sufficient to mediate bacterial attachment to human epithelial cells and both human and murine endothelial cells, and conclude that this function is critical for streptococcal virulence in vivo. We also report complement components C3a and C3 as novel substrates for ScpA, cleavage of which resulted in functional inactivation of both proteins, impairing the innate immune response by inhibition of C3 deposition on the bacterial surface, and effective neutrophil activation and function. Taken together we demonstrate that during infection ScpA promotes streptococcal pathogenesis via multiple complement independent and dependent mechanisms, which collectively dramatically impair host immune control during infection.

### Characterization of a novel tyrosine kinase and tyrosine phosphorylation in Streptococcus pyogenes

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The important role of Ser/Thr kinase and phosphatase in Streptococcus pyogenes (group A Streptococcus, GAS) physiology and pathogenesis is now well-established. Since GAS genomes do not contain an obvious gene encoding a typical bacterial-type tyrosine kinase (BY-kinase) but contain an orphan gene encoding protein Tyr-phosphatase (SP-PTP), it is presumed that Tyr-phosphorylation does not exist in GAS. Hence, its importance in GAS pathogenesis has remained completely unexplored. Our previous report demonstrating the ability of SP-PTP to dephosphorylate Ab1-tyrosine kinase-phosphorylated MBP and identification of phosphorylated Tyr101-residue in the autoprophosphorylated SP-STK prompted us to hypothesize that a novel putative tyrosine kinase and Tyr-phosphorylation exist in GAS. To identify a genuine Tyr-protein kinase, we performed a genome-wide search of kinases possessing a classical Walker motif. To that end, we first identified and purified a genuine but a non-canonical tyrosine kinase M5005_Spy_1476, a ~17 kDa protein (153 aa) (SP-TyK), which autophosphorylated in the presence of ATP. Both in vitro and in vivo phosphoproteomic analyses revealed two key phosphorylated tyrosine residues located within the catalytic domain of SP-TyK. An isogenic ΔSP-TyK mutant derived from the M1T1 strain grew poorly in THY-medium, and displayed defective cell division and long chains with multiple parallel septa, often resulting in aggregates. Additionally, the mutant displayed an altered Tyr-phosphorylated protein profile and attenuation of virulence in the mouse infection model. The sp-tyk-complemented strain
restored the lost functions. Together, these results indicated that SP-TyK-mediated post-translational modifications at Tyr-residues of certain targets directly play a critical role in GAS pathophysiology, morphogenesis, and pathogenesis.

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Group B Streptococcus in the urinary tract: infectious mechanisms and elements of pathogenesis
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Streptococcus agalactiae causes approximately 160,000 cases of urinary tract infection (UTI) in the United States annually. These infections present in various forms, including acute cystitis and pyelonephritis, as well as asymptomatic bacteruria (ABU). The host-pathogen interactions that occur during S. agalactiae UTI and mechanisms of pathogenesis are increasingly being defined based on in vitro and in vivo models of human infection, in addition to clinical case studies. In this presentation, I will provide an overview of these infections, including mechanisms of disease pathogenesis that stem from the interactions between bladder tissue and S. agalactiae. The dynamics of infection-induced cytotoxicity in human bladder cells, and the contributions of streptococcal virulence factors, including β-hemolysin/cytolysin (β-H/C), capsular polysaccharide, protein adhesins, and the global virulence regulator CovR, will be discussed. The biology of ABU-causing S. agalactiae strains, including how these bacteria might colonize the urinary tract by exploiting metabolic pathways that are novel for bacterial disease pathogenesis will also be discussed.

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Group B Streptococcus in sub-Saharan Africa
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Sub-Saharan Africa (sSA), accounts for 3.1 million of the 6.3 million child deaths worldwide each year, around a million of which are in neonates (0-27 days). The contribution of Group B Streptococcus (GBS), a well-established leading cause of early-onset GBS disease in high income settings, has, however, been uncertain in sSA. Recent data from Kenya suggest that GBS is an important pathogen, both in newborns and in stillbirths. This reflects a spectrum of early-onset ascending GBS infection resulting in stillbirth or early onset GBS disease, which through the use of molecular techniques (whole genome sequencing) is evidenced. Combining epidemiological and molecular analyses brings new insights into the clinical and molecular epidemiology of GBS, and helps understand why study findings differ. This has informed the methods for forthcoming estimates of the burden of GBS disease in pregnant women, stillbirths and infants worldwide. Understanding this burden is critical in establishing the case for GBS vaccine development.

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Genomic investigation of the rise in incidence of Group B Streptococcus neonatal invasive disease in the Netherlands
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GBS is a major cause of invasive infections in newborn babies. In the Netherlands, the incidence of GBS neonatal disease increased by 60% between 1987 and 2011, despite the introduction of disease prevention guidelines in 1999. This was associated with rise in number of cases caused by isolates belonging to clonal complex 17 (CC17). To investigate the changing genetic epidemiology of GBS neonatal infections in the Netherlands we whole genome sequenced isolates submitted to the Netherlands Reference Laboratory for Bacterial Meningitis between 1987 and 2016. A total of 1331 isolates derived from early and late-onset infections were analysed. The population consisted of 5 lineages representing CC17, CC19, CC23, CC10 and CC1. There was an increase in relative prevalence of CC17 and CC23 while CC19 remained stable. We inferred phylogenies for individual lineages and observed temporal trends for defined sub-lineages. The CC17 phylogeny revealed a major sub-population that increased rapidly in prevalence over time becoming dominant in early 2000s. Bayesian analysis of past population dynamics showed a major expansion in effective population size of this clade in mid 1990s. In contrast CC19 revealed a dropping prevalence of isolates representing a major clade while CC23 showed increasing frequency of isolates from a dominant cluster, which experienced population expansion in mid 1980s. Preliminary analysis shows emergence of a phage in GBS population in the mid 1990s, carrying a novel surface protein gene, that has spread rapidly in CC17 and was associated with clonal expansion events.

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Dual activities of group B Streptococcus capsular sialic acid provide resistance to platelet-mediated antimicrobial killing
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Group B Streptococcus (GBS) causes bacterial sepsis and meningitis in newborns. Platelets have important functions not only in thrombosis, but also in modulating inflammatory responses. However, the role of platelets in
innate immunity to bloodborne bacterial infection remains mostly unexplored. We found that purified human platelets rapidly kill Staphylococcus aureus, whereas GBS exhibits remarkable resistance. All human GBS serotypes express a polysaccharide capsule with terminal α2,3-linked sialic acid residues mimicking a common epitope present on human cells. Interestingly, we found that platelets express high surface levels of the inhibitory Sia-recognition immunoglobulin superfamily lectins (Siglecs). Our prior published work has shown that GBS capsular sialic acid binds inhibitory Siglecs to block phagocyte activation. Here we show platelets bind wild-type (WT) GBS more avidly than an isogenic sialic acid-deficient mutant strain ΔneuA. Furthermore, the GBS ΔneuA mutant activated platelet degranulation more strongly than WT GBS, with evidence of engagement and signaling through Siglecs. Simultaneously, the GBS ΔneuA mutant was killed by human platelets and releasates or platelet-derived antimicrobial peptides than the WT strain. In mouse IV challenge, antibody-mediated platelet depletion increased susceptibility to platelet-sensitive S. aureus, but did not change susceptibility to platelet-resistant GBS. Mice lacking the inhibitory Siglec-E had increased platelet activation and GBS clearance by avoiding sialic acid mimicry-induced platelet suppression. Thus GBS sialic acid has dual roles in evading platelet antimicrobial immunity: inhibiting platelet activation through Siglecs and conferring resistance to platelet-derived antimicrobial components. We report the first bacterial virulence factor for evasion of platelet-mediated immunity.

The two-component system response regulator LytR influences Group B Streptococcal colonization and disease

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Streptococcus agalactiae (Group B Streptococcus, GBS) is a commensal bacterium that colonizes healthy adults asymptptomatically and is a frequent inhabitant of the vaginal tract in women. However, in immune-compromised individuals, GBS may transition to an invasive pathogen and, despite currently recommended intrapartum antibiotic prophylaxis for GBS-positive mothers, GBS remains the leading cause of neonatal sepsis and meningitis. To adapt to various host environments encountered during its disease cycle, GBS possesses multiple two-component regulatory systems (TCS). Several TCS have been shown to affect GBS virulence and we hypothesize that the previously uncharacterized LytSR TCS in GBS may also play a role pathogenesis. We have found that infection with the ΔlytR mutant GBS strain lacking the LytR transcriptional regulator causes increased secretion of inflammatory cytokines from human endothelial and epithelial cells. Additionally, the ΔlytR mutant is hypervirulent in our murine hematogenous meningitis model. Interestingly, while wild type GBS can colonize and persist in the mouse vaginal tract, the ΔlytR mutant is rapidly cleared. Further characterizing LytR signaling in GBS infection can provide insight into how the bacteria regulates its interaction with the host immune system and the effects of the host inflammatory response on disease progression. Ongoing RNA-sequencing studies are underway to identify downstream gene targets of the LytR regulator in order to better understand the changes in gene expression that occur during the transition between colonization and disease states. Ultimately, uncovering how LytR signaling affects bacterial persistence and virulence may inform the development of more effective therapies to prevent GBS infections.

Do the benefits of universal group B Streptococcus (GBS) screening to prevent early-onset GBS disease (EOGBS) outweigh the harms? An evidence review for the UK National Screening Committee (NSC)

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Background: In the UK, there is much debate around the prevention of EOGBS, with regular calls for the introduction of universal antenatal screening. This review, undertaken for the UK NSC, summarised recent evidence to assess GBS screening against key criteria on epidemiology, test accuracy, and screening and treatment effectiveness.

Methods: We searched Medline, Embase, and Cochrane databases as well as grey literature from surveillance reports. Participants were pregnant women ≥35 weeks and neonates <7 days. The intervention was selective recto-vaginal culture at 35-37 weeks followed by intrapartum antibiotic prophylaxis (IAP) for those found positive. Reviewers independently conducted study selection, data extraction, and quality assessment.

Results: 73 studies were included from 6,287 references. EOGBS affects 0.57 per 1,000 livebirths, and has a case fatality rate of 5.2% in the UK. Screening would be offered to approximately 718,126 term pregnant women. Approximately 150,800 (21%) would be colonised with GBS at 35-37 weeks and offered treatment, however, around 40,716 (27%) would change to negative by labour. Of the GBS-colonised women at 35-37 weeks, approximately 0.2% would have a neonate with EOGBS (350/150,800). Therefore, many women would be overtreated. Not only are the consequences of widespread IAP are unknown, the observational evidence on the
effectiveness of GBS screening is limited.

Conclusion: EOGBS is an important health condition, but a more refined prevention approach is required. Antenatal culture screening is inaccurate at identifying mothers at risk of having a neonate with EOGBS, and currently the balance of harms and benefits of screening cannot be quantified.

Host genetic susceptibility to rheumatic heart disease
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Rheumatic heart disease (RHD) remains a leading cause of cardiovascular morbidity and mortality among children and young adults across much of the developing world. Current understanding of the disease’s pathogenesis is unrefined compounding limited preventative strategies.

We recently completed a genome-wide association study (GWAS) of RHD susceptibility in 2,852 individuals recruited across Oceania and identified a novel susceptibility signal in the immunoglobulin heavy chain (IGH) locus. We found that nonsynonymous IGHV4-61 variants tagged the IGHV4-61*02 allele and demonstrated that each copy of IGHV4-61*02 was associated with a 1.4-fold increase in the risk of RHD at genome-wide significance.

In addition, we have now undertaken a second GWAS in 854 individuals recruited in Lucknow, Northern India. In combining these data with those from 309 individuals of Fijian Indian ancestry, we not only confirm our earlier IGH finding but we further identify a signal at genome-wide significance located in the human leukocyte antigen (HLA) class III region. This appears to comprise two independent signals, the first spanning HLA class I (HLA-B) and the second HLA class II (HLA-DQA1/B1).

Overall, our study provides promising insight into the pathogenesis of this devastating disease and is a stepping-stone towards large-scale collaborative meta-analyses that will facilitate translational therapeutic discovery.

The AFROStrep Registry antad Biorepository for Streptococcus pyogenes: the first year in South Africa
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Introduction: The African group A streptococcal infection registry (the AFROStrep Study) was established to collect data on group A β-haemolytic Streptococcus (GAS), a gram-positive bacterium also known as Streptococcus pyogenes in Africa, where surveillance data on GAS are largely lacking. Launched in 2016 with a pilot project in South Africa, it aimed to provide an understanding of GAS disease on the continent. We briefly summarise below, progress on the project to date, including some preliminary results.

Methods: The AFROStrep Study is a collaborative multi-centre study of clinical, microbiological, epidemiological and molecular characteristics for GAS infection in Africa; the participating regions in Africa are shown in the figure. The AFROStrep registry comprises two components: (1) active surveillance of GAS pharyngitis cases from sentinel primary care centres (non-iGA S), and (2) passive surveillance of non-invasive and invasive GAS disease (iGAS) from diagnostic microbiology laboratories. Data were prospectively collected on a standardised questionnaire, using established case definitions. Samples, one per patient, were inoculated in sheep blood agar plates, and processed according to standard laboratory protocols. A biorepository was established to house laboratory-confirmed GAS isolates from patients. Isolates were subjected to DNA extraction to allow for emm gene characterization by molecular methods and cryo-preservation for long-term storage.

Results: The passive surveillance arm of AFROStrep commenced on 01 March 2016, with 561 isolates collected from the diagnostic laboratory over 18 months; mean age, 34 years (SD 19.4 years), % Male = 64%). Isolation sites of samples were reported as being from pus swabs n=185 (33%), aspirates n=114 (20.3%), deep tissue n=59 (10.5%); abscess n=33 (5.9%), blood n=30 (5.4%), and CSF n=6 (1%); not specified or designated as other n=134 (23.9%). Invasive samples made up 48% of the isolates with blood samples occurring mainly in patients with joint-related conditions. Of those sequenced, the emm type distribution of GAS was dominated by types 76 (17.44%), 53 (9.3%), 44, 49 and 80 (7%, respectively). Compared with non-invasive isolates, invasive isolates were dominated by types 44 (5 invasive isolates vs 1 non-invasive isolate), 53 (5 vs 3) and 76 (9 vs 6). Dominant among non-invasive isolates were types 49 (5 non-invasive vs 1 invasive), 81 (4 vs 1), 184 (3 vs 1). Compared with studies elsewhere, emm types associated with invasive disease, were absent or single isolates. Three of the dominant emm subtypes isolated in our cohort were not amongst the putative 30-valent GAS vaccine currently under development.

Discussion/Conclusion: The AFROStrep Study has managed to create a platform for epidemiological analysis of
and research into streptococcal disease in Africa. The early results indicate rates of isolation of GAS higher than was anecdotally believed. We were able to provide, for the first time, the GAS emm subtypes in isolates from Africa and showed, a noticeable difference in the prevalent emm subtypes. Nevertheless, we acknowledge the low numbers of recruitment to date; definitive conclusions will have to await further molecular analysis of the remaining GAS isolates. Of concern, from the point-of-view of prevention, is that the current candidate vaccine against GAS, mostly based upon the M protein, does not include dominant emm subtypes isolated in our cohort. We believe that the AFROStrep study will help quantify the burden of GAS infection, document the prevalent strains presenting in the respective communities and, provide information that could inform the development of locally sensitive guidelines, future research programmes and policy development, all of which have the potential to improve the management of individuals with GAS infection and GAS related diseases.

Measuring adherence to secondary prophylaxis for acute rheumatic fever and rheumatic heart disease: which indicators predict ARF recurrences and can be easily monitored?
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Background: Acute rheumatic fever (ARF) and rheumatic heart disease (RHD) cause significant burdens worldwide. Many RHD control strategies depend on secondary prophylaxis (SP) – regular long-term antibiotics - to prevent disease progression, but few studies have examined associations between adherence and clinical outcomes. Adherence to SP is typically reported as the percent of doses administered or, increasingly, as ‘days at risk’ (DAR). We sought to identify which measures of adherence predict ARF recurrences and are practical to monitor.

Methods: Associations between recurrences and adherence (percent, total annual DAR, annual maximum continuous DAR) were analysed using data from Australia’s Northern Territory. Logistic regression was used to estimate odds ratios (OR) for recurrence.

Results: ‘Non-adherent’ people (<80% doses) were approximately two and a half times more likely to have a recurrence than ‘adherent’ people (OR: 2.74, (95%CI: 1.22 – 6.16), p<0.05). People with ≥70 DAR were six times more likely to have a recurrence than people with <70 DAR (OR: 6.20 (95% CI: 1.40 – 27.56), p<0.05); people with >42 continuous DAR were twice as likely to have a recurrence than people with shorter DAR durations (OR: 2.17 (95% CI: 1.15 – 4.12), p<0.05).

Conclusions: We show for the first time that increased adherence to SP is associated with reduced risk of ARF recurrence in Australia. Several adherence measures significantly predict recurrences. Total DAR could be adopted for patient- and clinic-level monitoring. At jurisdiction level, percent adherence could be used, however programs should also report recurrence rates as a performance indicator.

The New Zealand familial echo study: High prevalence of Rheumatic Heart Disease in siblings of Acute Rheumatic Fever cases supports active case finding in family members
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BACKGROUND
The risk of Rheumatic Heart Disease (RHD) in relatives of Acute Rheumatic Fever (ARF) patients is poorly defined. This study aimed to determine RHD prevalence in first degree relatives of ARF patients using echocardiography.

METHODS
Between 2014 – 2016, ARF cases were recruited from Auckland, New Zealand. Parents and siblings ≥4years were offered echocardiography. Echocardiograms were reviewed by a panel of three cardiologists and interpreted according to WHF 2012 criteria. RHD prevalence in family members was compared to reference population rates.

RESULTS
70 index cases were recruited. The median age of cases was 11 years (range 4 – 15 years). 55/70 (79%) were Pacific peoples and 15/70 (21%) were Māori.

Echocardiography was performed in 94 parents and 132 siblings. 2 siblings had Definite RHD and 7 had Borderline RHD. 1 additional sibling had a known history of ARF/RHD. Echocardiography found 3 parents with Definite RHD and 3 with Borderline RHD. 2 parents had known clinically detected RHD.

Prevalence of RHD (Definite + Borderline) in siblings was 75 per 1,000 (95% CI 40 – 134 per 1,000) compared to 36 per 1000 (95% CI 30 – 42 per 1000) in NZ children from high-risk RF populations (p 0.02).

CONCLUSIONS:
RHD prevalence in siblings of ARF cases is approximately twice the background rate. When a child is diagnosed with ARF, consideration should be given to offering siblings echocardiography. Where echocardiography resources
is rheumatic heart disease screening by non-expert operators feasible and accurate? Lessons from the Fiji studies.
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Introduction:
Echocardiographic screening for rheumatic heart disease (RHD) has the potential to detect subclinical cases for secondary prevention, but is constrained by inadequate human resources. Training non-expert health workers to perform focused cardiac ultrasound (FoCUS) may be a feasible strategy to enable mass screening. We aimed to investigate the inter-rater reliability and diagnostic accuracy of FoCUS performed by briefly-trained health workers.
Methods:
Following an encouraging pilot study, we developed a highly-defined, eight-week training program in FoCUS for RHD. Trained nurses performed FoCUS on schoolchildren aged 5 to 15 years. An echocardiographer also performed a standard echocardiogram. A cardiologist reported all studies. The diagnostic accuracy of the index test (nurse measurement of valvular regurgitation) was compared to the reference standard (diagnosis by the cardiologist, based on the echocardiographer study). The assessment of the presence of regurgitation by the nurse and cardiologist was compared.
Results:
Seven nurses completed the training. 2004 children were enrolled. The diagnostic accuracy of the screening test (area under ROC curve) was 0.89 (95%CI, 0.83 – 0.94). The sensitivity and specificity at the primary cut-off were 84% and 86%. There was substantial agreement (κ=0.75) on the presence of regurgitation. Variation between nurse operators was noted.
Conclusion:
Briefly-trained health workers can reliably assess the presence and extent of valvular regurgitation. The diagnostic accuracy of this screening method is high, and warrants further investigation. The diagnostic burden of screen-positive cases may present a challenge in many resource-limited settings. Quality assurance measures are important to ensure acceptable performance of all operators.

Robust Evidence Finally Available for Prevention of First Presentation Acute Rheumatic Fever in a Community Setting: a school based intervention.
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BACKGROUND: Robust evidence is lacking for community initiatives to prevent first presentation acute rheumatic fever (ARF) by group A streptococcal (GAS) pharyngitis treatment. Significant NZ government investment centred on school clinics.
METHODS: We measured the effect of introducing a sore throat clinic program on first presentation ARF into 61 year 1-8 schools with students aged 5-13 years (population ~ 25 000). In Auckland, NZ. The study period was 2010-2016. A generalised linear mixed model investigated ARF rate changes before and after the staggered introduction of school clinics. Nurses/lay workers treated culture-proven GAS sore throats (including siblings) with 10 days amoxicillin. ARF cases were identified from a population-based secondary prophylaxis register. Annual pharyngeal GAS prevalence was assessed in a subset.
RESULTS: ARF rates (5-13 years) dropped from 88 (95% CI 79, 111)/100,000 pre clinics to 37 (95% CI 15, 83)/100,000 after 2 years of clinics, a 58% reduction.
No change in rate was demonstrated prior to the introduction of clinics (p=0.88, incidence risk ratio (IRR) for a one year change 0.98 (95% CI 0.63, 1.52)) but there was a significant decrease of first presentation ARF rates over time following the introduction of the sore throat program (p=0.008, IRR 0.61 (95% CI 0.43, 0.88)).
Pharyngeal GAS cross sectional prevalence fell from 22.4% (16.5, 30.5) pre intervention to 11.9% (8.6, 16.5) and 11.4% (8.2, 15.7) one and two years later (p=0.005)
CONCLUSIONS: ARF declined significantly following school-based GAS pharyngitis management using oral amoxicillin paralleled by a decline in pharyngeal GAS prevalence.
Genetic basis of serotype-specific phenotypic variation in the group A Streptococcus
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Epidemiological studies have identified non-random associations between certain group A Streptococcus (GAS) serotypes and disease phenotypes. For example, serotype M3 isolates are associated with particularly severe and lethal invasive infections, serotype M18 isolates are associated with outbreaks of acute rheumatic fever, and serotype M28 isolates are associated with cases of puerperal sepsis. In my talk I will review my lab’s work identifying the molecular basis behind why M3 GAS isolates are associated with severe invasive infections. We have identified that, since at least the 1920s, there has been a rewiring of regulatory networks in M3 isolates such that they have a serotype-specific virulence factor expression profile. In total we have identified four regulatory systems that, due to mutation or sequence variation, have altered activity in M3 isolates. I will discuss the regulatory and virulence consequences of each system, and how they impact disease potential. Insights into the functionality of these systems will also be provided. Our data are consistent with alterations in gene expression being the driving force behind the association of serotype M3 GAS isolates with particularly severe invasive infections.

The utility of Whole Genome Sequencing in Group A Streptococcus outbreaks in England – a perspective from the reference laboratory
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Streptococcus pyogenes infection may have serious outcomes for patients and clusters and require management and control of onward transmission. RVPPBRU routinely performs emm typing of isolates from invasive disease and also superficial isolates linked to clusters. Whole Genome Sequencing (WGS) is increasingly being requested by public health professionals during outbreak investigations. RPVBRU investigated 91 GAS/iGAS transmission events queries between 2016/2017, where two or more isolates were received for typing. Surveillance data for iGAS indicates that the five most common emm types in England are emm 1.0, 89.0, 12.0, 28.0 and 94.0. Interestingly, they were responsible for 36 of the 91 clusters (39.6%; 10, 9, 6, 6 and 5 clusters, respectively). Four prolonged outbreaks with iGAS/GAS strains (two emm 1.0; one emm 5.23; one emm 11.0) were investigated. The emm 1.0 strains from two care homes and one shared staff member displayed 0-2 SNPs difference (average SNP variation between sporadic, contemporaneous emm 1.0 isolates was 29.8), suggesting a transmission event. Analysis of another emm 1.0 cluster in a maternity unit revealed that the isolates were not related (20 and 24 SNPs difference).
Phylogenetic analyses of the emm 5.23 and emm 11.0 clusters revealed that all cluster-related isolates co-located within the same clade distinct from contemporaneous sporadic isolates of the same emm type, suggesting that persistence rather than strain reintroduction was responsible for these two cases. These results demonstrate that the implementation of WGS analysis is a viable alternative and provides finer resolution to outbreak investigations.

Recombination-related remodeling in the group A streptococcal genome
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Whole genome sequencing of group A Streptococcus (GAS) populations is challenging the traditional view of the GAS genome consisting of a stable unvarying core genome and an accessory genome controlled by mobile genetic elements (MGEs). We, and others, have previously demonstrated that horizontal gene transfer in the core genome can occur, leading to the emergence of new variants that drive GAS population shifts. Genomic analysis of GAS isolates dating from the pre-antibiotic era and modern isolates identified hot spots for recombination in a number of different emm-types. One identified region was the NADase/SLO locus. This locus has been associated with the success of modern emm1 and the new variant of emm89 lineages, where recombination events have led to variants with high NADase and SLO expression. Recombination of this locus is not however restricted to emm1 and emm89, and has also produced lineages of emm28, emm87, emm75 and emm76 with enhanced toxin production. In some cases, like emm89, these lineages are also acapsular. A modern UK emm75 lineage carried an NADase/SLO locus almost identical to that found in modern emm1 and the emergent emm89 lineages, likely gained through recombination as historical emm75 strains had a different NADase/SLO locus with features of low toxin expression. The dominance of the NADase/SLO locus among recombination hot spots underlines its likely role in the evolution of GAS in the modern era, in response to environmental and host pressures that are as yet unknown but could include antimicrobials and disinfectants.

What comes after M Conservation of the core mga regulon of Group A streptococcus
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Epidemiological studies have identified non-random associations between certain group A Streptococcus (GAS) serotypes and disease phenotypes. For example, serotype M3 isolates are associated with particularly severe and lethal invasive infections, serotype M18 isolates are associated with outbreaks of acute rheumatic fever, and serotype M28 isolates are associated with cases of puerperal sepsis. In my talk I will review my lab’s work identifying the molecular basis behind why M3 GAS isolates are associated with severe invasive infections. We have identified that, since at least the 1920s, there has been a rewiring of regulatory networks in M3 isolates such that they have a serotype-specific virulence factor expression profile. In total we have identified four regulatory systems that, due to mutation or sequence variation, have altered activity in M3 isolates. I will discuss the regulatory and virulence consequences of each system, and how they impact disease potential. Insights into the functionality of these systems will also be provided. Our data are consistent with alterations in gene expression being the driving force behind the association of serotype M3 GAS isolates with particularly severe invasive infections.
Whole-genome sequencing analysis of group A streptococcal isolates associated with an invasive disease outbreak in Alaska

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Background
In 2016, an outbreak of invasive Group A Streptococcus (iGAS) infections caused by a rare M protein gene (emm) subtype, emm26.3, was identified in Alaska, USA. We used whole-genome sequencing (WGS) analysis to describe relatedness of outbreak cases and to characterize genomic features of these iGAS isolates.

Methods
Case definition: isolation of GAS emm26.3 from a normally sterile site in an Alaska resident from February to November 2016. Paired-end, 250-bp WGS was performed using a MiSeq platform on 32 case isolates. Short reads were assembled using VelvetOptimiser2.2.5. Single Nucleotide Polymorphisms (SNPs) were called from assemblies using kSNP3.0. Reads were also analyzed by an in-house bioinformatics pipeline to detect important gene features.

Results
The 32 case isolates differed, on average, by 2 SNPs (range, 0-6) based on a total of 16 SNP loci across approximately 1.7 Mb shared genome. Four sets of isolates were genomically indistinguishable (n=2, 5, 13, and 3) and were correlated with temporal/geographic groups of cases. Five independent SNPs (all non-synonymous) were located in the regulator operon covRS. Eleven SNPs (7 non-synonymous) affected 11 other genes. Pipeline analysis identified identical gene features across all isolates, including pilus type T28, MLST type ST745, no detectable resistance genes, and positive for genes hasA, prtf2, sfb1, smeZ, speC, and speG.

Conclusions
WGS analysis confirmed the clonal nature of an emm26.3 iGAS disease outbreak, revealing likely transmission clusters. Within the outbreak lineage, virulence regulator covRS showed a disproportionately high number of mutations, which might be partially responsible for increased pathogenicity.
Island populations that are relatively small and remote are inherently interesting from an infectious disease evolution perspective. Geographic isolation and sparse populations can limit transmission and enhance independent evolutionary processes. This can provide unique insights into the dynamics of host-pathogen interaction. The Faroe Islands (population ~50,000) includes 17 inhabited islands located in the North Atlantic between Iceland, Scotland and Norway. With the exception of Torshavn (population ~20,000), only eight communities have >1,000 residents.

Methods:
Isolates from pharyngitis or asymptomatic carriage (n = 125, collected in 2009-2010), or invasive infections (n = 37, collected between 1998 and 2016) were studied. Genomes were sequenced using Illumina technology and analyzed using common bioinformatic methods.

Results:
Strains were sequenced to ~150 fold-coverage. Among the noninvasive isolates, 11 emm-types (and 11 multi-locus sequence types, MLSTs) were identified, the majority (>90%) being emm1, 2, 4, 6, 22, and 89. Thirteen emm-types (and 15 MLSTs) were identified among the invasive strains, most (>70%) being emm1, 3, 4, 12, 28, and 89. These emm-types are common in other developed countries of North America and Europe. Antibiotic resistance frequency was low (<7%) and was largely limited to tetracycline resistance in emm22 noninvasive isolates. Genome sequences were compared with and are presented relative to isolates of the same emm-types collected from other North American and European countries.

Conclusion:
Our study is the largest genomic analysis of a bacterial pathogen causing infections in the Faroe Islands. The resulting data provided new information about S. pyogenes infections in these islands.

Genetics, Genomics and the role of group G streptococcus in human disease

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Historically group G streptococcus (Streptococcus dysgalactiae subsp equisimilis, SDSE) has been considered a commensal organism, only causing opportunistic infections. Multiple studies have now shown SDSE to be the cause a spectrum of disease similar to group A streptococcus (GAS), including pharyngitis, invasive disease and PSGN. Circumstantial evidence also exists for a role for SDSE in rheumatic fever. In contrast to GAS, our knowledge of the epidemiology, molecular genetics and pathogenesis of SDSE is limited. Here we describe a comparative genomic analysis of 240 SDSE genomes, representing 40 different SDSE emm-types, collected from different geographic regions. Phylogenetic analysis divided the genomes into 18 different genomic clusters. While individual emm-types and genome clusters were largely concordant, evidence for recombination involving this gene was apparent. Our data also show a strong correlation between genome cluster and the expression of group C or group G carbohydrate in SDSE. When compared to GAS, the genome structure is largely consistent, with differences largely attributable to the presence of mobile genetic elements. Together this data suggests underscore the value of population genomic analyses in providing new insights into the biology of SDSE.

Serum antibodies to DRSG protein from Streptococcus dysgalactiae subsp equisimilis increase the risk for chronic kidney diseases: parallels with IgA nephropathy.

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Streptococcus dysgalactiae subsp equisimilis (SDSE) is closely related to S. pyogenes. Although SDSE is generally considered as commensal it could cause diseases, and the disease spectrum is similar to that of S. pyogenes. Some isolates of SDSE expresses a secretory protein called DRSG, which is similar to SIC and DRSG proteins expressed by some M types of S. pyogenes. SIC, DRG and DRSG proteins are proline-rich and have PXXP motifs. The main role of these protein antigens is to inhibit the function of antimicrobial peptides.

We earlier showed that antibodies to SIC or DRG are associated with post-streptococcal glomerulonephritis (PSGN). More recently we showed anti-SIC antibodies are also associated with chronic kidney diseases (CKD). Similar studies with DRSG revealed that significantly higher proportion of PSGN and CKD patients had antibodies to DRSG than their corresponding age-matched control subjects. We infer that infection with DRSG producing SDSE early in life is a risk factor for CKD in later years. We strongly propose sero-surveillance of people who have been infected with SIC, DRG and DRSG producing Streptococci.

We draw parallels with the most common cause of glomerulonephritis, IgA nephropathy, wherein the patients show elevated levels of antibodies to the proline-rich hinge region of IgA, and the antibody-IgA complex was found in the mesangium.
Novel genetic determinants of Streptococcus dysgalactiae subspecies equisimilis

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Streptococcus dysgalactiae subsp. equisimilis (SDSE) is currently recognized as a causative agent of about 60% of group C and G streptococcal infections.

Goal of this study was the search of novel SDSE genetic determinants acquired from other bacterial species. Four strains isolated from healthy children in Vietnam in 2013-2014 were selected and cultured in THY broth. Genomic DNA was isolated by phenol/chloroform extraction. Libraries were prepared using Nextera XT Kit and sequenced at Illumina MiSeq (USA) using MiSeq reagent Kit v3. The reads were edited using SPAdes and Genomics Workbench v8 (CLCBio, USA). The contigs were analyzed using BLAST software.

Numerous DNA fragments previously undescribed for SDSE were revealed. Most of them contained genes of migrating genetic elements such as S. pyogenes A25 and phi-m46.1 bacteriophage genes, S. agalactiae and E. faecalis transposon Tn916 genes, plasmid genes, S. pyogenes ICESP1108 element genes, etc. Integrate and recombinase genes involved in recombination similar to those of S. agalactiae, S. pyogenes, S. suis, S. pneumoniae, and genes involved in transcriptional regulation of streptococci (XRE, Cro/Ci family genes, DNA-binding protein genes, etc.) were discovered. For the first time the tetS, tetT and IsaE, InuB genes encoding resistance to tetracycline and lincosamides, respectively, were revealed in SDSE. Several genes of animal pathogens (S. suis, S. equi) were also found in SDSE.

Bioinformation analysis suggested an extensive genetic exchange between SDSE and other gram-positive cocci. Given that horizontal gene transfer is driving force of evolution, an emergence of novel highly virulent SDSE clones is expected.

Four consecutive multicenter external quality assessments to assess the quality of molecular amplification methods for the detection of vancomycin resistant enterococci

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Objectives:
The QCMD vancomycin resistant enterococci (VRE) pilot EQA study was introduced in 2013 and repeated yearly to assess the ability of laboratories to correctly detect and characterize vanA, vanB and vanC genes in VRE by molecular methods.

Methods: EQA panels contained samples with VRE at dilutions between 1.0x103 and 1.0x107 CFU/ml, one mixture of vanB and vanC positive VRE, vancomycin susceptible enterococci and an Enterococcus negative sample.

Results: The number of participants increased from 44 laboratories in 16 countries to 65 laboratories in 21 countries. The majority of results were generated using in-house developed assays: 74.4% (29/39), 70.3% (26/37), 63.3% (31/49) and 55.2% (37/67) from 2013 to 2016 . This represents an increase in the proportion of commercial assays used in these EQA studies from 25.8% in 2013 to 44.7% in 2016.

In all EQA’s participants could correctly determine the presence of vanA in ≥ 92.3%. The correct detection of vanB increased from 71.8-87.2% of datasets in 2013 to 96.6-95.5% of datasets in 2016. Detection and characterization of vanC genes ranged from 14.3% to 35.9%. False positivity rate decreased from 7.7% in 2013 to 1.5% in 2016.

Conclusion: The majority of participating labs returned results generated by in-house PCRs but commercially available kits are increasingly used. Most participants were able to correctly characterize the vanA or vanB vancomycin resistance genes. The detection of the vanC genes is not included in the majority of commercially available or in-house tests explaining the poor results on EQA samples containing vanC genes.

Beneficial health outcomes following use of probiotic Streptococcus salivarius K12

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Streptococcus salivarius is a commonly-occurring commensal bacterium found both exclusively and ubiquitously in the human oral cavity. The well-characterized S. salivarius strain K12 was originally selected for development as a probiotic on the basis of its particularly strong, megaplasmid-encoded inhibitory activity against the important disease- associated species Streptococcus pyogenes. As such, its initial application was to provide school-aged children with protection against streptococcal pharyngitis and its sequelae. More recently however a variety of additional health benefits have been linked to the regular use of probiotic preparations of strain K12. Reported benefits have included the reduction of acute otitis media episodes in young children and decreased severity of symptoms in halitosis-affected adults. We now report that the dosing of expectant mothers with strain K12 results...
in natural transmission of the probiotic bacteria to the mother’s baby in the first days of life. These observations, together with the previously-documented protection afforded by K12 against Streptococcus agalactiae vaginal infections in a mouse model provide a strong basis for further exploring the health benefits of perinatal applications of strain K12. Other studies conducted in Italian adults demonstrated an apparent protective effect of K12 dosing against upper respiratory tract virus infections and this has prompted us to investigate the anti-viral immunostimulatory activity of K12. A notable response detected has been the elevation of salivary levels of gamma interferon. In summary, a wide variety of potential and established beneficial health outcomes are now being linked to the use of probiotic S. salivarius strain K12.

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Atypical Streptococcus dysgalactiae subspecies equisimilis (SDSE) in England in Wales – a perspective from the reference laboratory

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Beta-haemolytic Streptococcus dysgalactiae subsp. equisimilis (SDSE) is most commonly classified into Lancefield groups C or G and is often considered non-pathogenic or only able to cause mild disease. However, a number of severe, invasive SDSE infections are reported annually in England and Wales. Furthermore, the PHE reference laboratory is receiving increasing numbers of SDSE isolates with the Lancefield group A antigen, with 86 atypical SDSE isolates referred from 2010 to 2017. The aim of this study was to characterise 25 atypical SDSE isolates from 2010-2017 using whole genome sequencing (WGS) and to compare these data with contemporaneous typical GAS and typical SDSE.

Phylogenetic analyses from WGS SNP data demonstrated that atypical SDSEs were more closely related to Lancefield groups C and G SDSE isolates compared to typical GAS isolates (Streptococcus pyogenes). MLST data derived from WGS indicated that 21/25 (84%) of the atypical SDSE isolates belonged to ST128, with single isolates of ST29 & ST134 identified and two isolates with novel STs, one of which is a single locus variant of ST128. eBurst analysis of the 21 ST128 isolates showed a founding population of eBurst group 4 indicating the atypical SDSE were an emerging clone. Interestingly, emm typing divided the isolates into 5 types, 14 (56%) STG495.0; 8 (32%) STG652.0; 2 (8%) STG245.0; and a single isolate of emm type STC46.1 and STG480. Phage sequence of 23/25 isolates were shown to contain the same incomplete phage sequence within their DNA (Streptococcus phage K13) according to PHAge Search Tool Enhanced Release.

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Lessons from the Stop Rheumatic Heart Disease A.S.A.P. Programme

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A spoon full of sugar: Understanding the role of host glycan recognition in GAS infection

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The oral epithelial tract is a niche highly abundant in glycosylated structures, particularly those of the ABO(H) blood group antigen family. Using a high-throughput approach, we determined that M1T1 GAS strain 5448 interacts with numerous, structurally diverse glycans. Recombinant M1 protein showed high affinity for several terminal galactose blood group antigen structures. Deletion mutagenesis shows that M1 protein mediates glycan binding via its B repeat domains. Association of M1T1 GAS with oral epithelial cells varied significantly as a result of phenotypic differences in blood group antigen expression, with significantly higher adherence to those cells expressing H antigen structures compared to cells expressing A, B, or AB antigen structures. Furthermore, defined glycan mixtures could be used to outcompete attachment of GAS to buccal epithelial cells. These data suggest a novel mechanism for GAS attachment to host cells and propose a link between host blood group antigen expression and M1T1 GAS colonization.

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Evaluation of the New Zealand Rheumatic Fever Prevention Programme – Updated Analysis

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And the Enterococci begat the Streptococci: Tracing the ultimate origin of the streptococci to the terrestrialization of animals >425 MYA

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The 16S tree of life shows that the streptococci and lactococci emerged from the enterococci, sharing a last
common ancestor with Enterococcus hermanniiensis. To understand the basic nature of this branch of microbial life, we investigated the origins of the enterococci and offspring. The enterococci are strongly associated with land animals, whereas ancestral lineages, the vagococci and carnobacteria, are associated with marine life. To determine when the enterococci arose, we sequenced and compared genomes of all major phylogenetic branches. Applying molecular clocks, we found that the enterococci originated between 600 and 400 MYA, around the time of the Cambrian explosion. The first fossil evidence of animal land life – the ecology in which enterococci are found – are of arthropods, dated to 425 MYA. All species radiations within enterococci date after this. After an initial burst of species radiation between 425 and 330 MYA, there was relative stability in the genus. Then, abruptly, after 250 MYA, a second wave of speciation occurs. This coincides perfectly with the radiation of new animal hosts following the Great Permian Extinction, 251 MYA. The enterococci possess about 100 traits that distinguish them from the ancestral marine vagococcal lineage, most encoding functions for hardening the cell wall to harsh conditions, precisely the traits needed for adapting to life on land. Discovering that terrestrialization led to the expansion of the Enterococcus genus, now ubiquitous in land animals, raises the fundamentally important question, what new ecologies gave rise to lactococcal and streptococcal genera?

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Group A Strep skin infection and Rheumatic Heart Disease in indigenous NZ children
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Streptococcal skin infections are a common occurrence in high risk groups of children in New Zealand who have considerable trouble accessing health services. Maori and Pasifika children in New Zealand have huge unmet health needs driven by environmental factors and poorly designed health services that make access difficult. If access to health services does occur then appropriate management of these problems is not always delivered by the health teams. Complications of delayed or no treatment of these conditions result in unnecessary pain, time off school and activities and financial and emotional impact on families. In addition to this immune mediated conditions such as Rheumatic Fever and Post Streptococcal Glomerulonephritis can result in catastrophic long term complications. Severe and all too common complications of untreated streptococcal skin infections include deep infections affecting joints and bones result significant morbidity, cost to the health system and grief to the child and their family.
Dr O’Sullivan has developed a community driven digital health service that serves to bring access to health services to children in their schools and homes. A cloud platform iMOKO aims to provide care to children in seconds and minutes rather than days and weeks.
Dr O’Sullivan is aiming to expand this programme into the Pacific and is excited to be forming partnerships with organisations like NZ's MFAT Cure Kids and international philanthropic organisations to make this happen. Come along to hear about what the future of healthcare looks like.

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A Sustainable Approach to the Prevention and Control of Rheumatic Heart Disease in the Fiji Islands
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Background/Objectives
Rheumatic Heart Disease (RHD) is a significant health problem and a leading cause of premature death in Fiji. Establishing a sustainable prevention and control program that is best-practice based is a challenge in developing countries.
Methods
The establishment of a program like the Fiji RHD Prevention and Control Program requires political will, leadership including expert technical oversight, funding, local program team and implementers. The challenge is in integrating and strengthening of existing health systems; and empowering people living with RHD.
Aligned to the 4 main pillars of the Fiji RHD Policy, Program activities include development of a unique Rheumatic Fever Information System (RFIS) linked to the MOHMS PATIS; best practice guidelines for Acute Rheumatic Fever (ARF) and RHD; early case detection model; along with primary prevention and health promotion.
Results & Conclusion
The Program has observed upward trends in adherence to secondary prophylaxis at monitored sites in two divisions and a mid-term training review has reported high rates of knowledge retention among nurses trained in ARF/RHD. The RFIS whilst in its infancy has enabled better visibility of patients including movement of patients between clinics and patients who are defaulting treatment.
The Program has duplicated activities from other countries and developed innovative approaches to improve visibility and outcomes for people living with RHD. While all these innovations are yet to be analysed fully, a few will be presented.

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Our stepped-wedge, community randomised trial to improve secondary prophylaxis delivery for Aboriginal people with acute rheumatic fever (ARF)/rheumatic heart disease (RHD) did not achieve intended outcomes... Why?

Māori and Pacific people living in New Zealand experience high rates of rheumatic fever and rheumatic heart disease. The Rheumatic Fever Information System (RFIS) was launched in 2016 following 18 months of development. A stepped-wedge, community randomised trial was implemented in Australia’s Northern Territory to test whether a clinic-level intervention could improve prophylaxis delivery for Aboriginal people with ARF/RHD. Ten clinics received a multifaceted intervention which supported them to develop and implement strategies to improve penicillin delivery and RHD care, aligned with themes of the Chronic Care Model. The proportion of patients receiving ≥80% of scheduled injections in the intensive phase (126/304 [41.5%]) did not improve compared with baseline (141/304 [46.4%]), odds ratio 0.78 (95% CI 0.54 to 1.11). An overarching theory-driven evaluation framework guided the analysis of qualitative data to explain the reasons for the study not achieving its primary outcome.

Methods: The intervention’s effectiveness, efficiency, process, fidelity, performance and context was evaluated. Pre and post interviews with participants (n=166), quarterly project officer observational reports (n=50), and detailed tracking of action plans (n=10) and implemented action items (n=252) provided primary qualitative data. Findings: Low level of intervention uptake and exposure was documented affecting causal processes, thus attainment of outcomes. Acceptability and completeness of the intervention and its components, barriers to implementation and organizational change strongly contributed to intervention effectiveness. All sites highlight the significance of context in assessing why an intervention may be implementable and effective in one setting but not another.

Conclusion: Despite implementing a comprehensive health system strengthening strategy, we did not find a significant improvement in adherence to ARF secondary prophylaxis. Other strategies for improved ARF prevention, including Group A Streptococcal prevention and treatment are required.

The Fiji rheumatic fever information system innovation to strengthen disease control
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Fiji has had a limited register based RHD programme since 2005. To expand and enhance capability of the programme to provide clinical and epidemiological reports nationally, a collaboration with Ministry of Health and external partners developed the first web enabled national disease register with the ability to link to the Ministry of Health electronic medical record system. The Rheumatic Fever Information System (RFIS) was launched in 2016 following 18 months of development. The RFIS provides health professionals with easy access to patient clinical data and disease status. The first quarter reporting period in 2017 has shown the programme is now able to provide up to date information that can assist the MoHMS to target medication adherence, ensure timely clinical review, describe trends in epidemiology and identify vulnerable and at risk sub-populations. The RFIS has 3689 patients registered nationally (ARF n=302, RHD n= 3387, 33 % aged <18 years), 60% are iTaukei (Indigenous Fijians) and 21% Fijians of Indian descent. In 2016 there were 46 deaths with RHD attributed as the causal factor. A 2012 audit found that only 12% of patients were receiving adequate adherence rates. The RFIS now collects and reports data on a quarterly basis from all clinics nationally. Results are reported back to each clinic allowing activities to be directly targeted at clinics and individuals to improve adherence and support patients to receive protective levels of adherence. The RFIS provides an enhanced ability to monitor adherence and epidemiological data in real time.

Reducing rheumatic fever in New Zealand through a multi-faceted, comprehensive prevention programme
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Māori and Pacific people living in New Zealand experience high rates of rheumatic fever and rheumatic heart disease. New Zealand Ministry of Health, Thorndon, WELLINGTON, New Zealand
Rheumatic fever is a preventable condition that results from an autoimmune response to a Group A streptococcal throat infection. The New Zealand Government committed to tackling this inequity and reducing the incidence of rheumatic fever by two-thirds by 2017.

To achieve the target the Rheumatic Fever Prevention Programme delivered a range of initiatives using three key strategies:

- Increasing awareness of rheumatic fever, what causes it and how to prevent it
- Improving access to timely, effective treatment for group A streptococcal sore throat in primary care and community settings
- Reducing household transmission of group A streptococcal bacteria

Since the beginning of the programme, there has been a 23 percent decrease in first episode rheumatic fever hospitalisations. There has been an almost 50% decrease among Māori but more work needs to be done to reduce rheumatic fever among Pacific people.

Although the target has not been reached, the Rheumatic Fever Prevention Programme has had a wider positive impact as a change programme and as an exemplar in relation to integrating primordial and primary prevention activities across government departments through to communities. This presentation will include an overview of the complex, multi-faceted rheumatic fever prevention programme and its successes. Lessons learned from the implementation of this programme will be shared in order to aid the reduction of rheumatic fever and rheumatic heart disease in the Pacific.

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**Rheumatic heart disease in pregnancy: cardiac outcomes in the New Zealand AMOSS cohort.**

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AMOSS (Australasian (Australia and New Zealand) Maternity Outcomes Surveillance System) studies rare and serious conditions in pregnancy, including rheumatic heart disease (RHD). Pregnant women with RHD were prospectively identified throughout New Zealand between October 1st, 2012 - December 31st, 2014, to determine the numbers affected by RHD in pregnancy, the degree of severity of RHD and outcomes for mother and baby.

147 pregnancies, occurred in 130 women, 64 (44%) Māori, 74 (50%) Pacific. Ages ranged from 15–43 years. 16 women (11%) were diagnosed with RHD for the first time. 41 (25%) women had severe RHD. 24 (16%) women had had previous cardiac surgery including 13 (9%) with mechanical valves.

Cardiac complications occurred in 24/147 (15.6%) pregnancies. 5 women with less than severe RHD were assessed for shortness of breath or palpitations and 1 (moderate mixed mitral valve disease) admitted for medical stabilisation. 19 women with severe RHD presented with acute pulmonary oedema or atrial fibrillation, managed medically apart from 2 requiring balloon mitral valvotomy and 2 urgent valve surgery. There were no maternal deaths.

Infant outcomes included miscarriage or termination 14 (9.6%), still birth 2 (1.5%) and preterm birth 15 (11.4%). Mean birth weight 3295g (410-5300g). There were 88 (60.3%) spontaneous vaginal deliveries, 42 (32.8%) inductions and 31 (23.5%) Caesarian sections.

Most mothers and babies did well. With known disease, echocardiography in pregnancy is required for risk stratification. In NZ, RHD occurs in pregnancy in 1/500 Māori and 1/200 Pacific women. Screening echocardiogram should be considered in these women.

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**Group A Streptococcus, A Master At Turning Its Host Against Itself**

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Group A streptococci (StrepA) have evolved a variety of macromolecules that mimic their host. Autoimmune responses to these molecules, tissue reactive antibodies, subsequent to infections are implicated in Kidney, heart and neuropsychiatric disorders. Mucosal infections by StrepA also induce a robust Th17 cellular response, which we showed is protective. Subsets of Th17 cells are directly involved in rodent models of autism spectrum, and multiple sclerosis, and are linked to rheumatic heart disease.

The potential of these cells to migrate into the brain and spinal cord focused our research toward understanding the autoimmune neuromuscular and behavioral StrepA sequelae. Sydenham’s Chorea and Pediatric autoimmune Neurologic disorders associated with group A streptococcus (PANDAS). Although mimic antibodies, directed at brain proteins are found in sera of patients, mechanisms that allow passage of IgG into the brain are unknown. Our experiments showed that CD4 IL17+ T cells are the dominant StrepA specific helper T cell in human tonsils. A mouse model of intranasal infection combined with a MHCII Tetramers reagent revealed that StrepA specific CD4 IL17+ T cells migrate from nasal associated lymphoid tissue into the olfactory bulb and other regions of the brain. Localization of T cells in the brain was accompanied by leakage of IgG across the blood brain barrier and microglial cell activation. The broader implication of these findings for exacerbations of streptococcal associated and other chronic autoimmune disorders will be considered.
Toll-like receptor 2-and 4-mediated reciprocal Th17 and antibody responses to group A streptococcus infection
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Background: The role of Toll-like receptors (TLRs) in adaptive immunity is incompletely understood. Recurrent human infections by Group A Streptococcus (GAS) and associated autoimmune conditions suggest that the immunity to GAS is intricately regulated and TLRs may be involved in the regulation.

Method: This study investigated adaptive mucosal immune responses to GAS in TLR2+/− and TLR4+/− mice with an intranasal infection model.

Results: FACS analyses of Nasal-Associated Lymphoid Tissue (NALT) cells showed that robust Th17 responses to GAS in WT mice were reduced in TLR2+/− mice by 50%. Conversely, antibody levels and follicular T (Tfh) and B cells in germinal center of NALT were significantly higher in TLR2+/− than in WT mice. However, antibody response to soluble antigens co-immunized with GAS was similar in WT and TLR2+/− mice. Moreover, the adaptive clearance of GAS in TLR2+/− mice was as efficient as in WT mice, whereas, it was significantly impaired in TLR4+/− mice, in which antibody responses were significantly lower than in WT mice.

Conclusion: Activation of TLR2 by GAS is responsible for massive Th17 activation and deficient antibody response, which may increase predisposition to GAS-related autoimmunity and reduce efficiency of protection.

Key words: Group A Streptococcus (GAS); Toll-like receptor 2 (TLR2); Toll-like receptor 4 (TLR4); Th17; Mucosal immunity.

LL-37 cleavage by the Group A streptococcus CXC chemokine protease eliminates its immunomodulatory activities thus promoting invasive infection
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Severe soft tissue infections caused by Group A streptococci (GAS) are characterized by rapid dissemination of GAS followed by massive necrosis of the infected tissue. The GAS serine protease ScpC (also known as SpyCEP) plays a central role in GAS virulence by cleaving the host CXC-chemokine, interleukin-8 (IL-8) and thus impairing recruitment, activation and formation of neutrophil extracellular traps (NETs).

IL-8 has been the only identified substrate of ScpC. Here, we report that the human cathelicidin anti-microbial peptide, LL-37, also serves as a substrate for ScpC. LL-37 cleavage by ScpC results in the loss of its immunomodulatory activity, namely the ability to recruit neutrophils directly, and to stimulate IL-8 production by skin keratinocytes. These activities seem pertinent to human invasive soft-tissue GAS infections as previous study revealed the coexistence of LL-37 with viable bacteria in soft tissues debrided from human patients. This study hinted that LL-37 does not necessarily act as an anti-microbial peptide but perhaps as immuno-modulator of the innate immune response. The identification of the LL-37 cleavage site by ScpC enabled us to produce LL-37-derived peptides that were resistant to ScpC cleavage and retained their immunomodulatory functions. Using the murine model of human GAS soft tissue infection, we demonstrated that the LL-37 analogs significantly affected the distribution of neutrophils in the infected tissues.

Validation of a rapid diagnostic assay based on antibodies to human cardiac myosin S2 epitopes to monitor rheumatic heart disease
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Introduction: Ongoing cardiac damage can be identified by the presence of high levels of autoantibodies to human cardiac myosin (HCM) in acute rheumatic fever and rheumatic heart disease (ARF/RHD). We hypothesize that these antibodies and in particular antibodies to immuno-dominant epitopes of the S2 region of HCM can be used for both sero-diagnosis of ARF/RHD and monitoring of the disease progression. Since India is a major contributor to global burden of ARF/RHD and is associated with different circulating M types of Group A streptococci and HLA types of patients, it is important that we validate the HCM immuno-dominant epitopes as has been determined in some other populations.

Methods: In our pilot study, ELISA was performed with serial samples of sera from patients with ARF/RHD and controls. Using 32, 25mer overlapping peptides from the S2 region of HCM (Mimitopes, Australia), we determined antibody titers.

Results: Our pilot study demonstrates that the mean reactivity of patient group is higher for epitopes S2-21 & S2-
22 in contrast to the other studies which suggest S2-1, 2 & 8 as immuno-dominant epitopes.

Discussion: ELISA performed on our patient serum samples (EC/GOVT-07/2012) from India is useful in identifying the immuno-dominant S2 epitopes. This could lead to the identification of a universal serological marker for the diagnosis and assessment of ongoing cardiac damage in patients with ARF/RHD. Our findings from the Indian cohort will be presented and it would add to the discussion for the need for an economically viable, rapid, point-of-care test for ARF/RHD.

Interaction of disease-causing Group A Streptococcus with innate immune pathways
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Introduction:
Streptococcus pyogenes (group A Streptococcus) commonly causes pharyngitis in humans, with severe invasive diseases and immune sequelae being an infrequent consequence. The ability of S. pyogenes to invade the host and establish an infection likely involves subversion of host immune defences, however the innate immune responses and signalling pathways that respond to S. pyogenes are not well understood.

Methods:
In this study, we used RNAseq and ELISAs to characterise the inflammatory responses of primary human tonsil epithelial cells to infection with a laboratory-adapted M6 strain (JRS4) and a clinical isolate of the globally disseminated M1T1 clone (5448), the leading cause of pharyngitis and severe invasive S. pyogenes infections globally.

Results:
Both strains induced the expression of genes encoding a wide range of inflammatory mediators, including IL-8. Pathway analysis revealed differentially expressed genes were enriched in transcription factor networks which regulate IL-8 expression, such as AP-1 and NFAT. Surprisingly, while M6 infection induced strong secretion of IL-6 and IL-8, M1T1 infection did not induce IL-6 secretion, and suppressed IL-8 levels below mock-treated cells. This suggests that M1T1 post-transcriptionally downregulates host IL-8 production. IL-8 secretion was restored by infection with M1T1∆cepA, a knockout mutant for the cysteine protease SPYcep, which degrades human IL-6 and related chemokines.

Conclusion:
Our results thus suggest that the pathogenic M1T1 clone induces a strong pro-inflammatory response in the human tonsil epithelium, but overcomes this host response by selectively degrading host-protective neutrophil-recruiting chemokines to benefit infection.

Anti-streptococcal antibodies and T-cells induce heart pathology in Lewis rats following adoptive transfer
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Acute rheumatic fever (ARF) and rheumatic heart disease (RHD) are autoimmune mediated diseases caused by group A streptococcus (GAS). Structural similarity between GAS antigens and host tissue proteins is considered to initiate ARF/RHD. Antibody-mediated immune response to GAS pharyngitis initiates autoimmune reactions followed by immune cells infiltration of the heart tissues that leads to permanent heart damage in RHD. However, the individual role of anti-GAS antibodies and T-cells in the pathogenesis of autoimmune carditis is under-explored. In this study, Lewis rats (recipient) were adoptively transferred independently with serum or together with T-cells from GAS M5 protein injected rats (donor). The antibody and T-cell response, histological changes in the heart and ECG and Echo finding of the recipient rats were found indistinguishable from the donor rats. Moreover, the changes in the independent serum or T-cell recipient rats were observed similar to the integrated serum and T-cell transferred rats. The results indicate that both B-cell and T-cell has individual potential to develop autoimmune reactions in rheumatic heart disease.

Peptide Signaling in Enterococcus faecalis
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Infections caused by enterococci are a serious threat to human health, as they are a leading cause of hospital-
associated infections in the United States and around the world. During infection, organisms rely on communication signals to ascertain population density, which enables a coordinated genetic response to assist in pathogenesis. Enterococcus faecalis relies on a variety of peptide signals to coordinate the appropriate response, enabling colonization and infection. Our lab has investigated two unique types of peptide signals that facilitate steps in pathogenesis. The Fsr quorum sensing system senses a cyclic peptide, termed GBAP, that activates expression of secreted proteases as well as a novel bacteriocin. We have also examined mechanisms related to cell-cell signaling as it pertains to horizontal gene transfer in E. faecalis and discovered a peptide transporter, termed PptAB, responsible for the active secretion of linear peptides used as pheromones to induce a conjugative mating response.

More recently, we have focused our attention on a family of transcription factors that are predicted members of the RNRPP family, which are thought to respond to linear peptide signals. I will discuss one such transcription factor that contributes to biofilm development and pathogenesis in a catheter associated urinary tract infection model.

Regulation of innate immunity to Streptococcus
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Bacterial pneumonia remains a leading cause of disease and mortality worldwide, and the most vulnerable patients are those with the weakest immune systems, such as neonates or the elderly. Approximately 25% of adults are colonized with group B Streptococcus (GBS), but typically do not experience symptoms. In contrast, GBS remains the leading cause of death in neonates, causing pneumonia, sepsis, and meningitis. Similarly, S. pneumoniae is the leading cause of bacterial pneumonia, and is disproportionately lethal in the elderly. To prevent disease, innate immunity in the lung must effectively limit bacterial replication and spread, while minimizing damage to the host. Disease outcomes are almost uniformly dictated by differential immune responses, however the mechanisms underlying these differences are not well understood.

Hypoxia Inducible Factor-1α (HIF-1α), a host transcription factor, was discovered by our laboratory to play a critical role in immune cell function. Genetic modifications or drugs that increase HIF-1α levels increase the antibacterial capacity of PMNs, macrophages and epithelial cells, and mice lacking HIF-1α are more susceptible to many infections. Here, we investigate the role of HIF-1α in resistance to Streptococcus in mice and compare HIF-1α function in neonatal and adult human neutrophils. In addition, we describe a role for murine cathelicidin (CRAMP), a HIF-1α regulated gene, in mediating progression of Streptococcal pneumonia. These data demonstrate how failure of innate immunity allows for progression to more severe forms of disease such as sepsis and meningitis.

Streptococcus pneumoniae evades host innate immunity through parallel β-helix protein PfbA
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Streptococcus pneumoniae, a leading cause of community-acquired pneumonia, often evades host immunity and causes systemic diseases, such as sepsis and meningitis. Pneumococcal pneumonia is accompanied by an excessive inflammatory response at infection sites. In the present study, we investigated the role of PfbA, a pneumococcal parallel β-helix protein, in infection.

A homology search indicated that the pfbA gene is highly conserved among pneumococcal strains, while other streptococci do not contain such an orthologous gene. In human neutrophil bactericidal assay findings, a pfbA mutant strain showed significantly reduced survival as compared to the wild-type strain, while exogenous addition of recombinant PfbA recovered survival of the mutant strain incubated with neutrophils. Next, we performed time-lapse microscopic analysis of the interaction between S. pneumoniae and human neutrophils. Within 1 minute, neutrophils phagocytosed the pfbA mutant strain, whereas after 5 minutes they were unable to capture the wild-type strain. In addition, flow cytometric analysis revealed that recombinant PfbA-coated beads were captured less often by neutrophils as compared to non-coated beads. Furthermore, a TLR2/4 inhibitor peptide significantly enhanced survival of the pfbA mutant strain incubated with neutrophils, whereas it had no effect on survival of the wild type. Finally, 24 hours after CD-1 mice underwent intratracheal infection with S. pneumoniae, the wild-type strain showed a significantly greater number of colony forming units in bronchial lavage fluid as compared to the pfbA mutant strain.

Our results indicate that PfbA is specific to S. pneumoniae and functions as an anti-phagocytic factor.

Identification of genes required for the virulence of Streptococcus equi in the natural host by barcoded transposon directed insertion-site sequencing (TraDIS)
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The host-restricted Lancefield group C pathogen Streptococcus equi infects the lymph nodes in the head and neck of horses, forming abscesses which may become so large that the horse’s airways are obstructed, lending to the disease’s name, strangles. We have developed a barcoded TraDIS system that identifies coding sequences (CDSs) required for the virulence of S. equi in a susceptible natural host. Twelve Welsh mountain ponies were each infected with two of three barcoded ISS1 libraries each containing 33,000-50,000 unique mutants. These libraries contained, on average, an ISS1 transposition every 56 bp, or an average of 19 unique mutants per CDS. Ponies were euthanased on developing early clinical signs, typically pyrexia and preference for haylage and water over dry-pelleted food. Viable mutants were recovered from the abscess material of 24 retropharyngeal and 14 submandibular lymph nodes. Sequencing across ISS1-genome junctions identified 492 CDSs, which were non-essential in vitro, but were significantly depleted in the abscess pools. Depleted mutants included insertions in lgt or prtM, which are known to reduce the ability of S. equi to cause disease in ponies. Significant decreases in fitness were also measured in sagA, csrS and SEQ_2190 (surface-anchored protein). Mutants in a further 239 CDSs, including those encoding 19 putative membrane proteins, were significantly enriched in the abscess pools. Our data provide an unprecedented insight into the mechanisms employed by S. equi to cause disease in the natural host. Our findings are likely to also shed light on pathways important for virulence in other streptococci.

### Insertion Sequence elements are drivers of diversification in the broad host range aquatic pathogen Streptococcus iniae

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Fish mortality caused by Streptococcus iniae is a major economic problem for fish aquaculture in warm and temperate regions globally. There is also risk of zoonotic infection by S. iniae through handling of contaminated fish. To date, a handful of complete genomes have been described but our understanding of the genomic diversity of this species is limited. Here we investigated the role of mobile genetic elements (MGEs) in the evolution of S. iniae. We first established a high-quality reference sequence by manually curating the MGEs in a complete reference genome of S. iniae (determined by PacBio long-read sequencing). The reference genome harbours 93 individual insertion sequence (IS) elements comprising 13 different types. We then used the Illumina sequencing to determine the draft genomes of a global collection of 112 S. iniae isolates from different hosts. A non-recombinant core genome maximum likelihood phylogenetic tree revealed separate clustering of human and fish isolates, as well as phylogeographic groupings, even within Australia. By analysing IS distribution across the entire phylogeny we discovered clade-specific expansion of certain IS types. For example, up to 17 additional insertions of an ISSag3-like element were identified in one clade compared to its nearest phylogenetic neighbour. Remarkably, we found several cases of convergent evolution driven by IS, including multiple lineages in which the CRISPR/Cas system had been independently disrupted by different IS types. By defining the role of MGEs in S. iniae diversity we have enabled a better understanding of its evolutionary trajectory and mechanisms of adaptation to different niches.

### Positive and negative control mechanisms of pathogenicity expression in Streptococcus intermedius

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【Purpose】Streptococcus intermedius (SI) is an opportunistic pathogen secreting a human-specific cytolysin: intermedilysin (ILY) as a major pathogenic factor. The Ily expression is suppressed with LacR: the repressor of lac operon, so that galactose (Gal) up-regulates the ily expression. Moreover, SI can degrade glycans into monosaccharides such as Gal and N-acetylneuraminic acid (NeuNAc) using multisubstrate glycosidase A (MsgA) and neuraminidase (NanA). Therefore, we investigated the pathogenicity regulation via sugars in SI.

【Results and Discussion】We detected ILY-overproduction in SI strain PC574 cultured in fetal bovine serum (FBS) as compared to the standard medium. FBS-cultured cells also showed higher MsgA and NanA activity although ILY-overproduction in FBS was undetectable in mutants nanA-null and msgA-null. In this study, it was revealed that purified MsgA and NanA produced 2.8 mM Gal and 4.3 mM NeuNAc in FBS which were sufficient to up-regulate the expression of ILY, MsgA and NanA. Interestingly, >10 mM Gal strongly inhibited ILY activity. Conversely, no increase of ILY was observed in human plasma, rather it inhibited the stimulation by FBS. We confirmed that human plasma contains immunoglobulins neutralizing ILY, MsgA, and NanA and results in reduction of cytolysin in FBS of S. intermedius toward human cell-line, HepG2. Overall, blood contains factors that stimulate and inhibit ILY expression and activity, which affect monosaccharide production. These results suggest that SI is in symbiosis with human on the balance of pathogenicity control mechanisms via mainly sugars.
and serious failure in negative control induces SI infectious diseases.

The INFECT-project; a multicentre prospective study on necrotizing soft tissue infections: from clinics to pathogenesis to intervention.

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The INFECT-project focuses on necrotizing soft tissue infections (NSTIs), which are rapidly spreading life-threatening infections. Diagnosis and management are difficult due to heterogeneity in clinical presentation, in co-morbidities and in microbiological aetiology. The project builds on eight work packages (WPs) including: WP2, recruitment of NSTI patients and patient samples, and experimental models; a murine NSTI model (WP1) and a human tissue model (WP6); Samples from infected individuals/models are analysed by host and pathogen traits by genomics, transcriptomics, proteomics and metabolomics (WP3-6). All data are analyzed in relation to clinical parameters by integrated computational modelling (WP4) to identify involved pathways/networks and disease traits that are used for development of novel diagnostic tests (WP7). WP8 aims to disseminate and exploit the results through implementation of improved guidelines and novel diagnostics and therapeutics. In INFECT, >400 NSTI patients have been enrolled and >6000 biobank samples collected. Beta-hemolytic streptococcus was the most common cause of infection. Through a forward systems genetics approach using BXD mice, the IL1β network was identified as a key network involved in modulating the susceptibility to infection. This was further supported by analysis of patient plasma and tissue biopsies as well as infected human skin tissue model. Other key findings of INFECT include, among others, identification of 1) bacterial biofilm in infected tissue which can contribute to reduced antibiotic efficacy, and 2) bacterial pore-forming toxins as central mediators of tissue necrosis.

New insights into SLO and NADase as co-toxins.

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The globally dominant, invasive M1T1 strain of group A Streptococcus (GAS) harbors polymorphisms in the promoter region of an operon that contains the genes encoding streptolysin O (SLO) and NAD-glycohydrolase (NADase), which results in high-level expression of these toxins. While both toxins have been shown experimentally to contribute to pathogenesis, many GAS isolates lack detectable NADase activity. Reduced or absent enzymatic activity can be associated with a variety of point mutations in nga, the gene encoding NADase. However, nga has not been observed to contain early termination codons or mutations that would result in a truncated protein, even when the gene product contains missense mutations that abrogate enzymatic activity. We found that expression of NADase, either enzymatically active or inactive, is associated with increased abundance of SLO in culture supernatants and increased SLO-mediated toxicity for keratinocytes. Conversely, production of SLO protects NADase from proteolytic cleavage. Analytical gel filtration chromatography demonstrated physical association of purified SLO and NADase in solution, a result confirmed by biolayer interferometry. After glutaraldehyde treatment of a mixture of the two proteins, a crosslinked species of 110.7 kDa could be detected by mass spectrometry, consistent with a heterodimer of 1:1 binding stoichiometry. Thus, SLO and NADase interact in solution, and both the translocation and catalytic domains of NADase are required for maximal binding between the two toxins. We conclude that expression of full-length NADase is under positive selection because binding of NADase to SLO stabilizes both toxins, thereby enhancing GAS survival in the human host.

Modulation of the immune response by encapsulated streptococci: elucidating the role of sialylated capsular polysaccharides

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Streptococcus suis and group B Streptococcus (GBS) are two encapsulated bacteria that induce similar pathologies, including sepsis and meningitis in animals and/or humans. Among several serotypes identified, S. suis types 2 and 14 and GBS types III and V are highly virulent and frequently isolated, and were used in our studies as a model. For both pathogens, the capsular polysaccharide (CPS), which defines the serotype, is considered as a major virulence factor. The four CPSs share structural features, including a side chain terminated by sialic acid, a unique characteristic of S. suis and GBS among Gram-positive bacteria. However, the interplay of CPS with components of the innate immune system, including antigen-presenting cells (APCs), seems to radically differ between these two streptococci. Experiments using nonencapsulated mutants have shown that, in contrast to GBS, S. suis CPS has a strong antiphagocytic effect and severely interferes with the activation and maturation of APCs and downstream modulation of NK and T cells. The anti-CPS antibody response of infected- or purified CPS-immunized mice also differs between these species/serotypes. The presence of sialic acid either contributes to CPS immunogenicity (GBS type III) or it has no impact on the antibody response depending on the CPS type. In conclusion, in spite of similar CPS biochemical features, including expression of sialic acid, S. suis and GBS differentially modulate host innate and adaptive immune responses.

### Role of zinc in group A streptococcal pathogenesis

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Streptococcus pyogenes (Group A Streptococcus; GAS) is a Gram-positive human pathogen responsible for a wide spectrum of diseases. Zinc is recognized as an important metal ion in relation to nutritional immunity and zinc deficiency is linked to increased susceptibility to bacterial infection. Zinc stress impairs glucose metabolism through the inhibition of the glycolytic enzymes. In the presence of zinc, a metabolic shift to the tagatose-6-phosphate pathway allows conversion of D-galactose to dihydroxyacetone phosphate and glyceraldehyde phosphate, partially bypassing impaired glycolytic enzymes to generate pyruvate. We analyzed the clinically important GAS M1T1 wild-type strain, and the phenotypes of two isogenic mutants and corresponding complemented mutants. The targeted GAS czcD gene encodes for a putative zinc efflux pump and the adjacent gczA gene encodes a putative Zn-dependent activator of czcD expression. Compared to wild-type and complemented cells, both mutants exhibited reduced ability to grow in the presence of zinc. Transcriptional analyses indicate that gczA up-regulates czcD in response to zinc. The gczA regulator also induces galactose metabolism, circumventing zinc-induced blockage of glucose uptake. Both czcD and gczA are up-regulated in contact with human neutrophils. Zinc efflux plays a critical role in GAS pathogenesis, as both czcD and gczA mutants displayed increased susceptibility to killing by human neutrophils and reduced virulence in a murine infection model. Taken together, these results demonstrate that zinc homeostasis is an important contributor to GAS pathogenesis and innate immune defense against infection. Strategies to manipulate zinc homeostasis in order to reduce GAS infection are discussed.
Methods: We synthesised and modelled over 65 data sources including; nationally health burden of GAS diseases.

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Background: Group A streptococcus (GAS) causes a high burden of severe invasive and immune-mediated diseases as well as superficial infections. The World Health Organization has highlighted that a vaccine could offer major health and economic benefits, however, these effects have never been studied. We aimed to establish the potential benefits of preventing GAS infections in Australia and New Zealand by estimating the economic and health burden of GAS diseases.

Methods: We synthesised and modelled over 65 data sources including; nationally-representative survey and...
What can a million throat swabs tell us about the distribution of group A Streptococcus and acute rheumatic fever?

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Acute rheumatic fever (ARF) produces an important burden of disease in New Zealand (NZ) Māori and Pacific populations. Operation of New Zealand’s Rheumatic Fever Prevention Programme (RFPP) resulted in large scale throat swabbing and testing for Group A Streptococcus (GAS) pharyngitis, which can trigger ARF. Our aim was to describe the distribution of GAS in Auckland and comment on its correlation with ARF incidence. Throat swab data collected from Auckland primary healthcare clinics (PHG) and schools were obtained (2010-2016). Descriptive epidemiological summary statistics were generated covering swab numbers, population incidences of swabbing and GAS, and GAS+ve swab proportion. Altogether 1,257,058 throat swabs were collected. Swabbing and GAS+ve proportion peaked in age groups with highest ARF risk (5-14 year olds). GAS+ve proportion was similar between ethnic groups (~19%), however GAS incidence was highest in Pacific (81.9/1,000 child-years) and Māori (60.4/1,000 child-years). Similarly, GAS incidence was highest in the most deprived group (84.7/1,000 child-years), yet GAS+ve proportion was similar across socioeconomic quintiles. GAS incidence peaked in winter and was lowest in summer, yet GAS+ve proportion was highest in summer (%).

The RFPP greatly increased swabbing and testing of populations at high risk of ARF, but dramatically increased swabbing (and antibiotic treatment) in low-risk groups. There is a broad correlation between GAS population load and ARF risk, however ethnic and socioeconomic disparities in ARF are much more pronounced. Therefore, factors besides the distribution of GAS culture-positive pharyngitis influence the epidemiology of ARF in NZ.

The IgdE family of streptococcal Immunoglobulin degrading enzymes - Are you my host?

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Streptococci are highly intertwined with specific host species, despite their ability to cause zoonosis or anthroponosis in other uncommon hosts. Only few factors linking streptococci to their natural host have so far been described. We have identified and characterized a novel family of IgG degrading enzymes designated IgdE. IgdE of the endemic pig pathogen S. suis is the founding member of the new C113 cysteine protease family in the MEROPS peptidase database. Interestingly, IgdE proteases exhibit pronounced substrate specificities that were found to mirror adaption of the bacteria to their hosts. Through a computational approach we identified putative IgdE family proteases in nine different streptococcal species. The proteolytic capacities of IgdE proteases of S. agalactiae, S. porcinus, S. pseudoporcinus and S. equi were assessed. Proteolytic activity was found to be restricted towards IgG of the pathogens main hosts. Moreover these proteases also showed pronounced specificity towards IgG subtypes, as IgdE from S. agalactiae and S. pseudoporcinus only cleaved human IgG1, while IgdE from S. equi was subtype specific for equine IgG7.

An igdE in-frame deletion strain in S. suis was found to be attenuated in piglet blood survival assays ex vivo, suggesting an important role during infection. Prolonged and reocurring infections require streptococcal immune evasion mechanisms to circumvent detection and eradication by the host's immune responses. Several streptococcal species evade antibody mediated immune defences by secretion of IgD-degrading enzymes. However, the importance for streptococci to neutralise certain IgG subtypes indicates decisive roles of these subtypes in counteracting infection or colonization.
FasX is a virulence-regulating small regulatory RNA in the group A Streptococcus
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Small regulatory RNAs (sRNAs) are a class of regulatory RNA molecule utilized by a majority of bacterial species to regulate gene expression; they are typically non-coding, and commonly function through post-transcriptional base-pairing mechanisms. sRNAs are essential to the virulence of many pathogens, performing crucial tasks such as regulating stress responses and virulence factor expression. Despite the importance of sRNAs in many pathogens, and the public health challenge posed by GAS, the contribution of sRNAs to GAS virulence has been studied only superficially. We have characterized the 205 nucleotide sRNA FasX and identified that it both positively (the thrombolytic agent streptokinase) and negatively (the collagen-binding pilus, and fibronectin-binding PrtF1 and PrtF2 adhesins) regulates the expression of GAS virulence factors. This regulation occurs post-transcriptionally through the direct binding of FasX to target mRNAs, modulating their stability and/or rate of translation. The importance of FasX-mediated regulation to GAS virulence is highlighted by our findings that FasX reduces GAS adherence in a tissue culture-based model, and enhances disease severity in a bacteremia model of infection using humanized-plasminogen-expressing mice. Our data are the first to mechanistically characterize sRNA activity in GAS, as well as the first to show these mechanisms contribute to the virulence of this pathogen, revealing the possibility of inhibiting FasX activity as a novel anti-infective strategy. We propose that FasX functions as a molecular switch, governing the transition between colonization and dissemination during infection.

Decoding the serological typing genes of Group A Streptococci into an ecological framework
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The primary ecological niche for group A streptococci (GAS) is the superficial epithelium of the human throat and skin, wherein the organism can reproduce, cause a localized infection (pharyngitis or impetigo), and readily transmit to new hosts. Distinct “throat” and “skin” strains, as defined by M-serotype, are widely recognized. Yet, core housekeeping genes point to widespread genetic exchange between strains of the GAS population. A central question of interest: What is the molecular basis for tissue site preferences for infection among GAS? Genes giving rise to M- and T-serotypes are likely to provide important clues. A meta-analysis of 29 population-based surveys, involving >5,000 pharyngitis and impetigo isolates of known emm type, shows that throat and skin specialist strains are distinguished by their emm genes/products, including functional domains that bind fibrinogen and plasminogen, respectively. The emm pattern-defined throat specialists dominate GAS harboring the FCT-1 pilus region encoding T-serotype (71%), whereas 86% of GAS with FCT-3 are skin specialists, and 80% with FCT-4 are generalists. Phylogenies of the pilus adhesin (Cpa) and backbone (FctA) subunits (FCT-3/FCT-4 regions) reveal 10 and 16 sequence clusters, respectively. Extensive genetic recombination is evidenced via 44 unique Cpa-FctA cluster combinations among GAS of 88 emm types. Despite ample genetic mixing, the association between plasminogen-binding emm cluster D4 and certain Cpa clusters is highly non-random (p = 0.0001), as expected for genes conferring key adaptive traits. Mapping of serological typing genes onto a clinical-ecological framework can lead to the generation of well-supported hypotheses that can be experimentally tested.

Mathematical modelling of Group A streptococcal infection and transmission
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A general understanding of the factors driving Group A streptococcus (GAS) epidemiology remains elusive. In part, this is due to the complexity of the pathogen itself, with fundamental principles of transmission, carriage and immunity still not well defined. In high-burden settings such as remote Australian indigenous communities, this lack of understanding is compounded by limited knowledge about host population characteristics that facilitate transmission. Mathematical models of infectious diseases can help to elucidate drivers of transmission and infection by providing a framework within which to incorporate existing data from observational and intervention studies. Models then enable us to extrapolate from current epidemiological information to inform thinking about effective interventions to control transmission. In this presentation I will briefly introduce the concept of mathematical modelling, and provide an overview of modelling research being undertaken as part of project seeking to improve our understanding of GAS transmission in remote communities. The prevalence of GAS-associated skin sores in these communities is extremely high and, despite successful community-based interventions over recent decades, sustained control has not been achieved. We are using models to address a number of knowledge gaps in this area, including the role played by scabies as a driver of GAS infection, the effect of GAS carriage for epidemiological dynamics, and how household, community and regional mobility can increase reinfection risk and undermine control efforts.
**Epidemiology of invasive Group A streptococcal disease in Alberta, Canada, 2007-2016.**

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Invasive Group A Streptococcal (iGAS) disease is a reportable disease to the Ministry of Health in the province of Alberta (pop in 2016; 4.25 million), Canada. This requires that all GAS isolates collected from cases of invasive disease in the province to be sent to the Provincial Laboratory for Public Health for emm typing. This process allows for identification of cases iGAS in Alberta.

From 2007 to 2016 (a 10 year period), the incidence rate of all iGAS ranged from a low of 4.8/100,000 in 2010 to a high of 7.6/100,000 in 2016. Rates of severe iGAS (necrotizing fasciitis and/or streptococcal toxic shock) ranged from a low of 0.8/100,000 in 2010 to a high of 1.63/100,000 in 2015. Interestingly, the increase in rates has not been driven by any single emm type but rather by an overall increase in a collection of emm types. The top 5 emm types in 2007 by case numbers were: emm1 (36 cases), emm82 (30), emm59 (29), emm83 (22) and emm28 (18) and in 2016: the top 5 emm types were emm1 (60), emm101 (37), emm82 (28), emm59 (21) and emm83 (21). The increase in emm101 began in 2015 (26 cases) whereas in the previous 5 years, the number of cases averaged only 4.8/year.

In summary, incidence rates of iGAS disease have been increasing in Alberta, Canada over the last ten years. This increase has not been driven by any single emm type but rather a collection different emm types.

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**The epidemiology of invasive and non-invasive group A streptococcus in Cape Town, South Africa: The AFROStrep Registry**

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**BACKGROUND** Group A β-haemolytic streptococcus (GAS), is responsible for a wide range of diseases which include pharyngitis, Rheumatic Fever and Rheumatic Heart Disease. Primordial prevention efforts include a vaccine based on thirty variants of the M protein product of the emm gene. We report the distribution of invasive (iGAS) and non-invasive (non-iGAS) isolates obtained from the National Health Laboratory Services. In addition, we wished to compare emm subtypes of GAS isolates against those incorporated in the putative vaccine.

**METHODS** This hospital-based study forms part of the AFROStrep study, designed to collect clinical, microbiological and molecular data on GAS in Africa. iGAS was defined as GAS isolated from a sterile site, while non-iGAS was isolated from a non-sterile site. We performed DNA extraction and PCR on our isolates. Sequencing is currently underway so as to determine potential vaccine coverage of the 30-valent vaccine formulation.

**RESULTS** Between March 2016 to February 2017, 440 GAS isolates were recovered from patients aged 3 months – 89 years. iGAS infection comprised 40%(n=176), majority being male, (65%). Clinical presentation of cases included bacteraemia, septic arthritis and necrotising fasciitis, while common sites of isolation were from pus swabs, abscesses, aspirates, blood and deep tissue. Sequencing results are pending and will be presented at the conference.

**CONCLUSION** This study, the first of its kind in South Africa confirms that GAS is a significant cause of disease, frequently isolated from patients with invasive disease in Cape Town. Increased awareness amongst healthcare personnel and vaccine efforts are urgently warranted. We anticipate our sequencing results will further contribute to informing vaccine initiatives.

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**The epidemiology of Streptococcus pyogenes infection among children in Blantyre.**

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**Objective:** To determine the proportion of children with or without pharyngitis in Blantyre with a positive throat swab culture for Streptococcus pyogenes (Group A Streptococcus)

**To measure the age-stratified (<5, 5+ year olds) prevalence of Group A Streptococcus (GrAS), Group C Streptococcus and Group G Streptococcus detected in the oro-pahrynx of children attending the QECH with or without symptoms of pharyngitis.**

**Methods:** 1000 throat swabs from children presenting to Queen Elizabeth Central Hospital, Blantyre, Malawi were collected.

**Results:** A total of 22 (2.2%) samples had laboratory confirmation of presence of bacteria: 20 grew GrAS, 1 Group C streptococcus and 1 Group G streptococcus. 82% of the which were greater than 5years. 36.5% of the participants had symptoms of pharyngitis during the time of swab collection and 13(3.6%) of them had laboratory confirmed presence of bacteria with 11 (3%) being GrAS. 4.8% of the participants with the presence of GrAS had Tonsillar erythema, Tonsillar swelling, Pharyngeal exudate and Tonsillar exudate. On the multivariable analysis, the significant predictors for GrAS presence was presence Tonsillar erythema, Tonsillar swelling, Pharyngeal exudate or Tonsillar exudate.

**Conclusion:** This is the first study in Malawi to look into the prevalence of GrAS in the oro-pahryn of children. The prevalence rate is lower than expected and the data highlights the need to look into the wider community to determine the community prevalence and carriage rate.
Investigation of Group A streptococcus immune responses in an endemic setting with a particular focus on J8
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Background: Candidate Group A Streptococcal (GAS) vaccines based on the J8 peptide have demonstrated promising immunogenicity in mice. Bridging assessment of the likely protective efficacy of vaccine-induced antibodies requires a more robust understanding of their role in the human immune response to GAS infection. We investigated the age-specific profile of J8 antibodies and the association between infection exposures and related antibody responses in a series of complementary studies conducted in the Central Division of Fiji.

Methods: Three complementary studies were undertaken in the Fijian population: 1) Cross sectional population serosurvey of 424 individuals without GAS infection; 2) Paired serum collection for assessment of M-specific and J8 antibody responses in 53 GAS-infected schoolchildren; 3) Longitudinal assessment of GAS infection (6 measurements) and immunity (3 measurements) in a cohort of 459 schoolchildren over a 10 month period.

Results: Median J8 antibody levels reached their peak in the 5–14 year age group, decreasing with age thereafter. Elevated antibody levels were observed in the 20–30 year age group, consistent with child/parent exposure. While M-specific antibody increases confirmed antigenically significant exposures, similar increases were not observed for J8 antibodies. Neither J8 antibody responses nor current J8 antibody levels varied by the time since last skin infection or the number of infections experienced.

Conclusion: Our results suggest that natural skin infection does not induce significant J8 antibodies, and that J8 antibodies have a very short half-life following infection. Further investigation is warranted to better understand differences between J8 responses following infection and vaccination.

Integration of Host and Bacterial Signals Leads to Production of Group A Streptococcal Bacteriocins Contributing to Niche Control.
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Group A Streptococcus is not the sole agent contributing to the pathogenesis of rheumatic heart disease
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Introduction: Acute rheumatic fever and rheumatic heart disease (ARF/RHD) have long been described as autoimmune sequelae of Streptococcus pyogenes or group A streptococcal (GAS) infection. Although in some ARF/RHD endemic regions, Streptococcus dysgalactiae subspecies equisimilis (SDSE), also known as β-hemolytic groups C and G streptococci (GCS/GGS) have been implicated in the pathogenesis of ARF/RHD, to date there has been no experimental evidence supporting this proposition.

Methods: We used the Rat Autoimmune Valvulitis (RAV) model to investigate multiple mechanisms involved in initiating and potentiating cardiac damage including the potential of streptococci other than GAS contributing to the development of ARF/RHD.

Results: We discovered that GGS does indeed cause both myocarditis and valvulitis, hallmarks of ARF/RHD. Furthermore histological, immunological, echocardiographic and electrocardiographic changes in the hearts of rats exposed to GGS were identical to those exposed to GAS. T and B cell responses and antibody cross-reactivity to cardiac myosin were comparable in both GGS and GAS exposed animals providing additional evidence that GGS can induce and/or exacerbate ARF/RHD.

Discussion: Our experimental observations confirm that repetitive infections with GAS and/or GGS have the potential to initiate and/or exacerbate ARF/RHD. Therefore, ARF/RHD should no longer be described as a disease solely triggered by GAS infection.

Group A Streptococcal M protein activates the NLRP3 inflammasome and triggers programmed cell death in macrophages
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Group A Streptococcus (GAS) is among the top 10 causes of infection-related mortality in humans. M protein, the most abundant protein on the GAS surface, is one of the best studied of all Gram-positive bacterial virulence factors. M protein extends from the GAS surface as hair-like fimbriae, and its structure, function, and immunology are unique among known virulence molecules. M1 serotype GAS strains are associated with invasive infections including necrotizing fasciitis and toxic shock syndrome. M proteins, including M1, can be released during infection by the action of neutrophil-derived granule proteases. Under physiological conditions, M1 protein is also naturally released from the GAS surface and can be detected at high concentrations in the extracellular medium. Here we report a novel property of released, soluble M1 protein in triggering programmed cell death in macrophages. M1 served as a second signal for caspase-1-dependent NLRP3 inflammasome activation, inducing maturation and release of proinflammatory cytokine IL-1β and macrophage pyroptosis. The structurally dynamic B-repeat domain of M1 is critical for inflammasome activation, which involves K⁺ efflux and M1 protein internalization by clathrin-mediated endocytosis. Comparisons in the production of IL-1β during infection in vivo between GAS and GASΔM1-infected mice demonstrated that when M1 is present, the production of IL-1β is higher. Accordingly, mouse intraperitoneal challenge showed that GAS soluble M1 is sufficient and specific for IL-1β activation, which may represent an early warning to activate host immunity against the pathogen. Conversely, in systemic infection, hyperinflammation associated with M1-mediated pyroptosis and IL-1β release could aggravate tissue injury.

Using Human Organotypic Skin to Model Severe Streptococcal Infection
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Necrotizing soft tissue infections (NSTIs) are severe infections that destroy skin and underlying tissue and are often caused by the bacterium Streptococcus pyogenes. In this EU-funded project, INFECT, the aim is to define host and bacterial derived components that contribute to the pathogenesis of NSTIs. Using an organotypic model of human skin, we explore host and bacterial-derived components that contribute to the pathogenesis at the local site of infection. The skin tissue model was exposed to different Group A streptococci isolates collected from NSTI patients enrolled in INFECT. Histological and immunofluorescence analyses of infected tissue models revealed structural damage of the stratified epithelial cell layer, and underlying tissue. Gram staining of the infected model tissue revealed bacterial dissemination throughout the entire tissue at 24h to 48h after infection. Analysis of tissue model supernatants revealed increasing IL-1β levels over the infection period, confirming results obtained in patients and a murine model of NSTIs. The further analysis of infected skin tissue models revealed the capability of GAS isolates to form biofilm, allowing studies on the requirements for biofilm formation in NSTIs. The human organotypic skin model has been further developed, involving studies on the role of macrophages and adipocytes in NSTIs. In summary, exploitation of the human organotypic skin model provides a novel powerful tool to study the pathogenesis of NSTIs and has contributed to the identification of IL1β as a potential key regulator in NSTIs, and biofilm to be considered as a potential complicating microbiological feature of GAS NSTI.

Nasopharyngeal infection by Streptococcus pyogenes requires superantigen-responsive Vβ-specific T cells
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The globally prominent pathogen Streptococcus pyogenes secretes potent immunomodulatory proteins known as superantigens (SAgs) which engage lateral surfaces of major histocompatibility class II molecules and T cell receptor (TCR) β-chain variable domains (Vβ). These interactions result in the activation of numerous Vβ-specific T cells, which is the defining activity of a SAg. Although streptococcal SAgs are known virulence factors in scarlet fever and toxic shock syndrome, mechanisms by how SAgs contribute to the life cycle of S. pyogenes remain poorly understood. Herein, we demonstrated that passive immunization against the Vβ8-targeting SAg streptococcal pyrogenic exotoxin A (SpeA), or active immunization with either wild-type or a non-functional SpeA mutant protects mice from nasopharyngeal infection; however, only passive immunization, or vaccination with inactive SpeA, resulted in high-titer SpeA-specific antibodies in vivo. Mice vaccinated with wild-type SpeA rendered Vβ8+ T cells poorly responsive, which prevented infection. This phenotype was reproduced with staphylococcal enterotoxin B, a heterologous SAg that also targets Vβ8+ T cells, and rendered mice resistant to infection. Furthermore, antibody-mediated depletion of T cells prevented nasopharyngeal infection by S. pyogenes, but not by Streptococcus pneumoniae, a bacterium that does not produce SAgs. Remarkably, these observations suggest that S. pyogenes utilizes SAgs to manipulate Vβ-specific T cells to establish nasopharyngeal infection.
Examining the effects of host-glycan expression patterns in Group A streptococcal disease

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Colonisation of the oropharynx is the initial step in Group A streptococcal (GAS) pharyngeal infection, facilitating continual asymptomatic carriage and disease spread. Over 600 million cases of pharyngitis are reported each year with approximately 15% of school children suffering from GAS pharyngitis in developed countries. The incidence rate in developing countries is 5 times higher. We have previously reported that the highly virulent M1T1 GAS clone mediates attachment to oral epithelial cells via M1 protein interaction with blood group antigen carbohydrate structures. Here, we have identified that like M1-type GAS, throat tropic GAS serotypes M3 and M12 have distinct and varying binding affinities for ABO blood group antigen and Lewis antigen structures. Investigation of GAS binding to oral epithelial cells suggests susceptibility to oral tract infection by GAS may correlate with phenotypic differences in host blood group antigen expression. Fucos, galactose, N-acetylgalactosamine and sialic acid are fundamental structures of blood group and Lewis antigen synthesis. Exoglycosidase treatment of primary human oral epithelial cells identified that terminal β1-3,4 galactose and α2-3 sialic acid structures mediate colonisation of M1, M3 and M12 GAS. Furthermore, the presence of α1-2 fucose and α1-3 N-acetylgalactosamine, determinants of H- and A-blood group antigens respectively, significantly alters the level of GAS attachment to oral epithelial cell surfaces. These data highlight how differences in host glycosylation patterns may affect GAS colonisation. Overall, this work may facilitate future studies to design multi-valent mimetics for blocking glycan mediated GAS colonisation of the upper respiratory tract.

Are you Streptococcus suis? : Characterization of streptococcal strains from ruminants

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Although S. suis attracts public attention as a major swine and human pathogen, this bacterium is sometimes isolated from other animals. In the diagnostic service of NIAH-NARO, we have encountered isolates from ruminants, which were identified as S. suis by commonly used methods for the identification of S. suis. However, in the isolates, S. suis-like bacteria were actually included, and this caused confusion in veterinary diagnostic laboratories. For accurate diagnosis of streptococcal diseases in ruminants, we re-identified 64 “S. suis” isolates from ruminants and their environments by S. suis-specific recN-PCR and 16S rRNA gene sequencing. We found that only 10.9% of them were authentic S. suis and that 94.6% of the isolates from the lesions of diseased animals were closely related to S. suis serotype 33 reference strain, which has recently been proposed as a novel species Streptococcus ruminantium (Tohya et al., in press). Because prevalence of S. suis and S. ruminantium in the tonsils of healthy cattle was comparable (29.7% and 18.8%, respectively) in our investigation in a meat inspection center, frequent S. ruminantium isolation from diseased ruminants implies that S. ruminantium more preferentially causes diseases in ruminants than S. suis. Unlike S. suis, potentially virulent clonal groups were not found by PFGE analysis. Draft genome sequence analysis of representative S. ruminantium isolates revealed the diversity of the cps gene clusters. In this study, we also developed a S. ruminantium-specific PCR. These tool and information will contribute to a deeper understanding and accurate diagnosis of streptococcal infections in ruminants.

Human Streptococcus suis infections in South East Asia: Do we have molecular evidence for transboundary zoonotic infections?

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Streptococcus suis is a major emerging zoonotic pathogen which causes over 1600 systemic infections in humans globally. Clinical syndromes include meningitis and sepsis which can be fatal due to septic shock in 13% of patients. Occupational exposure to pigs or pork and consumption of high risk pork dishes are reported risk factors for human S. suis infections in Asia. S. suis serotype 2 strains were detected in more than 65% of 188 tested samples of fresh blood pudding. S. suis isolated from these samples carried the virulence genes cps2J, mrp, epf, and sly. Serological studies in Vietnam suggested the existence of mild or sub-clinical S. suis infections in healthy people who may or may not have a history of pig exposure. Over 90% of human systemic infections with S. suis have been reported from Asia, mainly sporadic cases in Thailand and Vietnam. In recent years, more than a dozen systemic S. suis infections have been microbiologically confirmed in both Cambodia and Laos. Molecular analysis using multi-locus sequence typing (MLST) and pulse field gel electrophoresis (PFGE) suggest that S. suis isolates from patients from Cambodia (ST1) and Laos (ST104) share identical PFGE band patterns with those from Vietnam and Thailand, respectively. Cross-national pig trade between these countries exists in both formal and informal forms and may be correlated to these pig-related zoonotic infections. Analysis of whole genome sequence data may help to reveal the genetic relationships of these strains to provide further evidence for potential transboundary zoonotic transmission of S. suis.

Type I interferon induced by Streptococcus suis serotype 2 is strain-dependent and may be beneficial for host survival
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Streptococcus suis serotype 2 is an important porcine bacterial pathogen and zoonotic agent mainly responsible for sudden death, septic shock, and meningitis, with exacerbated inflammation being a hallmark of the infection. However, serotype 2 strains are genotypically and phenotypically heterogeneous with varying virulence levels. Though type I interferons (IFNs) are traditionally associated with important anti-viral functions, recent studies have demonstrated that they may also play an important role during infections with extracellular bacteria. Herein, the implication of IFN-beta in the S. suis serotype 2 pathogenesis, which has always been considered a strict extracellular bacterium, was evaluated using strains of varying virulence. This study demonstrates that intermediate virulence strains are significantly more susceptible to phagocytosis than virulent strains. Hence, subsequent localization of these strains within the phagosome results in recognition of bacterial nucleic acids by Toll-like receptors 7 and 9, leading to activation of the interferon regulatory factors 1, 3, and 7 and production of IFN-beta. Type I IFN, whose implication depends on the virulence level of the S. suis strain, is involved in host defense by participating in the modulation of systemic inflammation, which is responsible for clearance of blood bacterial burden. As such, when induced by intermediate, and to a lesser extent, virulent S. suis strains, type I IFN plays a beneficial role in host survival. Meanwhile, the highly virulent strain responsible for the 2005 human outbreak hastily induces a septic shock that cannot be controlled by type I IFN, leading to rapid death of the host.

Genomic changes in S. suis associated with clinical disease isolates
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Streptococcus suis is known as a pathogenic organism that causes respiratory disease and central nervous system disease in pigs, as well as meningitis in humans; however, it also lives without causing disease as a carriage species in pig tonsils. We used a bioinformatics approach to examine the genomes of 1,018 S. suis strains, which consisted of both clinical and non-clinical strains. Both types of strains are spread throughout the core genome phylogeny, which suggests that the evolution to cause clinical disease has occurred multiple times. We examined whether 24 independent instances of transitions to clinical disease were repeatedly accompanied by the acquisition of virulence genes, as well as examining related shifts in genome size and gene number. We found the transition to clinical disease agent is correlated with the amount of repetitive DNA, as well as with a loss of phage-related genes. As extensive changes to gene content and gene number implicate horizontal gene transfer, we examined gene phylogenies for each of over a thousand core genes to reveal the pattern of gene exchange within this
species. Our genomics approach provides insight into the divide between carriage and clinical isolates in S. suis; these patterns of genomic changes may help us understand a generalised evolutionary path to pathogenicity.

**Prediction of antimicrobial resistance phenotype from whole genome sequences in streptococcus suis**

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Streptococcus suis is an important zoonotic pathogen of pigs that has significant economic impact on the pig industry, animal welfare and human health. In UK pigs, it is the second largest cause of systemic disease. This coupled with the lack of effective vaccines has resulted in the heavy use of antimicrobials within the industry, especially in the water or feed of piglets and, is likely to increase the selection pressure for the development of resistance against antimicrobials.

Development of antimicrobial resistance (AMR) is an increasingly global concern and as such, monitoring of antimicrobial resistance in zoonotic pathogens such as S. suis is of relevance to infection control. However, unlike other streptococcal pathogens, no systematic characterisation of AMR genes and their role in AMR phenotype has been carried out. The current study aims to determine the genetic basis of antimicrobial resistance in S. suis and to build a predictive tool of antimicrobial resistance phenotype from whole genome sequencing data.

Using whole genome sequences from 667 isolates from the UK and Canada, along with minimum inhibitory concentrations for 17 antibiotics widely used antibiotics in the agricultural industry, we show that rates of antimicrobial resistance in S. suis are high. However, in many cases, the resistance phenotypes could not be fully explained by the known resistance genotypes. Therefore we have used genome wide association studies and have uncovered novel resistance mechanisms, which subsequently increased the effectiveness of the prediction tool.

**The prevalence and diversity of Streptococcus suis in the production and supply chain of pork in China and the UK**

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Streptococcus suis (S. suis), a global zoonosis of pigs, shows regional differences in prevalence of human associated disease for Asian and non-Asian countries. The isolation rate and diversity of S. suis on tonsils of healthy slaughter pigs in China and the UK were studied for effects of geography, temperature, pig age and farm type. The contamination of S. suis in Chinese markets were evaluated with the MPN-PCR method. S. suis isolates underwent analysis of molecular serotype, multilocus sequence type, minimum core genome grouping, and virulence-associated genotyping. Although we found no significant difference in positive isolation rates between Chinese and UK farms, the prevalences of serotypes associated with human disease were significantly greater in the Chinese collection (p = 0.002). A significant effect of temperature was found on the positive isolation rate and prevalence of human disease associated serotypes in both countries (China, p = 0.011; UK, p = 0.05). In the MPN results, the contamination rate of the samples from small abattoirs was up to 90%, higher than that from wet and super-markets (84% vs. 70%). The similar effect of temperature was found on the MPN value of S. suis in the pork samples. This study highlighted the widespread existence of S. suis in PSCP of China and UK. The significant effect of temperature on S. suis in living pigs and pig products sheds new light on geographic variations in human S. suis associated disease.

**Risk of acute rheumatic fever and acute post streptococcal glomerulonephritis following streptococcal infections of the throat and skin**

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The risk of acute rheumatic fever (ARF) and acute post streptococcal glomerulonephritis (APSGN) following streptococcal infection is still poorly quantified. While the elevated risk of ARF following group A streptococcus (GAS) pharyngitis appears certain, there are many unresolved questions, including the timing of this elevated risk; the relative importance of Group C/G infections; and the role of skin infections versus pharyngeal infections.
in initiating ARF and APSGN.

This study takes advantage of New Zealand's linked health data to investigate these relationships using two large patient datasets: Hospitalisation data for 2000-15 for a wide range of conditions, including pharyngitis and skin infections and laboratory test data for 2010-16 for throat and skin swabs that are positive and negative for GAS and Group C/G detections. These exposure data are then individually linked, via an encrypted patient number, to initial ARF and APSGN case data to detect these subsequent events and estimate rates and rate ratios. Preliminary analysis shows a strong positive relationship between having a GAS positive throat swab and the risk of ARF over the following 8-90 days (RR 5.53, 95%CI 3.96-7.72). There is no significant rise in risk during this same time-period following Group C/G positive isolates (RR 1.31, 95%CI 0.57-3.01).

Hospitalisation data show an increased risk of ARF and APSGN in the 90 days following hospitalisation for acute upper respiratory tract infection. The association with skin infection hospitalisations is equivocal. Comprehensive results will be presented at the meeting.

Necrotizing fasciitis: overview with special regards to new perspectives
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Necrotizing soft tissue infections (NSTIs) are life-threatening infections leading to destruction of fascia, ie, necrotizing fascitis (NF), and sometimes fat or muscles. NF incidence is estimated to 2-4 cases per 100 000 inhabitants per year. NF has a 20-30% mortality (28% in our 1996-2012 retrospective database) and heavy burden - 15% amputation and long-term disability in 30% of survivors -. Although GAS is the main agent involved, most of NF are considered polymicrobial (70-80% according to a 2010 classification). We performed a NGS prospective study of bacterial flora comparing culture, targeted metagenomic (TM) and Shotgun metagenomic (SM) in 34 patients who underwent surgical procedures: type I (digestive) NSTIs was predominant but types II and III were represented too. Combination of SM and culture seems the most valuable. We also found that « healthy areas » were colonized with the same bacteria than necrotic areas. Management is based on IDSA guidelines including prompt surgical debridement and broad spectrum antibiotics. The results of our still in revision Cochrane systematic review should not change those conclusions. Clindamycin is widely used in an attempt to better control toxic manifestations and the value of IVIG is still controversial with a negative RCT just published. Delay to surgery is a key factor of survival and surgery should be performed in centers experiencing NSTIs management (at least 3 NSTIs per year in our nationwide multilevel study). Since 2013, we organized a NF referral center in Paris area with a decreased mortality in our preliminary results.

A new fluorescent test system for the rapid detection of Group A streptococcus from throat swabs in a Point-of-Care setting
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Ellume has developed a digital test system for the rapid detection of both infectious and non-infectious disease, including Group A Streptococcus. The system is designed for use at the point-of-care i.e. physician’s office or pharmacy. The system has three distinct elements including a hand-held graphical user interface, reagents for sample extraction and neutralisation, and a disposable cassette that performs all of the diagnostics i.e. immunoassay, data acquisition and computation. The core of the technology within the cassette is an inexpensive but highly sensitive fixed point fluorometer that is coupled with exceptionally bright and highly functionalised multi-quantum dot fluorescent microparticles. We use an immunochromatographic test strip as the solid phase, with the fluorescent particles immobilised onto a polyester release pad that is connected to a porous nitrocellulose membrane with a test zone that is coated with specific antibodies and a reference zone. Using a nitrous acid extraction we have been able to detect less than 500 CFU of S. pyogenes in 7 min, which is best-in-class for rapid antigen-based detection. We have shown reactivity to a range of M types of S. pyogenes and no cross-reactivity to other Streptococcus species examined. In retrospective testing against 156 culture-confirmed throat specimens we demonstrated clinical sensitivity of 100% (64/64) and a specificity of 97.8% (90/92).

A large prospective clinical trial of the device is currently underway in 6 clinical sites across Australia. We expect to have performance data relative to both culture and an FDA-approved PCR by late 2017.

Clinical features of necrotizing soft tissue infections caused by beta-hemolytic Group A, C and G streptococci: analysis of the Scandinavian INFECT study cohort
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Methods: analyze their genetic diversity.

Background and Objective: The concept that a minority of Group A streptococcus (GAS) emm types are more “rheumatogenic” than others is widely disseminated. The objective of this study is to provide a comprehensive list of ARF-associated strains and analyze their genetic diversity.

Results: In total, around 400 patients were included. Approximately 1/3 was caused by GAS and 1/10 by GCS/GGS. The GAS cases were associated with younger age and less comorbidity. Preliminary data on the streptococcal cases indicate a 90-day mortality rate of 15-20%, a clear association of toxic shock syndrome and mortality and predominance of emm1 and other emm types previously associated with invasive disease. Details on the whole cohort of streptococcal cases will be presented. These include molecular and clinical characteristics and interrelations of comorbidity, clinical presentation, treatment and outcome.

Conclusions This large prospective multicenter cohort study confirms the high severity of streptococcal NSTIs observed in previous surveillance studies and retrospective single-center studies. The diversity of disease and possible prognostic factors are described.

Community-based performance of a molecular test for streptococcal pharyngitis

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Timely treatment of GAS pharyngitis prevents ARF. Accurate diagnosis limits antibiotic overuse. Throat culture (TC), the diagnostic standard, takes 18-48 hours. Rapid antigen tests (RTs) have inadequate sensitivity (Sens) to use alone. The Illumigene® Group A Streptococcus assay (Meridian Bioscience) uses loop-mediated isothermal amplification (LAMP) to identify GAS in ≤1 hour. Both Sens and specificity (Spec) were ≥95% in 3 hospital studies.

We enrolled children (3-18 yrs) at 5 community sites: 2 intervention sites (Ofc) used LAMP: Site1- LAMP alone; Site2- LAMP for back-up of negative RTs. Duplicate throat swabs went to our lab for TC and LAMP. Sens, Spec, PPV, NPV of Ofc and lab LAMP were calculated using lab TC or lab LAMP as reference. 3 control (Ctrl) sites used RT with TC back-up. Ofc and Ctrl time to diagnosis (TTD) and time to treatment (TRx) were measured.

Site1 enrolled 366 patients, Site2 31, Ctrl sites 232. 19.4% of patients had positive LAMP or TC. Using lab TC as reference, lab LAMP had Sens .97, Spec .95, PPV .88, NPV .99; and Ofc LAMP had Sens .75, Spec .88, PPV .71, NPV .90. Using lab LAMP as reference, Ofc LAMP had Sens .68, Spec .88, PPV .73, NPV .86. TTD and TRx for Ofc LAMP were significantly shorter than for Ctrl TC (each p<.0001).

LAMP results were available and used more rapidly than TC results. Compared to lab TC and LAMP, Ofc LAMP did not perform as well in published hospital studies.

Is the concept of rheumatogenic Group A Streptococcus a myth? A systematic literature review from 1944 to 2016 and a molecular analysis of the M-protein

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Background and Objective: The concept that a minority of Group A streptococcus (GAS) emm-type are more “rheumatogenic” than others is widely disseminated. The objective of this study is to provide a comprehensive list of ARF-associated strains and analyze their genetic diversity.

Methods:
All articles reporting ARF-associated strains or ARF-associated emm-type-specific antibody responses were identified in Pubmed from 01/01/1944 (first publication of Jones criteria) to 31/12/2016. The revised Jones Criteria (American Heart Association, 2015) were used to define ARF and a maximum time-period of four weeks between microbiological characterization and ARF onset was accepted. A database of 175 M-protein sequences was used to analyze the genetic diversity of ARF-associated strains in a PhyML phylogenetic tree. Geneious software was used to search for the presence of putative ARF-associated motifs (PARF motif and two proposed rheumatogenic peptides).

Results:
Thirty-six relevant studies were identified among 677 publications. 440 ARF-associated isolates belonging to 66 different emm-types were included in the analysis. The classical "rheumatogenic" emm-types represented 41% of the 440 ARF-associated isolates and 14% of the 66 identified emm-types. When the classical rheumatogenic emm-type were mapped by specific clade onto the emm-cluster-type phylogenetic tree, ARF-associated emm-types were disseminated along the tree suggesting ARF-associated strains belong to various genetic backgrounds. ARF-associated motifs (PARF or rheumatogenic peptides) were present in only 20 and 12% of the ARF-associated strains and emm-types, respectively.

Discussion:
The concept of "rheumatogenicity" should probably be extended to include strains other than those classically described. Further work is needed to understand the physiopathology of ARF.

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**Vaccine against S. pyogenes: where we are**

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StreptInCor, a candidate vaccine against S. pyogenes is based on protective 55 amino acids residues of C-terminal portion of the M protein. Experimental assays have demonstrated that the StreptInCor peptide induces high titers of opsonic and neutralizing and protective antibodies in outbred immunized mice. Using HLA class II transgenic mice, it was possible to evaluate the immunogenicity and safety of the StreptInCor vaccine epitope for a period of one year. Specific and non-auto reactive antibodies were produced and no autoimmune or pathological reactions were observed in the heart or other organs of these animals. We also performed several studies in mini-pigs in order to evaluate the immune response as well as safety by submitting these animals to echocardiogram examination before immunization and after the four doses treatment. No alterations were observed. StreptInCor vaccine also induces regulatory T cells (Treg) that strongly indicate that the vaccine peptide may have therapeutic potential to control both inflammatory and autoimmune response in RF/RHD patients.

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**Constrained strain-specific immune induction underlies the epidemic of streptococcal pyoderma: overcoming immune resistance through vaccination**

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The immunobiology underlying the slow acquisition of immunity to group A streptococci (GAS), is not understood.
but attributed to specific virulence factors impeding innate immunity and significant antigenic diversity of the type-specific M-protein, hindering acquired immunity. This results in extreme endemicity and very high rates of streptococcal-associated serious pathology, including rheumatic heart disease, post streptococcal glomerulonephritis and invasive GAS disease. We used a number of epidemiologically distinct GAS strains to model the development of acquired immunity to pyodermia. We show that infection leads to antibody responses to the serotype-specific determinants on the M protein and profound protective immunity; however, memory B cells do not develop and immunity is rapidly lost. Two sequential infections with the same strain within a short time frame were required to induce enduring strain-specific immunity. Sequential infections with different strains resulted in partial immunity only to the last strain to which they had been exposed and not to any previous strains. Mice exposed to multiple strains, either sequentially or simultaneously, did not develop antibodies to a conserved M protein vaccine peptide, J8, demonstrating that this epitope is cryptic to the immune system. However, in contrast to the lack of strain-specific immunity that follows infection, immunity following vaccination with J8 protects against multiple strains delivered sequentially or as a co-infection. Moreover, vaccine-induced immunity could be boosted by sequential heterologous infections. This highlights a major difference in how naïve and memory B cells respond to cryptic epitopes which could have important implications for vaccine research.

### Development of an opsonophagocytic killing assay for group A streptococcus

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Group A Streptococcus (GAS) or Streptococcus pyogenes is responsible for an estimated 500,000 deaths worldwide each year. Protection against GAS infection is thought to be mediated by phagocytosis, which can be greatly enhanced by bacteria-specific antibody. There are no licenced GAS vaccines, despite many promising candidates in preclinical and early stage clinical development. Progress has been hindered, in part, by the lack of a standardised functional assay suitable for vaccine evaluation. Current assays, developed over 50 years ago, rely on non-immune human whole blood as a source of neutrophils and complement. Variations in complement and neutrophil activity between donors result in variable data that is difficult to interpret. We have developed an opsonophagocytic assay (OPA) for GAS that utilises DMF-differentiated HL-60 cells and baby rabbit complement. As such, we have removed the major sources of variation in current assays. We have standardised the OPA for several clinically relevant isolates including emm-types 1, 6, 12, 53, 75, 89 and 100. In the presence of both baby rabbit complement and differentiated HL-60 cells, we have shown antibody-specific killing for each strain using a series of M-protein-specific sera. Antisera was generated through the immunisation of rabbits with recombinant full-length M-protein. This antibody-specific killing can be blocked through pre-incubation of the antisera with homologous antigen. This OPA assay has the potential to provide a reliable and reproducible platform to assess GAS vaccine candidates.

### In vitro and in vivo screening of potential vaccine candidates against prevalent Indian and Israel Streptococcus pyogenes serotypes

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Streptococcus pyogenes (GAS) is a pathogenic bacteria. It causes various suppurative diseases (like pharyngitis, impetigo, etc) and invasive diseases (like necrotizing fasciitis, toxic shock syndrome etc). There is heavy burden of GAS diseases in tropical region worldwide. It causes 616 million of pharyngitis and 1.78 million of severe disease each year. A 2013 survey stated that developing countries are suffering more severely than developed countries. Our strategy is to develop a universal vaccine against GAS using reverse vaccinology approach. First by combining Bioinformatics, Proteomics and Microarray data, we have identified 52 potential vaccine candidates which are either surface or secretory proteins. These 52 vaccine candidates expression was checked using qRT-PCR for both prevalent Indian serotypes (M1 and M49). Invasion assay along with qRT-PCR data revealed that M49 is more invasive as compare to M1. M49 is further used for in vitro Neutralization assay on HEP2 cells. Forty five sera were raised in mice against each recombinant protein candidate. Out of 45 sera candidates, 18 showed inhibition in adherence (>75%). FACS was used to determine whether the candidate is exposed on bacterial surface or not. Out of 18 sera, 10 protein candidates corresponding to sera were showing significant surface exposure in FACS. Four candidates selected by in vivo study are cloned, expressed and purified. Purified protein protection was checked in mice by in vivo survival assay using both Indian and Israel GAS. Functional immunization assays was used to determine immune response developed in mice immunized by vaccine candidates.

### Protocol for a group A streptococcal pharyngitis human challenge study

Joshua Osowicki¹,², Kristy I Azzopardi³, Ciara Baker¹, James B Dale⁴, Michael F Good⁴, Manisha Pandey⁴
Group B Streptococcus and the vaginal microbiome: dysbiosis, co-infection, and pathogenesis of Group B Streptococcus in pregnancy
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Group B Streptococcus (GBS) is a common member of the vaginal microbiome that can become pathogenic during pregnancy, causing invasive infections of the uterus, placenta, and fetus. GBS is more likely to colonize women who have an abnormal vaginal microbiota characterized by low numbers of healthy lactobacilli and overgrowth of other diverse anaerobic bacteria, most often Gardnerella vaginalis. African American women are more likely to be affected by an abnormal vaginal microbiota and are also at much higher risk of having a fetus/infant affected by invasive GBS disease. We developed a mouse co-infection model to investigate the hypothesis that G. vaginalis may play a role in GBS pathogenesis during pregnancy. Briefly, 10⁷ GBS was inoculated vaginally in the presence or absence of 10⁶G. vaginalis at e14.5 in mice carrying allogeneic pregnancies. Co-infected dams were more likely to be vaginally colonized with GBS and were also more prone to developing ascending infections of uterine and placental tissue. Interestingly, co-infected animals exhibited significantly smaller placentas and evidence of placental dysfunction, a phenotype that was independent of whether animals developed ascending infection. Together, the data show that G. vaginalis encourages vaginal colonization by GBS during pregnancy and suggests that G. vaginalis may lower the threshold for GBS pathogenesis. These data suggest that further investigation into the potential influence of the vaginal microbiota on GBS disease in women is warranted.

Mechanisms of ascending Group B Streptococcus infection
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Reduced incidence of neonatal early onset group B streptococcal infection after promulgation of guidelines for risk-based intrapartum antibiotic prophylaxis in Sweden: analysis of a national population-based cohort.
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Group B Streptococcus biofilm regulatory protein A (brpA) contributes to host cell adherence and resistance to innate immune clearance
neonatal disease by these pathogenic microbes. This work shows for the first time that C. albicans and GBS may interact synergistically to enhance colonisation of vaginal epithelium, and that surface proteins belonging to two major adhesin families play a critical role in this interaction.

In vitro assays show that C. albicans promotes association of GBS with vaginal epithelial cells, and vice versa. Furthermore, utilising knockout, complemented and heterologous expression strains, GBS antigen I/II family adhesins (BspA/BspC) and candidal hypha-specific adhesin Als3 are identified as critical molecular determinants of this process. Modulation of host responses by this association has also been explored.

This work shows for the first time that C. albicans and GBS may interact synergistically to enhance colonisation of vaginal epithelium, and that surface proteins belonging to two major adhesin families play a critical role in this association. Such interkingdom interactions may serve as novel targets to combat risk of both maternal and neonatal disease by these pathogenic microbes.
Public health response to an evolving Group A Streptococcal landscape in England
Theresa Lamagni, Manisha Pandey, Mehfuz Zaman, Therese Nordstrom, Victoria Ozberk, Yun Shi, Ainslie Calcutt, Emma L Langshaw, Jessica Powell, Mei Fong Ho, Zachary N Phillips, Thomas Haselhorst, Mark von Itzstein, Istvan Toth, Michael R Batzloff

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The development of vaccines for GAS will require technologies that enable induction of immune responses to multiple polymorphic epitopes or to a conserved epitope/s that are targets of protective antibodies. Synthetic peptides enable the use of minimal epitopes significantly reducing the chance of autoimmune pathology; however, peptides suffer from poor immunogenicity. Antibodies to a conserved minimal epitope, J8, from the M protein can kill multiple strains of GAS and protect from skin and mucosal infection even though J8 is cryptic and immunologically inert following infection. We defined an additional conserved sub-dominant epitope from SpyCEP, which, when combined with J8 provides enhanced protection, particularly against CovR/S mutants. We can deliver these immunogens via either injection or intranasal delivery and show that they protect against skin and URT infections and invasive GAS disease but require three immunizations to do so.

To enhance the immunogenicity of J8, we have undertaken a series of rational mutations leading to a novel peptide which is highly immunogenic, inducing protection after only a single immunization. Surprisingly, sera from vaccinated mice recognize the non-mutated parent peptide and bind the surface of GAS significantly better than sera from mice vaccinated with the parent peptide. Molecular dynamic simulation suggested that the mutations resulted in enhanced helical folding of the vaccine peptide, which may explain the its efficacy as a vaccine.

The results demonstrate a generic strategy that can enhance the utility of peptides as vaccines and strongly support the development of a GAS vaccine based on minimal epitope synthetic peptides.

Enhanced protection from skin, mucosal and invasive Group A Streptococcal disease following vaccination with native and mutated cryptic epitopes
Michael Good, Manisha Pandey, Mehfuz Zaman, Therese Nordstrom, Victoria Ozberk, Yun Shi, Ainslie Calcutt, Emma L Langshaw, Jessica Powell, Mei Fong Ho, Zachary N Phillips, Thomas Haselhorst, Mark von Itzstein, Istvan Toth, Michael R Batzloff

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Defining Group A Streptococcal Fitness Determinants Within In Vivo Environments
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Genome sequencing and transcriptome profiling have helped establish the genetic determinants expressed by a pathogen during infection; however, revealing genes that are “functionally required” for survival and fitness within the host is key to understanding pathophysiology. We have been applying a powerful genetic approach, transposon-sequencing (Tn-seq), to identify en masse genes important for fitness using relevant models of in vivo infection in the strict human pathogen Streptococcus pyogenes (Group A Streptococcus, GAS). GAS causes a wide array of disease manifestations ranging from self-limiting superficial infections of the skin & throat to severe invasive diseases of soft tissues & other normally sterile sites. Colonization of epithelia, followed by infection of the sub-epithelial tissue represents initial events during GAS infections. Using a near-saturation mariner (Krmit) transposon mutant library of the globally disseminated M1T1 GAS 5448, we performed Tn-seq to define essential (non-mutable) genes and assessed the genetic determinants of increased and decreased fitness during in vitro growth in rich media. We have now used Tn-seq to investigate the genes important for fitness and survival in lesion formation during soft tissue infection. The 5448 Krmit mutant library was inoculated subcutaneously into immunocompetent hairless mice and fitness was monitored by Tn-seq during abscess (24 h) and subsequent ulcerative lesion (48 h) formation in vivo. After comparison to our in vitro Tn-seq dataset, we were able to define and validate GAS 5448 genes required for fitness in lesions. One category of emphasis were genes annotated as “unknown function” that we renamed subcutaneous fitness (scf) genes. A two-gene operon (scfAB) encoding putative membrane proteins was identified in the screen and was found to be conserved amongst many important Gram-positive pathogens. Defined GAS 5448 mutants in scfAB were outcompeted by wild type GAS in vivo, were attenuated for lesion formation in vivo, and exhibited reduced survival in human blood. Continuing Tn-seq studies are exploring different GAS disease-relevant environments in vitro (minimal media, metals), ex vivo (blood, phagocytes, biofilm), and in vivo (colonization, invasive sites). By comparing and contrasting our Tn-seq screens, we can begin to reveal key pathways and genes that play a functional role during GAS infections as potential new therapeutic targets.

Recent outbreaks of Group A Streptococcal infection in North America: challenges and new opportunities

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Rates of invasive group A streptococcal (GAS) infections in the United States (U.S.) have increased from approximately 10,000-13,000 cases each year to nearly 19,000 and 1700 deaths in 2016. Concurrent and likely contributing to this upsurge has been an increase in previously uncommon strains such as emm types 26, 49, and 89—strains which may have been amplified through outbreaks among the homeless and illicit drug users in multiple urban areas. The proportion of persons with invasive GAS reported to the Centers for Disease Control and Prevention’s (CDC) laboratory- and population-based surveillance who are identified as using intravenous drug use has doubled from an average of 5% of cases over the last 10 years to 11% in 2016, concurrent with a national epidemic of opioid use. The U.S. has also seen an increase in reported small clusters and extensive outbreaks in long-term care facilities (LTCFs). Outbreaks among this vulnerable population and among illicit drug users are often initially unrecognized and difficult to control. CDC has begun using whole genome sequencing (WGS) as a tool to supplement outbreak investigations and our public health response to invasive GAS infections. We have developed an outbreak detection tool for use in our national invasive GAS surveillance covering a population of approximately 34 million persons; refinement and validation of the tool is ongoing. WGS has been used to help elucidate potential transmission chains in persistent LTCF outbreaks and may be useful in outbreaks among marginalized populations.

Identification of glycan receptors for streptococcal cholesterol dependent cytolysins.

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The cholesterol-dependent cytolysins (CDCs) are key virulence factors expressed by many pathogenic Gram-positive bacteria, including the Streptococci. The defining feature of CDCs is the ability to form pores in cholesterol containing membranes. Because the CDC’s cytolytic mechanism depends on the presence of cholesterol in the target cell membrane it was believed that cholesterol served as the cellular receptor for these toxins. Using glycan microarrays and surface plasmon resonance we have identified high affinity interactions between all CDCs examined (n=7) and glycan structures. These include the streptococcal CDCs: pneumolysin (PLY), streptolysin O (SLO), intermedilysin (ILY), lectinolysin (LLY) and suilysin (SLY). Using human red blood cell hemolytic assays we demonstrate that the identified glycan targets can inhibit CDC mediated lysis when provided in solution as free oligosaccharides, thereby supporting their role as cellular receptors. ILY is unique among the CDCs in that it has previously been reported to use the glycoprotein CD59 as a receptor. We demonstrate that removal of the O-linked glycan from CD59 reduced binding of ILY to CD59, indicating that the glycan component of CD59 is key for receptor recognition. Lectin activity is a widespread feature among the
Group A Streptococcal research in Fiji: 10+ years of data
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Background
The Fiji Group A Streptococcal Project (Fiji GrASP) began in 2005 with funding received from the National Institutes of Health (NIH) National Institute of Infectious and Allergic Diseases. It is a long standing collaborative research project between the Fiji Ministry of Health and the University of Melbourne Centre for International Child Health and other institutions. Subsequent funding has come largely from Cure Kids. Fiji GrASP has been the focal point for group A Streptococcal (GAS) related research in Fiji, with a focus on rheumatic heart disease (RHD) and acute rheumatic fever (ARF). Fiji GrASP has had a close relationship with the Fiji RHD Control Program.

Methods
Initial studies sought to describe the epidemiology (both clinical and molecular) of GAS disease in Fiji with a view to vaccine development. Subsequent studies have focused RHD epidemiology using echocardiograph as first line screening; a pilot study to teach nurses to undertake basic RHD echocardiography; evaluating the diagnostic accuracy of health workers briefly trained in focused cardiac ultrasound screening and secondary prophylaxis adherence and clinical outcomes of young people with screening-detected RHD. Other non-GrASP studies include skin and scabies related GAS and control strategies.

Conclusions
Fiji GrASP has been successful in collaborating with the Fiji RHD Program and other partners in conducting research to answer clinically relevant questions for the Fiji and has resulted in more than twenty publications since its inception and informed the Fiji RHD policy and best practice guidelines.

Functional conservation hidden within antigenic variability
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The antigenic variability of the M protein is attributable to selective immune pressure. However, within this variability, functional conservation also seems to be at play, as sequences vary among M types but are stable within the type. With >220 M types, it is difficult to imagine an equivalent number of functionally indispensable host factors with which M protein variable regions interact. Recent structural studies examining the interaction of four M protein HVRs with human C4b-binding protein (C4BP) offer an answer to this puzzle. These structures revealed that the α-helical coiled coils of the four M protein HVRs present a pattern of amino acids that are chemically complementary to a uniform and tolerant ‘reading head’ present on C4BP. Significantly, the C4BP-binding sequence pattern identified in the four structurally characterized M protein HVRs were found to be conserved among a much larger set of M protein HVRs. This conserved sequence pattern appears to be masked from immune recognition by divergence. The large number of variable amino acids surrounding the C4BP-binding pattern appears to divert the attention of the antibody response, resulting in a type-specific rather than a type-promiscuous response. However, suggestive evidence exists that certain antibodies can mimic the M type-promiscuous binding mode of C4BP, thereby providing broad neutralization. These results show that hidden within M protein variability are sequence patterns conserved for interaction with indispensable host factors.

Progress in Group B Streptococcal vaccine development
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Group B streptococcus (GBS) is a leading cause of perinatal and early life invasive infections. Existing control strategies based on bacteriologic or clinical screening-guided intravenous antibiotic prophylaxis are only partially effective, seldom implemented in low and middle income countries, and drive a high volume of perinatal antibiotic use. Evidence from observational studies about the role of maternal antibodies acquired following natural GBS exposure suggests that maternal immunization with multiple serotypes of protein-conjugated GBS capsular polysaccharides may reduce the risk of invasive disease. Protein-based vaccine candidates are also under evaluation. The World Health Organization recently highlighted priority activities in GBS vaccine development, strategic goals and preferred product characteristics (1, 2). Ongoing candidate vaccines development activities will be reviewed, and potential pathways to licensure and policy decision for global use will be discussed.

Group A Streptococcal disease: past, present and future
Andrew Steer

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Our understanding of group A streptococcal disease epidemiology, pathogenesis, diagnosis, management and public health control continues to expand. This presentation will highlight key milestones on the path to our current understanding, and will discuss future research areas necessary to achieve control of group A streptococcal disease including priorities for vaccine development. The presentation, with a focus on clinical group A streptococcal disease, will provide a bookend to the 20th Lancefield Symposium, incorporating information presented over the course of the meeting where possible and where appropriate.

Expression of the Group B Streptococcus cyl operon in Lactococcus lactis confers hemolysin production
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Group B Streptococcus (GBS) are β-hemolytic Gram-positive bacteria frequently associated with fetal injury, preterm birth, spontaneous abortion, and neonatal infection. GBS disease remains a major global health problem, as current antibiotic-based prevention strategies have limitations. In order to develop improved, rationally designed prevention strategies for GBS infection and disease, a comprehensive understanding of GBS virulence factors is essential. A key virulence factor for GBS is the unique pigmented ornithine rhamnolipid, which confers hemolysis and induces cytotoxicity in a number of host cells, including neutrophils, monocytes, macrophages, and amniotic cells. Here, we add to the body of knowledge about the GBS hemolytic pigment toxin by showing that heterologous expression of the GBS cyl operon in non-hemolytic Lactococcus lactis results in hemolysis, pigmentation, and cytotoxicity to neutrophils. We isolated the hemolytic pigment toxin from cyl-complemented L. lactis and performed NMR analysis and hemolytic assays, which confirmed the presence of the active hemolytic ornithine rhamnolipid in this strain. Together, these findings provide evidence that the cyl operon is sufficient for the production of the active GBS hemolytic pigment toxin in a non-pathogenic Gram-positive bacterial strain. Further studies are in progress and will reveal new information about this unique toxin.

Dendritic cells are susceptible to death in response to hyperpigmented Group B Streptococcus
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Group B Streptococcus (GBS) is the leading cause of bacterial infections in utero and can cause preterm birth, fetal injury, stillbirth and infections in newborns. The mechanisms by which GBS causes these outcomes are largely unknown. We have previously reported that purified GBS pigment toxin causes hemolysis and cytolysis of certain immune cells such as mast cells and neutrophils, which contributes to disease. Previous studies have demonstrated GBS-induced dendritic cell (DC) death by pyroptosis, but little is known about whether this effect is mediated by the GBS pigment toxin.

DC are professional antigen presenting cells (APC) which provide a crucial link between the innate and adaptive immune systems and ensure that adequate immune responses are initiated in response to foreign antigen. Their role is especially important in maintaining healthy pregnancy.
Here, we show that GBS infection in vitro induced death in murine bone marrow-derived DC in a pigment toxin-dependent manner, as evidenced by lactate dehydrogenase (LDH) and IL-1β release. When phagocytosis was blocked by cytochalasin, DC death increased. Interestingly, stimulation of DC with purified pigment toxin in the absence of bacteria led to propidium iodide (PI) but not annexin V uptake or LDH or IL-1β release. It is possible that this discrepancy is due to DC plasma membrane repair, therefore allowing DC to resist pigment-mediated lysis. Further studies will investigate the impact of pigment toxin-induced DC death on GBS pathogenesis, as well as roles of caspase-1 and cell death signaling pathways in this process.

Vaginal viridans streptococci and Lactobacilli have synergistic inhibiting effect on group B streptococci
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Background: The complex vaginal flora is not only harbouring lactobacilli but also viridans group streptococci (VGS). Some lactobacilli have an inhibitory effect on group B streptococci (GBS) and other pathogenic bacteria in vitro. If other commensal vaginal bacteria also have inhibiting effect on GBS is less well known.
Material and methods: Clinical isolates of vaginal lactobacilli and VGS from healthy women were collected. In vitro methods like agar over-lay, PBS supernatant test and co-culturing in broth was tested under different pH conditions. A pilot test on two women with vaginal colonization with GBS using a vaginal crème with Lactobacillus plantarum and Streptococcus oralis during a three week period was also performed.
Results: Several isolates of Lactobacillus sp. could inhibit growth and also kill GBS in the in vitro test. However, when pH was higher than pH 6.0 this inhibiting effect was less effective. Some isolated VGS could also inhibit growth of GBS and the results were not depending on pH. There was also a synergistic killing effect of Lactobacillus plantarum strains together with several VGS strains (S.sanguinis, S.oralis, S.salivarius). The two GBS colonized women had negative GBS cultures in vaginal samples after using a cream with Lactobacillus plantarum and Streptococcus oralis.

Conclusion: Commensals like Lactobacilli are important for a healthy balance in the vaginal microbiota. Should also VGS be considered in this respect? Some of these strains can inhibit growth of GBS in vitro and together with Lactobacillus plantarum have synergistic killing effect.

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Although more than 30% of the Egyptian females carry SGB in the vagina, E.coli and Enterobacteriaceae species are the leading cause of neonatal infections. SGB strains are found now to be an important invasive pathogen in the Egyptian adults between community acquired patients.

A total of 99 SGBs were isolated from 51 male and 48 female patients, serotypes 1a, 11, 111, and V were the predominant types accounting for 83% of the isolates, of which 25% were type V. The 17% rest were belonging to types 1b, 1V, V1, V11 and V111. 31 SGB serotypes 1a, 1b, 11, 111 and V were isolated from urinary tract infection patients. 25 SGB serotypes 1b, 11, V and V11 were isolated from male patients suffering from prostatitis and urethritis. 12 SGB serotypes, 1111, V and V1 were isolated from male patients with diabetic foot infection and 10 SGB serotypes 1a, 11, 111, V and V1 from the female patients, of which 80% from both patients were types 11 and V.

Antibiotic susceptibility test for SGB was performed by disc diffusion method and the zones of inhibition were measured according to the CLSI regulations. All strains were sensitive to penicillin, ampicillin, vancomycin and linezolid. 4 strains were resistant to macrolides only and 10 were MLS resistant. 17% were resistant to quinolones and 94% were resistant to tetracyclines.

The presentation and analysis of results will be presented.

Erythritol, a polyol (sugar alcohol), enhances Group B Streptococcus virulence
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Group B streptococci (GBS) causes invasive disease in neonates and adults. A cofactor we hypothesize to be associated with GBS virulence in neonates are polyols. Polyols (of which erythritol is a member) are sugar alcohols that have been found in the amniotic fluids of humans. The effect erythritol has on GBS virulence was investigated. GBS were grown in varying concentrations of erythritol (0%, 1%, 2% and 4%), bacteria, harvested and a collection of virulence phenotypes assayed. The expression of phosphoglycerate kinase (PGK) compared to 0% erythritol (120%, 122 and 134%, respectively). The antiphagocytic activity of GBS showed no significant difference in growth in the various concentrations of erythritol used (P=0.69 with 1%, P=0.63 with 2% and P=0.51 with 4%). GBS growth in the presence of 1%, 2% and 4% erythritol enhanced the expression of GBS-PGK compared to 0% erythritol (120%, 122 and 134%, respectively). The antiphagocytic activity of GBS in 1%, 2% and 4% erythritol was significantly increased compared to 0% (P=0.026, P=0.019 and P=0.0002, respectively). Also, GBS invaded the HeLa cells at a higher rate (164%) in 1% erythritol than in 0% erythritol.

In summary, the presence of erythritol (a polyol) in culture media enhances the virulence of GBS. This suggests that erythritol may play a role in GBS pathogenesis during neonatal infection.
Results: The overall prevalence of GBS carriage was not statistically different between HIV-infected and uninfected pregnant women (PW). Methods: GBS isolates were serotyped and genome sequenced. Case reviews and enhanced surveillance for additional GBS LOD cases were undertaken, coupled with weekly rectal screening, supported by genome sequencing.

Results: Over 2 years, 12 cases of GBS LOD were detected. The first cluster comprised serotype V GBS isolates of multi-locus sequence type (ST)1 carrying tetM and ermB resistance genes. Comparison with contemporaneous ST1 data showed neonatal and adult invasive GBS to be intermixed. Subsequent clusters of GBS LOD were identified and confirmed genomically, due to serotype Ib, serotype III, and serotype la. Overall, genomic analysis revealed that 11/12 (>90%) GBS LOD cases were linked to at least one other LOD case, highlighting that horizontal transmission in the neonatal setting was the most common mode of acquisition for GBS LOD.

Conclusion: Acquisition routes for GBS in the nosocomial setting are poorly understood. Large-scale genomic databases that include longitudinally collected data from both disease-associated and carriage isolates can provide context for outbreak investigation and allow more certainty regarding transmission events. Our experience suggests that a single case of LOD arising in hospital should be considered as a potential nosocomial transmission event warranting immediate investigation with heightened infection prevention vigilance and action where required.

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Quantifying the burden of Group B Streptococcus as a cause of surgical site infection in England
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Background
Population surveillance of invasive GBS infection has identified an increasing incidence of adult disease in many countries. As a means to understand the risk factors for adult disease, we undertook a study to quantify the burden in patients undergoing surgery in England.

Methods
Public Health England National surgical site infection (SSI) surveillance data for patients undergoing 18 categories of surgery were extracted and analysed to assess the contribution of GBS to the overall burden of SSI. Data were prospectively collected by hospitals using standardised active surveillance methodology identifying infections within 30 days of surgery (1y for surgery for prosthetic implants). This study was supported by a grant from Pfizer Inc.

Results
Surveillance data on 961,414 patients undergoing surgery between 2008 and 2015 were submitted by 239 participating hospitals. Preliminary findings identified 215 SSIs due to GBS of which 44% were superficial incisional infections, 37% deep incisional and 19% organ/space infections. The median time to onset of GBS infection in surgery with implant was 21 days (IQR 13-48d) and 10 days for surgery without prosthesis (IQR 6-14d). Of the SSIs with microbiological diagnosis (n=11,409), GBS was most common in Caesarean section accounting for 48.4% of SSIs followed by abdominal hysterectomy (4.70%) and hip replacement (2.58%).

Conclusion
Our preliminary findings have highlighted the relative importance of GBS as a cause of post-surgical infection, including in several high volume categories of surgery. Further assessment to assess the proportion potentially preventable should be undertaken.

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Prevalence and characteristics of group B Streptococcus colonization in HIV-infected pregnant women in Belgium
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Objectives: High incidence of GBS sepsis has been reported in HIV-exposed but uninfected (HEU) infants in both developed and developing countries, particularly late-onset diseases. We aimed at determining the prevalence, the characteristics and the risk factors of GBS carriage in HIV-infected and HIV uninfected pregnant women (PW).

Methods: Between 1/01/2011 and 31/12/2013, HIV-infected (n=132) and uninfected (n=123) PW had recto-vaginal swabs for GBS detection performed at 35-37 weeks of gestation and at delivery. Demographic, obstetrical and medical data related to HIV-infection were prospectively collected. Serotyping of GBS strains was performed on a limited number of randomly selected samples (26 from HIV-infected and 13 from uninfected PW).

Results: The overall prevalence of GBS carriage was not statistically different between HIV-infected and uninfected
Update of the characteristics of Group B Streptococci (GBS) colonizing pregnant women in Belgium: capsular-type distribution, pili characterization, antimicrobial susceptibility profile and Multiple Locus Sequence Types.

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Aim: Improving knowledge and characterization of GBS strains colonizing pregnant women in Belgium.

Methods: In 2013, collection of 387 strains of GBS from 80 laboratories participating in a national survey among pregnant women. For each strain, determination of capsular-polysaccharide type by agglutination and PCR, of pili-type by PCR and of antimicrobial susceptibility by disk-diffusion, broth-microdilution and detection of resistant genes by PCR. For serotype III strains, determination of sequence-type by Multiple-Locus Sequence-Typing (MLST).

Results: Serotype III was the most prevalent (28.5%) followed by serotypes V, Ia, II, IV and Ib (20.4%, 19.9%, 17.8%, 7%, 5.4%). Serotypes VI, VII and IX were found each once. All strains remained susceptible to penicillin (MICs: 0.03-0.125 mg/L) and other beta-lactams tested: 28.7% were resistant to erythromycin and 26.7% to clindamycin. With regards to pili, all 387 strains harboured one of the PI-2 variants alone or in combination and 70.3% contained PI-1. The 110 serotype III isolates were resolved into 18 STs. The most common were ST-17 (35.5%) followed by ST-19 (30%) and ST-529, ST-27, ST-23 (<5%).

Conclusion: Among GBS from colonized pregnant women in Belgium: capsular-type and pili distributions, and MLST profile among type III strains were quite similar to reported data from Europe and USA during the last decade. As showed in this study, penicillin remains the first line drug of choice. On the contrary, resistance rates against macrolides/lincosamide, has reached a plateau since a decade, but it is noteworthy to notify the emergence of strains with isolated resistance to clindamycin.


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Methods: A multiplex-PCR, using a set of specifically designed or already described (Kataja, 1999; Malbruny, 2011) primers was developed and used to detect, in GBS, three genes for erythromycin-resistance, ermB, ermTR, mefA and one gene for clindamycin-resistance lsaC. AdhP gene amplification was used as control for GBS identification. All(219) GBS isolates from invasive infections in newborns and adults received by the Belgian National Reference Center for GBS in 2015, and control strains were tested for erythromycin/clindamycin susceptibility (disk-diffusion/broth-microdilution) and for detection of resistance genes.

Results: PCR products demonstrated the expected respective sizes. The method has been validated successfully according to ISO15189 analytical requirements. Of the 219 isolates, 67(30.67%) were resistant to erythromycin and/or clindamycin: 44/67(65.78%) showed a constitutive-MLS phenotype and 10/67(14,9%) the inducible-MLS phenotype. Among the constitutive-MLS strains, 73% harboured ErmB gene, 13% ErmTR, 7% ErmB+mefA and 7% ermB together with LsaC gene. The inducible-MLS strains harboured mostly ErmTR gene (89%) and the others the ErmB gene. Among the 10/67(14,9%) GBS strains with an M-phenotype (isolated resistance to erythromycin), the MefA gene was exclusively detected. Among the 3(4,48%) strains showing an isolated resistance to clindamycin (L-phenotype), the LsaC gene was detected.

Conclusion: The developed multiplex PCR is able to detect simultaneously four genes involved in MLS resistance in GBS. In 2015, 30.6% of the invasive GBS strains isolated in Belgium were resistant to macrolides and/or lincosamides. The emergence of the L-phenotype in GBS described since 2010, justifies the relevance to also detect LsaC gene together with ErmB, ErmTR and MefA.
Conclusions: Higher quality studies compared with light colonisation. Higher in heavily colonised mothers, and EOGBS was up to 15 times higher in heavily colonised neonates, with a pooled risk ratio (RR) = 1.51, 95% confidence interval (CI) 1.12-2.03, and serotype III had a higher risk of developing EOGBS than other serotypes (RR = 1.95, 95% CI 1.10-3.45). In twelve studies, neonatal colonisation was approximately 2% of women and newborn babies. In neonates, GBS causes early onset and late onset disease characterised by sepsis, pneumonia, septicaemia and meningitis. Ten to forty percent of pregnant women in the world are colonised by GBS. Intrapartum antibiotic prophylaxis has led to a significant reduction in early onset disease. However, increase in drug resistant microorganisms has become a major threat. Vaccine development has been elusive and slow hence need to explore new and safer alternatives to treatment. Benzylpenicillin, Ampicillin, Cefotaxime, Ceftriaxone, Levofloxacin, Erythromycin, Clindamycin, Linezolid, Vancomycin, Tetracycline, Cotrimoxazole and Olea europaea plant extracts have exhibited significant potential for application as alternative treatment options for GBS.

Results: Among the 888 swabs, 111 were positive for GBS, that is a prevalence of colonisation of 12.5%. A total of 90 strains were available for typing: 91.1% could be serotyped by latex agglutination and all the strains, including the 8 phenotypically non-typable strains, were successfully genotyped. CPS type V was the most prevalent (36.7%) followed by CPS types Ib (25.6%), III (21.1%), VI and VII (8.9% and 4.4%). CPS type II was found twice and serotype Ia was found once. CPS types IV, VIII and IX were not present in this population.

Conclusion: With predominance of types V, Ib and III, this distribution of CPS-types of GBS colonizing pregnant women in Hanoi, Vietnam, differs from distributions described in Europe and in other Asian countries. This study provides useful information for the development of a universal vaccine that could contribute to improve the prevention of neonatal GBS infections.

Susceptibility Testing of Streptococcus agalactiae isolates from pregnant women to indigenous and conventional antimicrobials

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Streptococcus agalactiae also known as Group B streptococcus (GBS) is a major cause of disease in pregnant women and newborns. In neonates GBS causes early-onset and late-onset disease characterised by sepsis, pneumonia, septicaemia and meningitis. Ten to forty percent of pregnant women in the world are colonised by GBS. Intrapartum antibiotic prophylaxis has led to a significant reduction in early onset disease. However, increase in drug resistant microorganisms has become a major threat. Vaccine development has been elusive and slow hence need to explore new and safer alternatives to treatment. Benzylpenicillin, Ampicillin, Cefotaxime, Ceftriaxone, Levofloxacin, Erythromycin, Clindamycin, Linezolid, Vancomycin, Tetracycline, Cotrimoxazole and Olea europaea plant extracts and essential oils were tested against GBS isolates, isolated from pregnant women. All isolates showed >100% sensitivity to benzylpenicillin, ampicillin, ceftriaxone, levofloxacin, linezolid and vancomycin. Only one isolate was resistant to cefotaxime. Thirty six isolates and sixteen isolates were resistant to clindamycin and erythromycin respectively. All isolates tested negative for the genes coding for beta lactamases. GBS isolates showed sensitivity to Olea europaea extracts at low minimum inhibitory concentrations. Beta lactams are still the drugs of choice for treatment of GBS disease. However, the extracts of Olea europaea have exhibited significant potential for application as alternative treatment option for GBS.

Key words: Olea europaea, Streptococcus agalactiae, antibacterial, Beta-lactams.

Bacterial load and antibiotic resistance of neonatal Group B Streptococcus (GBS): A systematic review

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Background: GBS is the leading cause of morbidity and mortality from neonatal sepsis. Vaginal GBS colonisation during labour can cause GBS transmission to neonates in 36% of women, and this can progress to early-onset GBS disease (EOGBS, <7 days) in 1-3% of colonised neonates. We conducted a systematic review investigating whether bacterial load and antibiotic markers are associated with GBS vertical transmission, neonatal GBS colonisation, and EOGBS.

Methods: We searched Medline, Embase, Cochrane, and Science Citation Index from inception to 10th October 2016 for observational studies in English. We also hand-searched reference lists of publications and experts cross-checked included studies. Two reviewers independently conducted study selection, data extraction, and quality assessment. Random-effects meta-analyses were conducted where possible.

Results: 15 studies were included from 1107 records. In addition to bacterial load, molecular markers investigated were c-protein antigens, serotypes, and sequence types. Most evidence was published before 2000 and at risk of bias. Meta-analyses showed that neonates colonised by serotype III had a higher risk of developing EOGBS than neonates colonised by serotype Ia (pooled risk ratio [RR] = 1.51, 95% confidence interval [CI] 1.12-2.03) and serotype II (RR = 1.95, 95% CI 1.10-3.45). In twelve studies, neonatal colonisation was approximately 2-3 times higher in heavily colonised mothers, and EOGBS was up to 15 times higher in heavily colonised neonates, compared with light colonisation.

Conclusions: Higher quality studies are needed to assess the predictive value of pathogen subtype and heavy colonisation.
bacterial load; they might be suitable for better-targeted prevention.

### Adverse outcomes in women and children who have received intrapartum antibiotic prophylaxis (IAP) treatment: A systematic review

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**Background:** Adverse outcomes from IAP are essential to inform clinical practice for neonatal group B Streptococcus (GBS) prevention, yet they are poorly documented. This review synthesised evidence on adverse outcomes in the mother and/or her child after IAP.

**Methods:** Medline, Embase, Cochrane, and Science Citation Index were searched from inception to October 16th 2016. Reference lists of relevant studies were hand-searched. Primary studies in English that reported any adverse outcomes from intrapartum antibiotics for any prophylactic purpose compared to controls were included. The search was not restricted to GBS prophylaxis but excluded symptomatic women and caesarean sections. Two reviewers independently conducted study selection, data extraction, and quality assessment. Results were narratively synthesised in text and tables.

**Results:** 30 studies were included from 2,364 records. A wide range of adverse outcomes were reported in 17 observational studies and 13 randomised controlled trials (RCTs). However, the evidence was at high risk of bias and inconsistent. One RCT investigated the long-term outcomes of IAP reporting serious outcomes such as cerebral palsy; however, it had uncertain biological plausibility and limited applicability. In seven observational studies, IAP for GBS colonisation altered infant microbiota. However, study populations were not followed to clinical outcomes, thus clinical significance is unknown. Finally, observational evidence showed increased antimicrobial resistance, however studies were at risk of bias.

**Conclusions:** The evidence base to quantify adverse outcomes from IAP for neonatal GBS prevention is limited. Better-quality and longitudinal observational studies across countries with widespread IAP are needed, as RCTs might not be feasible.

### A comparison in the international trends of neonatal group B Streptococcus (GBS) under different GBS prevention strategies

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**Objective:** We compared trends in incidence rates (per 1,000 livebirths) of early-onset GBS (EOGBS), late-onset GBS (LOGBS), and percentage of GBS cases resistant to clindamycin and erythromycin under different GBS prevention strategies across countries.

**Methods:** We collected data on annual GBS rates (outcomes), current prevention strategy (predictor), and contextual factors (confounding variables) in a survey sent to institutions across countries. Some contextual data was supplemented from international websites and some observations for contextual variables were imputed using multiple imputation. We analysed the association between prevention strategy and trends of outcomes using linear regression models, where prevention strategy was interacted with year, and along with the contextual variables, was regressed on each outcome. We also summarised the outcome trends by world region using unadjusted regression.

**Results:** Overall, data covered >40 areas from 1989-2015. We found a difference in GBS trends by current prevention strategy. EOGBS decreased under screening or both screening and risk-based prevention, and increased under risk-based or no prevention. The largest increase of LOGBS was under no followed by risk-based prevention, while screening had the smallest increase. These findings were finely balanced as sensitivity analyses produced different results. There were no differences in resistance trends possibly due to a lack of power, even though the percentage of neonatal GBS cases resistant to erythromycin increased higher under screening compared to other prevention strategies.

**Conclusions:** Adjusting for contextual differences, trends of neonatal GBS are internationally affected by prevention strategy. However, findings should be treated cautiously as results depend on many factors.

### The role of Streptococcus agalactiae CovR in urinary tract infection

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**Background:** Adverse outcomes from intrapartum antibiotics for any prophylactic purpose compared to controls were included. The search was not restricted to GBS prophylaxis but excluded symptomatic women and caesarean sections. Two reviewers independently conducted study selection, data extraction, and quality assessment. Results were narratively synthesised in text and tables.

**Methods:** We collected data on annual GBS rates (outcomes), current prevention strategy (predictor), and contextual factors (confounding variables) in a survey sent to institutions across countries. Some contextual data was supplemented from international websites and some observations for contextual variables were imputed using multiple imputation. We analysed the association between prevention strategy and trends of outcomes using linear regression models, where prevention strategy was interacted with year, and along with the contextual variables, was regressed on each outcome. We also summarised the outcome trends by world region using unadjusted regression.

**Results:** Overall, data covered >40 areas from 1989-2015. We found a difference in GBS trends by current prevention strategy. EOGBS decreased under screening or both screening and risk-based prevention, and increased under risk-based or no prevention. The largest increase of LOGBS was under no followed by risk-based prevention, while screening had the smallest increase. These findings were finely balanced as sensitivity analyses produced different results. There were no differences in resistance trends possibly due to a lack of power, even though the percentage of neonatal GBS cases resistant to erythromycin increased higher under screening compared to other prevention strategies.

**Conclusions:** Adjusting for contextual differences, trends of neonatal GBS are internationally affected by prevention strategy. However, findings should be treated cautiously as results depend on many factors.
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Up to 50% of humans experience urinary tract infections (UTI) during their life. Group B Streptococcus (GBS; Streptococcus agalactiae) can colonize the urinary tract and cause UTI in healthy adults, elderly and immunocompromised individuals. The mechanisms and virulence factors that influence GBS UTI are not fully elucidated. The CovRS two-component system regulates hundreds of genes in GBS, comprising a response receiver (CovS) and the DNA binding protein (CovR). Regulatory targets of CovR include cytolysins, adhesins and capsule polysaccharide. Prior studies associate mutation in covR with hyper-virulence, partly due to the overexpression of the GBS β-hemolysin/cytolysin (β-h/c).

We show that CovR enhances the ability of GBS to cause acute UTI. Mutation in covR perturbed GBS colonization of the bladder and urine in a mouse UTI model, and reduced the levels of adherence towards and invasion of human bladder uroepithelial cells. Mutation in covR also caused increased b-h/c activity, death of uroepithelial cells and caspase-3 activation. We saw altered immune responses during our in vitro and in vivo experiments for a number of pro-inflammatory cytokines and chemokines, including IL-6, IL-17A, GM-CSF and KC/IL-8, in a covR-dependent manner. Examination of several known CovR-regulated virulence factors revealed the adhesin gene hvgA partly explains the complex role of CovR in promoting UTI.

Collectively, these data highlight CovR as a central regulator of GBS virulence that influences the pathogenesis of UTI. We conclude the mechanisms underlying CovR-dependent phenotypes in UTI are complex and multifactorial due to the pleiotropic nature of the CovRS system in GBS.

Isolation and Partial Biochemistry Characterization of Surface Immunological Protein from a Chilean-isolated Bacterial Strain of Group B Streptococcus

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Background: The Group B Streptococcus (GBS) is the major etiologic agent of neonatal sepsis and meningitis around the world. Our laboratory isolated a Chilean GBS bacterial strain from a newborn with septicemia (NCBI code KU736792). We cloned the Surface Immunogenicity Protein (SIP) gene and expressed it in E. coli cells. The SIP is present in all GBS serotypes and could be a good target for vaccine and detection methods. Aim: Purify and perform partial biochemistry characterization of SIP.

Methods: The DNA of the SIP gene was sequenced and the SIP purification was carried out by low and high-pressure Liquid Chromatography (LPLC and HPLC). Purified SIP was analysed by SDS-PAGE, Western blot, MALDI tag chromatograph and the molecular weight was estimated as 53 kDa. The MALDI-TOF MS analysis corroborated the protein’s identity. The SIP was analysed by SEC and we observed a homodimer, but in presence of 6M urea, it was dissociated to a monomer. Conclusion: We purified the SIP isolated from a Chilean GBS bacterial strain. The preliminary experimental observation indicates that the protein was expressed like a homodimer and that the real molecular weight is lower than reported to date. The biologic function of this protein is not yet understood and a study of the secondary protein structure is under development.

The Streptococcus suis serotype 2 lipoproteins, but not the lipoteichoic acid, are important activators of the innate immune response

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Streptococcus suis serotype 2 is an important porcine bacterial pathogen and zoonotic agent responsible for sudden death (pigs), septic shock (humans), and meningitis (both species). Alongside peptidoglycan, lipoteichoic acids (LTA) are a major constituent of the Gram-positive cell wall and have been previously suggested to contribute to the virulence of S. suis. Moreover, lipoproteins (LP), which are often co-purified with LTA, are considered to be major activators of the innate immune response. However, the immunostimulatory properties of the S. suis LTA, taking into account the potential presence of contaminating LPs, have not been investigated in detail. Herein, LTA preparations from S. suis were extensively purified and used to stimulate dendritic cells (DCs), which are sentinel innate immune cells implicated in the S. suis infection. Results demonstrated that LTA preparations induce important and dose-dependent levels of the pro-inflammatory mediators TNF, IL-6, CCL3, and CXCL1 from DCs. In order to evaluate the role of contaminating LPs, preparations were treated with H2O2, which oxidizes LPs to render them biologically inactive. Treatment resulted in a significant reduction of pro-inflammatory mediator production, indicating that contaminating co-purified LPs are the main source of DC activation. In accordance, DCS deficient for the Toll-like receptor 2 (TLR2), which is the main receptor involved in the recognition of bacterial LPs, produced significantly lower levels of cytokines. In conclusion, this study demonstrates that the S. suis surface LPs are important inducers of pro-inflammatory mediators by DCs through TLR2 activation, unlike the LTA.
The prevalence of Streptococcus suis in tiet canh, a traditional raw pig blood dish in Vietnam
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S. suis is an emerging zoonotic agent capable of causing severe invasive disease in human. As consuming raw pig products is the most important risk for human infection in Vietnam, this study was carried out to investigate the existence of viable S. suis cells in tiet canh – a popular raw pig blood dish - and to examine the potential virulence of all isolates and their resistant profile toward critical antimicrobials. A total of 188 tiet canh samples bought from local food shops in 4 cities around Vietnam and prepared at home for traditional family celebrations were collected. The result indicated that 180 (95.7%) were found positive for S. suis of which 124 (65.9%) were positive to S. suis serotype 2. The Ct value ranged from 23 to 39 (median=36), corresponding to the predicted target bacterial load in enriched samples of 1x10^6 to 21.4x10^8 cells/ml. A total of 32 S. suis strains, six of which were serotype 2, were successfully isolated from 29 samples (15.4%). For strain characterization, 21/32 (65.6%) possessed at least 4 virulence factors indicating some level of virulence in causing diseases in human and for the first time, full resistance toward antimicrobials used to treat S. suis in human (penicillin and ceftriaxone) were reported. These findings provide concrete practical evidence confirming tiet canh is a high-risk dish for human S. suis infection and demonstrate the role of raw food as a reservoir of antimicrobial resistant bacteria that can be transferred to and cause diseases in humans.

Comparison of Streptococcus suis in pig farms and the swine oral microbiota between Japan and Vietnam
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Streptococcus suis occasionally causes invasive diseases in pigs, but the main source of infection remains still unclear. To understand the dynamics of S. suis in pig farms, we compared the distribution of S. suis in pig farms and the swine oral microbiota between Japan and Vietnam. Clinically healthy pigs, enrolled from each of two farms in two countries, were categorized by their growth stages as follows: suckling, post-weaning, growing and their sows. Samples were collected from their bodies (saliva, feces, vagina swabs) and surroundings (swabs of water dispenser and feed box). Relative numbers of total bacteria, S. suis of all serotypes, and S. suis serotype 2, which is predominantly isolated from diseased pigs, were estimated by Real-time PCR. The swine oral microbiota was investigated by high-throughput sequencing of 16S rRNA gene. S. suis were detected from all saliva. Relative numbers of S. suis in the saliva were the same order irrespective of the growth stages, farms, or nations, while the other samples showed lower detection rate and numbers of S. suis than those of saliva. S. suis serotype 2 was detected in the samples from a few farms where S. suis infection occurred frequently. The oral microbiota seemed to be more similar among the growth stages than between two countries. These results suggested that swine saliva could be a potential reservoir of S. suis regardless of the growth stages; however, the oral microbiota would be affected by the growth stages rather than geographical and/or environmental differences between two countries.

Emerging high virulent strain Streptococcus suis serotype 9 from brain meningitis epidemic outbroken in piglets
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Streptococcus suis (S. suis) is an emerging zoonotic pathogenic bacterium capable of infecting piglets and human. Here, we isolated a S. suis strain WH1609 from piglets brain with obvious bacterial meningitis symptoms in China, which belongs to serotype 9 according to multiplex PCR and agglutination test. The strong pathogenicity of strain WH1609 was reproduced successfully in BABL/C mouse model. The LD_{50} in mice reached 179 CFU/mouse, which means at least 1000 times virulence more than any strain of S. suis. To elucidate the genetic determinants of meningitis caused by the strain, whole genome sequencing was undertaken. Comparative genomic analysis revealed that WH1609 genome exhibits a high level gene gain and loss, especially in multi drug resistance and genomic islands (GI) addition. A candidate GI was investigated at genomic level which shares low homogeneity with other GIs of S. suis, consistent with its potential role in streptococcal toxic shock syndrome (STSS) and high virulence.

STK regulating cell division by modulating DivIVA phosphorylation in Streptococcus suis
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The virulence. genomic islands (GI) addition. A candidate GI was investigated at genomic level which shares low homogeneity revealed that WH1609 genome exhibits a high level gene gain and loss, especially in multi drug resistance and meningitis caused by the strain, whole genome sequencing was undertaken. Comparative means at least 1000 times virulence more than any strain of S. suis. To elucidate the genetic determinants of WH1609 was reproduced successfully in BABL/C mouse model. The LD_{50} in mice reached 179 CFU/mouse, which means at least 1000 times virulence more than any strain of S. suis. To elucidate the genetic determinants of meningitis caused by the strain, whole genome sequencing was undertaken. Comparative genomic analysis revealed that WH1609 genome exhibits a high level gene gain and loss, especially in multi drug resistance and genomic islands (GI) addition. A candidate GI was investigated at genomic level which shares low homogeneity with other GIs of S. suis, consistent with its potential role in streptococcal toxic shock syndrome (STSS) and high virulence.
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Introduction: Streptococcus suis (S. suis) is an important swine pathogen that causes a wide range of diseases and is also a occasionally pathogen for humans leading life-threatening diseases. A number of studies have shown that Serine/Threonine Protein Kinase (STK) is commonly found in bacteria and has a diversity of STK substrates involving in important life processes of bacteria, including cell division and morphology. Studies from our and other groups have shown that inactivation of stk greatly affected normal cell division of S. suis, however, there is no report on the phosphorylated substrate of STK in S. suis. Objective: Identification of S. suis STK phosphorylated substrate proteins, and getting insight into the underline mechanism. Results: Seven differentially phosphorylated proteins were identified by phosphoproteomics analysis for wild type and stk gene knockout S. suis strains. One of them was annotated as cell division initiation protein (DivIVA), which have been reported to regulate cell division in several bacteria. Western analysis using an anti-threonine phosphorylation antibody revealed that the recombinant STK protein could successfully phosphorylate DivIVA protein in vitro. To determine the specific amino acid residue that phosphorylated by STK, mass spectrometry was performed, which identified a threonine at position 199 of DivIVA as a potential phosphorylation site. In support of this finding, replacing threonine at position 199 of DivIVA with alanine completely abolished its phosphorylation by STK. Conclusion: Both in vivo and in vitro experiments support that DivIVA is a phosphorylated substrate of STK in S. suis. The results suggest that STK may regulate cell division in S. suis through modulating DivIVA phosphorylation.

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**Antimicrobial susceptibility and common virulence genotype of Streptococcus suis serotype 2 isolate from patient in Cambodia, Laos and Viet Nam**

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We characterized antimicrobial susceptibility profile and common virulence genes of S. suis isolated from blood or CSF samples of patients in Cambodia, Laos and Southern Viet Nam. A total of 13 patients from Cambodia, 13 from Laos and 52 from Southern Vietnam were identified with the mean age of 58.8 years (67.0% female), 46.3 years (23.3% female) and 48.6 years (20.0% female), respectively. All isolates were serotype 2. Isolates from Cambodia and Vietnam belonged to ST1 while 46% of Laos isolates belonged to ST104. Resistance to at least three drug classes macrolides, clindamycin and tetracyclin was detected in 30.8%, 87.5% and 92.9% of isolates from these three countries, in previous order. Tetracyclin resistance was correlated with tetM (100.0%, 12.5%, 94.7%) and tetO (16.0%, 87.5%, 22.8%) in Cambodia, Laos and Viet Nam isolates, respectively while TetL was only found in 7.0% of Viet Nam isolates. The macrolide-resistant gene ermB was present in 100.0%, 10.5% and 25.0% in isolates from Cambodia, Laos and Vietnam. Only genes sly was found in ST104 isolates while epf were found in 57.9%, 77.0% and 98.0% in Cambodia, Laos and Vietnam. S. suis caused meningitis and sepsis in human in Cambodia, Laos and Viet Nam, were associated with complex multi-antibiotic resistance, but still susceptible to ceftrizone and penicillin, two currently used antibiotics for human S. suis infection treatment.

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**Immunological response of Streptococcus suis infected patients admitted to Hospital of Tropical Diseases in Southern of VietNam (2015-2016)**

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Publish consent withheld

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**The differential ribosome profiling approach presents the complexity of Streptococcus suis translational landscape**

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Publish consent withheld

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**Insight into the specific pathogenicity islands and Serine-Rich Repeat Protein SssP1 in the Streptococcus suis novel serotype Chz**

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In our previous works, strain CZ130302, as a representative of the new serotype Chz of Streptococcus suis, has a high potential to be virulent and associated with meningitis in animals. However, the pathogenic mechanism of the serotype Chz SS is not known. In this study, we sequenced and annotated the genomes of strain CZ130302 and two avirulent strains (HN136 and AH681). The results of comparative genomics showed that two avirulent strains HN136 and AH681 were much closer to each other and two unique 50 K and 58 K genomic islands were revealed in strain CZ130302. To investigate the functions of the two islands, mutant strains (Δ50K-CZ130302 and Δ58K-CZ130302) were constructed, and then both islands, especially the 50K island, were confirmed to attenuate the virulence of S. suis CZ130302 in BALB/c mice. In addition, a set of SecY2/A2 secretion system and
the effector protein, named Streptococcus suis Serine-rich Repeat Protein 1 (SssP1), were demonstrated to fulfill critical roles in pathogenicity, which was an essential component of the 50K island. Furthermore, two recombinant proteins containing two Ig-like fold subdomains of SssP1 respectively were identified to have the ability to bind HBMEC cells by the indirect immunofluorescent assay. Meanwhile, strain ΔSssP1 showed a significant reduction both in HEp-2 and HBMEC cells compared with wild-type strain. Both the transmission electron microscope and immunofluorescence assays showed that the SssP1 can form fimbriae-like structures extending outwardly from the bacterial surface.

**Defining the ABC of gene essentiality in streptococci**
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Utilising next generation sequencing to interrogate saturated bacterial mutant libraries provides an unprecedented means to determine genome-wide gene essentiality. We developed a barcoded transposon directed insertion-site sequencing (TraDIS) system to define an essential gene list for Streptococcus equi subsp. equi, the causative agent of streptococcal meningitis. Six barcoded variants of pGh9:ISS1 were designed and used to generate mutant libraries that each contained between 33,000-66,000 unique mutants. Combining these data into a master library of 208,531 mutants detected an ISS1 transposition on average every 9 bp in coding sequences throughout the genome. These data identified 422 essential genes, representing 19.5 % of the S. equi genome. The gene essentiality data for this Lancefield group C Streptococcus was compared to that of group A and B streptococci, identifying concordances of 90.2 % and 89.4 %, respectively and an overall concordance of 83.7 % between the three species. Our data provides further evidence of the close genetic relationships between these important pathogenic bacteria and provides a solid foundation towards reporting the functional genome of streptococci.

**Streptococcus pneumoniae Ccs4 involved in penetration across blood-brain barrier and virulence in meningitis**
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Streptococcus pneumoniae is a pathogen known to be a leading cause of pneumonia, sepsis, and meningitis. Although ccs4, encoding candidate combox site 4, has been reported as a late competence gene, its function remains unknown. We examined the role of pneumococcal Ccs4 in the pathogenesis of S. pneumoniae-induced disease.

We constructed a ccs4 deletion mutant of S. pneumoniae TIGR4 and the mutant strain complemented with a shuttle vector harboring Ccs4. Despite prediction that Ccs4 possesses the positively charged extracellular region, susceptibility to LL-37 was comparable among strains. In addition, Ccs4 deletion had no effect on pneumococcal survival in mouse blood. On the other hand, wild-type and complemented strains exhibited significantly higher rates of association and invasion to human brain microvascular endothelial cells as compared to the ccs4 mutant strain. Additionally, we investigated the role of Ccs4 in vivo. In an intravenous infection model, ccs4 mutant strain-infected mice showed prolonged survival, whereas there were no differences seen in an intranasal infection model. Moreover, we examined bacterial burden in brain and blood samples obtained 24 hours after the intravenous infection. Wild-type strain-infected mice showed a significantly higher bacterial burden in the brain as compared to the mutant strain-infected mice, whereas that in blood was comparable between those strains. The median ratio of brain/blood colony forming units of the wild-type strain was also significantly higher than that of the ccs4 mutant strain.

These results indicated that Ccs4 is involved in pneumococcal penetration across the blood-brain barrier and contributes to its virulence.

**Group G streptococcus induces autoimmune mediated carditis in the Lewis rat model of Rheumatic Heart Disease**
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Echocardiography studies of mini pigs immunized with repeated doses of StreptInCor a candidate unknown function identified on the plasmid are currently under investigation. The presence of the toxin replication. In addition, the stable maintenance of this plasmid in strain SAA0430 containing two important elements suggested to function during replication: ORF1 was predicted to encode and composed of 10 ORFs including ORFs encoding a putative toxin protein RepB homolog, and iteron. Because none of the transformants carrying variant plasmid contains these proteins in the zebrafish infection model will provide novel insights into the virulence mechanisms that might be correlated to the in-vivo functions of the orthologue GAS proteins in the human host.

Characterization of a novel plasmid discovered in a clinical isolate of Streptococcus anginosus subsp. anginosus

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[Objectives] Mobile genetic elements (MGEs) are known to be involved in a number of genetic events in bacteria. However, there are few reports on MGEs in Streptococcus anginosus subsp. anginosus (SAA), a commensal strain in the human oral cavity. Recently, in our search for MGEs in SAA clinical isolates, we discovered a novel plasmid in strain 0430-08. In the present study, we examined the genetic structure and characteristics of this novel plasmid.

[Methods] The nucleotide sequence of the purified plasmid from strain 0430-08 (pSAA0430-08) was determined and the annotation of its predicted ORFs was performed. Studies were carried out to identify the function and participation of translational products of the predicted ORFs in plasmid replication and maintenance.

[Results and Discussion] We found that pSAA0430-08 is a circular plasmid with 7,038 bp and ~31% G/C content, and composed of 10 ORFs including ORFs encoding a putative toxin-antitoxin system. In addition, pSAA0430-08 contains two important elements suggested to function during replication: ORF1 was predicted to encode the replication protein RepB homolog, and iteron. Because none of the transformants carrying variant plasmid constructs lacked ORF1 or iteron, we conclude that both of these are essential elements for pSAA0430-08 replication. In addition, the stable maintenance of this plasmid in the SAA strain is thought to be due to the presence of the toxin-antitoxin system found on the plasmid. The characteristics of the remaining ORFs with unknown function identified on the plasmid are currently under investigation.

Echocardiography studies of mini pigs immunized with repeated doses of StreptInCor a candidate

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Acute rheumatic fever and rheumatic heart disease (ARF/RHD) have long been described as autoimmune sequelae of Streptococcus pyogenes or group A streptococcal (GAS) infection. Both antibody and T cell responses against immunodominant GAS virulence factors including M protein, cross-react with host tissue proteins triggering an inflammatory response leading to permanent heart damage. However, in some ARF/RHD endemic regions, throat carriage of GAS is low. As Streptococcus dysgalactiae subspecies equisimilis (SDSE), also known as β-hemolytic groups C and G streptococci (GCS/GGS) does indeed cause both myocarditis and valvulitis, hallmarks of ARF/RHD. Remarkably the histological, immunological and functional changes in the hearts of rats exposed to GGS are identical to those exposed to GAS. Furthermore, antibody cross-reactivity to cardiac myosin was comparable in both GGS and GAS exposed animals providing additional evidence that GGS can induce and/or exacerbate ARF/RHD.

Functional Analysis of Streptococcal Virulence Factors

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Background: Streptococcus pyogenes (Group A Streptococcus, GAS) causes a variety of diseases in humans ranging from pharyngitis and impetigo to more severe invasive diseases including cellulitis, necrotising fasciitis and toxic shock. Investigations of GAS pathomechanisms are hindered by the lack of suitable animal infection models. Objectives: To characterise the mechanisms of two important GAS virulence factors, Streptococcus pyogenes nuclease A (SpnA) and streptococcal 5'-nucleotidase A (S5nA), by investigating the orthologues SpnAi and S5nAi in the fish pathogen Streptococcus iniae using a zebrafish infection model. Results: SpnAi and S5nAi were cloned and expressed in E. coli. Biochemical analysis of purified rSpnAi and rS5nAi showed that both proteins have very similar reaction conditions compared to SpnA and S5nA. rSpnAi is able to digest linear double-stranded DNA and chromosomal DNA with highest activity at pH 6.5–7.5 and between 32°C–37°C in the presence of Ca2+ and Mg2+. rS5nAi is able to generate immunomodulatory molecules adenosine and macrophage toxic deoxyadenosine with highest activity at pH 7 and 42°C in the presence of Mg2+. A. S. iniae spnAi gene deletion mutant has been generated by allelic replacement and is ready for analysis in a zebrafish infection model. Conclusion: SpnAi and S5nAi have been confirmed as true orthologues of the GAS proteins SpnA and S5nA, respectively. The study of these proteins in the zebrafish infection model will provide novel insights into the virulence mechanisms that might be correlated to the in-vivo functions of the orthologue GAS proteins in the human host.
vaccine to prevent S. pyogenes infections as a safety control
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The aim of this preclinical study is to evaluate the safety of StreptInCor, a candidate vaccine to prevent S. pyogenes infections. A dose of 200 μg/500 μL of StreptInCor onto aluminum hydroxide and placebo (prepared under GMP condition) were used in this study. The repeated-dose toxicity studies in mini pigs were performed by a certified and independent company (Tecam Laboratórios). An additional non-manipulated group was included. All animals were submitted to echocardiogram examination before immunization and after the four doses treatment (28 days). Systolic function, mitral and aortic valves and cardiac blood flow were observed by a veterinarian and a cardiologist. The images obtained showed no significant changes. In addition, no macroscopic and microscopic alterations of heart and its valves in the animals from any group were observed. These results indicate that StreptInCor is a safe and promising candidate vaccine against S. pyogenes infections and therefore it will allow the conduction of a phase I/IIa clinical trial.

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PilVax: a novel peptide carrier for the development of vaccines against tuberculosis
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PilVax is a peptide delivery strategy for the generation of highly specific mucosal immune responses. The food-grade bacterium Lactococcus lactis is used to express selected peptides engineered within the group A streptococcal pilus, thereby allowing for peptide amplification, stabilization, and enhanced immunogenicity. A pilot study showed that mice immunised with PilVax expressing the model peptide Ova224-337 presented strong IgG and IgA responses to ovalbumin. The present study aims to demonstrate the suitability of PilVax for the generation of novel peptide vaccines against tuberculosis. Selected peptides (B cell and T cell epitopes), derived from tuberculosis vaccine targets, were genetically engineered into loop regions of the pilus backbone subunit and expressed in L. lactis. Western blots confirmed pilus formation on L. lactis. Antibody responses from mice immunised intranasally with recombinant L. lactis were strong against the pilus backbone, but weak against the target peptide. However, the poor antibody responses to the two peptides tested thus far were expected, due to being primarily T cell epitopes. We are currently also testing PilVax expressing selected B cell epitopes, and analysing the T cell responses of all constructs.

TeeVax – a multivalent T-antigen-based vaccine against group A streptococcus
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The surface of group A streptococcus is decorated with hair-like appendages known as pili. These are involved in adhesion and colonisation of the host during infection. The major protein component of the pilus is the T-antigen which multimerises to form the pilus shaft. Recent genomic analysis of tee genes from strains circulating in New Zealand revealed that inclusion of 18 different T-antigens in a vaccine should provide over 90% coverage (Steemson et. al. Journal of Medical Microbiology (2014), 63, 1670–1678). The T-antigen therefore represents an attractive target for vaccination. Our aim is to fuse protein domains from up to 6 different T-antigens, and combine up to 3 fusion proteins in the final multivalent vaccine. The first 2 of these fusion proteins have been successfully expressed and purified. Antibody responses were measured by ELISA and an in vitro bioluminescent bactericidal assay developed in our lab. Our results show that rabbits immunised with these TeeVax proteins elicit strong antibody responses that are able to mediate opsonophagocytotic killing of group A streptococcus.

Toxicity and skin sensitization assays as preclinical evaluations of StreptInCor a candidate vaccine to prevent S. pyogenes infections
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Aiming the development of a vaccine to prevent Streptococcus pyogenes infection and autoimmune disorders we...
analyzed a large panel of sera and T cells from human blood against 79 overlapping C-terminal synthetic peptides that allowed us to define both T and B epitopes with potential to induce protective immune response. Through this approach, we constructed by chemical synthesis a 55 mer peptide identified as StreptInCor, a candidate vaccine. We tested the ability of StreptInCor to induce protective antibodies by immunizing inbred (BALB/c), outbred (Swiss) and HLA class II (DR2, DR4, DQ6 and DQ8) transgenic mice. Our results showed strong humoral as well as cellular immune responses. To assess the safety of the candidate vaccine, three formulations were prepared under good manufacturing practice conditions: 50, 100 and 200 μg/500 μL of StreptInCor and placebo, onto aluminum hydroxide. These studies were performed by a certified and independent company (Tecam Laboratórios). The vaccine formulations were evaluated in animals for acute and repeated dose toxicity (Wistar rats) and local tolerance (Dunkin-Hartley guinea pigs). Toxicological studies showed that StreptInCor did not induce toxicity. Furthermore, StreptInCor was classified as non-sensitizing to the skin of guinea pigs.

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Using structural biology to inform the design of T-antigen based vaccines for Group A Streptococcus
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Group A Streptococcus (GAS) is a globally important pathogen causing a broad range of human disease and significant morbidity and mortality. There are no vaccines for GAS currently available but vaccine candidates based on the T-antigen, which forms the pilus backbone, are in pre-clinical development. T-antigens have previously been shown to have protective properties in mice and relatively low antigenic variation (GAS cluster into 18 major -tee-types). This project aims to investigate antibody-T-antigen interactions and provide 3-dimensional structural data of T-antigens to inform structure-led, vaccine design efforts.

FVB/n Mice and NZ white rabbits were vaccinated with recombinant T18 antigen (found in M18, M71 and M217 strains). Sera from these animals were screened by ELISA using an array of T-antigens that covers all major circulating tee-types to elucidate cross-reactivity patterns. The dominant antibody cross-reactivity observed was to T3.2 and T13. To visualise antibody cross-reactivity at a molecular level the structures of the three T-antigens (T3.2, T13 and T18.1) were solved using X-ray crystallography. Each T-antigen features two immunoglobulin-like domains and, despite low overall sequence identity, show significant structural homology with the previously published T1 antigen. Structural overlays reveal that T-antigens share a highly conserved core decorated with variable loop regions. Purification of T-antigen specific IgG from the animal sera, together with the isolation of high affinity (~50 nanomolar) monoclonal antibodies from the same animals, has enabled the patterns of antibody specificity to be mapped onto T-antigen structures. These structural maps of antibody-T-antigen interactions will inform the design of T-antigen based vaccines.

Distribution of collagen binding PARF Motifs in M Proteins of Group A, C and G Streptococcal Strains from North India
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Streptococcal strains, upon binding to host collagen form auto-antigen complex which generates anti-collagen antibodies, lead to development of RF/RHD. PARF(Peptide Associated with Rheumatic Fever) motif of M proteins helps strains to bind collagen. It is hypothesized that streptococci having PARF(AEYLKALN) may have higher tendency to cause RF/RHD. Besides GAS, there are indications that GCS/GGS strains may also cause RF/RHD. Therefore distribution of PARF was explored in GAS, GCS/GGS strains and level of anti-collagen antibodies was also estimated in patient sera.

Strains were isolated from children of age group 5-15 years in school health survey. Serum samples were collected from GAS positive, RF/RHD patients. emm typing and identification of PARF was done by emm gene sequencing. Anti-collagen IV antibodies were estimated by ELISA.

Out of 5000 children examined, 201 isolates(43GAS, 33GCS, 125GGS) were collected. From 990(19.8%) pharyngitis cases, 9(0.90%) were GAS, 3(0.3%) were GCS and 22(3.4%) were GGS positive. Out of 158 GCS/GGS isolates, 57 belonged to emm gene positive dysgalactiae group. Out of 100, 93 strains (37GAS,56GCS/GGS) were further characterized. From 37 GAS, belonging to 23 types(most prevalent: emm100, emm85, emm71), none of isolate was PARF positive. Among 56 GCS/GGS strains(most prevalent: stG245, stG653), PARF was identified from only two GGS(stG10.0) strains. PARF like motifs were identified from 20 GAS and 20 GCS/GGS strains. Anti-collagen IV antibodies were high in GAS positive(6), RF(8), RHD(14) samples in comparison to healthy controls(10).

PARF is rare in North Indian streptococcal strains but high level of anti-collagen antibodies in patient’s sera was observed.
PilVax – a novel peptide antigen delivery strategy for vaccine development
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Vaccine development has evolved from killed or live attenuated microorganisms to well-defined synthetic vaccines based on individual proteins or peptides. However, peptide antigens are usually poorly immunogenic and sensitive to proteolytic degradation, and thus require conjugation to carrier proteins, and administration with potentially toxic adjuvants. Lactic acid bacteria have become promising vectors to deliver antigens to mucosal tissues. Combining these two research trends, we have developed a novel peptide delivery system by utilising the group A streptococcus (GAS) pilus structure as a carrier for antigenic peptides, and expressing the modified pil on the surface of the non-pathogenic surrogate Lactococcus lactis. Advantages of this new technology, termed PilVax, include increased peptide immunogenicity and stability, higher safety and low production cost. In this proof-of-concept study, we identified several regions within the backbone pilin FctA (Spy0128) of a serotype M1 strain that can be replaced with the model peptide OVA324-339 without affecting pilus assembly and display on the surface of L. lactis. Intranasal immunisation of mice with the resulting recombinant L. lactis strain produced strong Ova-specific antibody responses in serum and bronchoalveolar fluid. Further modification of the PilVax design has been carried out, in order to improve adjuvanticity and surface antigen display. Oral vaccination as an alternative immunisation route is also being investigated. The PilVax technology provides a novel system for developing peptide vaccines for mucosal delivery.

Liposomal peptide vaccine delivery system induces protective humoral and cellular immune responses against group A streptococcus in the upper respiratory tract
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The most common disease following GAS colonization of the upper respiratory tract (URT) is pharyngitis. Rheumatic heart disease (RHD) can follow untreated pharyngeal infections. Globally, there is estimated to be 62–78 million individuals with RHD that results in over 300,000 deaths per year. Established correlates of protection show that IgA-specific mucosal immunity prevents pharyngeal colonization. To develop a vaccine that induces an IgA response, we constructed a liposomal vaccine consisting of a GAS-specific conserved B-cell epitope from the surface M protein (‘J8’) and a source of T-helper cell stimulation provided by diphtheria toxoid and delivered this intranasally (‘J8-Lipo-DT’).
Post immunization, we observed a significant IgA mucosal antibody response. Post-challenge with GAS the bacterial load in the nasal discharge was significantly lower. Mice also showed significant protection against infection of the pharyngeal surface and nasal associated lymphoid tissue. We measured the cytokine responses of spleen cells from immunized mice following in vitro stimulation. Antigen-specific IFN-γ and IL-6 from spleen cells from immunized mice was observed. IL-6 is a signal for neutrophil production and neutrophils are essential for IgA-mediated opsonization of GAS. We thus immunized IL6−/− and control mice with J8-Lipo-DT and observed that the IL6−/− mice had significantly higher total streptococcal tissue bioburden post-challenge in comparison to wildtype mice. The results demonstrate a generic strategy based on a liposomal vaccine delivery system that can enhance the utility of peptides as vaccines and strongly support the development of a GAS vaccine based on minimal epitope synthetic peptides.

Genome-wide association study of susceptibility to rheumatic heart disease in South Asians: Preliminary results
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Introduction
Rheumatic heart disease (RHD) remains a leading cause of morbidity and premature mortality in developing countries. Recently, large-scale genetic studies have begun to provide much needed insight into the underlying pathogenesis. Methods
We undertook a genome-wide association study of RHD susceptibility in 1163 individuals of Indian ancestry recruited in Fiji (n=309) and Northern India (n=854). Patients with incident or prevalent RHD were recruited as cases, while members of the general population were recruited as controls. We genotyped all individuals at ~250,000 variants using the Illumina HumanCore platform before estimating the genotypes of an additional ~8,000,000 variants by imputation. We used linear mixed models to analyse genotyped and imputed variants
before combining association statistics from the two datasets using fixed-effects meta-analysis.

Results
We observed a single signal at genome-wide significance, located in the human leukocyte antigen (HLA) class III region (OR=1.91, P=6.8 x 10^-6). Using conditional analyses, we demonstrated this was comprised of two independent signals, the first spanning HLA class I (HLA-B) and the second HLA class II (HLA-DQA1/B1). While the IGHV4-61 signal was apparent, as previously reported in the Fijian Indian data (P=0.006), the signal was essentially absent in the Northern Indian data (P=0.25), leaving only negligible signal in the pooled analysis (OR=1.21, P=0.084).

Conclusion
By combining new data from India with previously reported data from Fiji, we provide the first insight into genetic architecture of RHD in South Asians with a promising finding in the HLA locus. We also highlight the challenge of heterogeneity between populations.

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Demonstration of Antibody Dependent Enhancement (ADE) like phenomenon in cases of pyoderma & pharyngitis caused by Group A Streptococci (GAS) & Streptococcus dysgalactiae subspecies equisimilis (SDSE)
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Background: GAS & SDSE are significant human pathogens sharing similar disease spectra. Commonly seen infections like pyoderma & pharyngitis may lead to serious immune sequelae like RF/RHD & PSGN. Streptococcal Inhibitor of complement (SIC) & its orthologue, distantly related to SIC (DRS) are virulence factors expressed by over 4 M types of GAS. DRSG (orthologue of DRS) is a virulence factor expressed by some strains of SDSE. In the past we had observed sero-positivity to these three antigens in chronic kidney disease (CKD) & end stage renal disease (ESRD) patients.
Methods: GAS & SDSE isolates recovered from pyoderma & pyoderma cases were subjected to emm typing. Sero-positivity for SIC, DRS & DRSG was studied by ELISA test. In this study, immune response against all the 3 antigens from above mentioned cases is compared with age & sex matched controls who had no history of streptococcal infections in last 6 months. The results were checked statistically by two-tailed P Test.
Results: We found positive association between the SIC/DRS antibodies and pyoderma with diverse M types. Similar observations were also seen in pharyngitis when compared with respective controls. The mechanism of increased predisposition to pyoderma & pharyngitis among SIC/DRS antibody-positive population by diverse isolates is not clear.
Conclusions: Although only 4 types express SIC/DRS antigens, they elicit persistent antibody response. Therefore, antibody prevalence in a population is cumulative result of past infections by one of these four “core” types. Our observations could be explained by a phenomenon akin to ADE.

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Streptococcal Inhibitor of Complement (SIC) was detected in mesangium and glomerular capillary walls in kidney biopsies of patients having glomerular diseases
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Introduction: Growing number of studies suggest that PSGN and glomerular diseases as a strong risk factor for chronic kidney disease (CKD). Earlier studies suggests that the secretory streptococcal antigens such as streptococcal inhibitor of Complement (SIC) have a role in pathogenesis of CKD or End stage renal disease (ESRD) based on serological evidence. In current study, we investigated whether deposits of SIC and its variants such as Distantly related to SIC (DRS) and DRSG (orthologue of DRS) are present in kidney tissues from glomerular disease patients.
Methods: 25 patients diagnosed with glomerular diseases and for control 25 age-matched autopsies whose deaths were unrelated to renal disorders were recruited for this study. Sections of kidney tissues were tested for deposition of SIC, DRS and DRSG by immunofluorescence. Antibodies to these antigens were also tested.
Results: In the glomerular disease study cohort, 28% and 16% were positive for anti-SIC and anti-DRSG antibodies respectively as opposed to only 4% in the cadaver cohort (p=0.0007 and 0.0182 respectively). Also, a significant number of patients suffering from glomerular diseases (7/25; 28%, p = 0.0486) were positive for deposition of SIC in kidney sections in comparison to cadavers. No significant differences were found for deposition of DRS and DRSG between these cohorts.
Conclusion: This immunohistological and serological study suggests a role for SIC in pathogenesis of glomerular diseases. The findings of the present work strongly favour serology for anti-SIC antibodies as an important tool for poor prognostic marker in the management of glomerular diseases.
A Systematic Analysis of Immunoglobulin and Complement Pathways in Patients with Acute Rheumatic Fever
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Acute rheumatic fever (ARF) is an auto-inflammatory sequela that can develop after a Group A Streptococcus infection. Carditis, the most severe ARF manifestation, can lead to permanent heart valve damage and rheumatic heart disease. While immunoglobulin and complement were first observed in the myocardium and mitral valves of children who had died from cardiac failure following ARF over 50 years ago (Kaplan 1964), contemporary investigations of complement pathways in ARF have been lacking. The aim of this study was to determine how complement drives inflammation in ARF. The concentration of 17 complement factors spanning the classical, lectin and alternative pathways, together with six immunoglobulin isotypes and subclasses were measured in participant serum using bead-based immunoassays (BD510 Cytometric Bead Array and Luminex xMAP52). An integrative statistical approach was utilised to analyse relationships among immunoglobulin and complement in ARF patients stratified by concentration of C-reactive protein (CRP).

Patients with high CRP had significantly elevated levels of several complement factors and immunoglobulin types compared with patients with low CRP and healthy controls. Key features contributing to ARF inflammation were identified by multidimensional analysis combining feature selection (least absolute shrinkage and selection operator; LASSO) and partial least-squares discriminant analysis (PLSDA). Just 5 of the 23 analytes accounted for 71% of the variance between high and low CRP patients. Notably, patients in the high CRP group exhibited linked IgG3/C4 responses. This, together with an absence of any lectin pathway features, suggests a dominant role for the classical complement pathway in the immunopathogenesis of ARF.

Development of a high throughput HL-60 opsonophagocytic killing (OPK) assay for group A streptococci
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Detection of opsonic antibodies against group A streptococci (GAS) has previously relied on the classic indirect bactericidal test of Lancefield, which uses “non-immune” human blood as a source of phagocytes and complement. The immune status of the blood donor and variable results among donors often affected the reproducibility and robustness of the assay. Based on previous studies with S. pneumonia and group B streptococci, we have adapted the HL-60 assay for use with GAS in a 96-well plate format. Optimal growth of GAS in media containing active complement and transformed HL-60 cells was supported by the addition of human fibrinogen, low levels of heparin, and pig serum. While not all emm types required all components, the goal was to develop a universal buffer that could be used in all assays. A reference serum pool was generated in rabbits immunized with the 30-valent vaccine. Experiments were performed to determine the optimal concentrations of fibrinogen, pig serum, and cells. Methods were developed to plate replicate samples that could be quantitated by capturing digital images. The specificity of antibody-mediated opsonization was assessed by peptide inhibition studies. OPK activity mediated by HL-60 cells against multiple emm types of GAS correlated with that observed using human blood with rabbit and human sera against the 30-valent vaccine. We believe the HL-60 OPK assay will facilitate the future clinical development of GAS vaccines by yielding consistent and reproducible results.

Development of surrogate of protection assays for group a streptococcus
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There is currently no vaccine available against group A Streptococcus (GAS) infections but a large number of virulence factors have been identified and M-protein based vaccines have reached clinical trials. To assess the effectiveness of antibodies raised from vaccination trials, an opsonophagocytic assay (OPA) can be used as a surrogate of protection. This assay determines whether antibodies raised are able to encourage uptake and killing of GAS by neutrophils, mimicking infection clearance by the adaptive immune system in the body. Currently the Lancefield whole blood assay is used as a surrogate of protection OPA, but this has a number of limitations including the need for fresh human whole blood as a source of phagocytes and complement, leading to high variability between experiments due to heterogeneity of the donors and the difficulty in controlling the pre-existing background level of anti-GAS antibodies prior to addition of test sera.

In our work we have been developing an OPA based on neutrophil-differentiated HL60 cells and baby rabbit complement, for potential use as a surrogate of protection in the evaluation of efficacy of GAS vaccine candidates, using a GAS serotype M1 strain. Pooled human intravenous immunoglobulin (IVIG), identified to have a high level of anti-GAS M1 antibodies, was used as a source of human anti-GAS antibodies.

The development of a cell culture based standardised OPA will be highly beneficial to the GAS community to ensure consistent assay results between research groups to measure the functional activity of immune sera.
The GAS “Tetradigm” of pharyngeal symptoms and immune responses: Implications for ARF?
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The recent report by Hysmith et al (JPIDS March 2017) of unpredictable immune responses to symptomatic and asymptomatic pharyngitis acquisition creates 4 clinical circumstances, a GAS “Tetradigm.” (1) Symptoms/Immune Response (IR); (2) Symptoms/no IR; (3) no Symptoms/IR; (4) no Symptoms/no IR. Symptoms/IR corresponds to bona fide classic GAS pharyngitis. No Symptoms/no IR is consistent with chronic carriage; carriers are not routinely identified or treated. Classically, ARF follows bona fide GAS pharyngitis but probably not chronic carriage. Symptomatic patients can be identified and treated for GAS, the current approach to ARF prevention. Patients with Symptoms/no IR are not distinguished clinically from those with Symptoms/IR; their risk for recurrent infection and GAS sequelae is unknown. Development of IR without Symptoms might explain ARF in patients without history of pharyngitis but the extent of risk is unknown and identification before ARF occurs is problematic. Confirming antecedent GAS illness requires identifying IR to GAS, but Hysmith’s report highlights the unpredictability of IR.

Conundrums and Challenges: 1. M protein-specific IR protects against homologous M type acquisition but is variable and may not develop; 2. Non-M IRs are unpredictable and their role in protection against infection and sequelae is unclear; 3. Identifying GAS IR supports the diagnosis of ARF and/or evidence of vaccine response, but which IRs protect against, or create risk for, ARF?; 4. What is the meaning of GAS acquisition without anti-M IR: is there risk for ARF? Studies are needed to clarify unpredictable immune responses to GAS pharyngitis and subsequent risk for ARF.

Identifying Autoantibody Targets as New Biomarkers for Acute Rheumatic Fever using Mass Spectrometry and High Content Protein Arrays
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Acute rheumatic fever (ARF) and associated rheumatic heart disease (RHD) are serious sequelae of Group A Streptococcus (GAS) infection. Rates of ARF/RHD remain unacceptably high in the developing world and in indigenous populations in certain developed counties such as Australia and New Zealand. Specific diagnostic tests for ARF are lacking, with diagnosis instead relying on a set of clinical criteria. This presents a major hurdle in disease control efforts, with an accurate diagnosis requiring a series of assessments over a period of days. Our aim is to identify novel human targets of auto-antibodies in patients with ARF that can serve as biomarkers for diagnosis. Firstly, an optimised western blot protocol has been developed to test for heart reactive antibodies from the sera of ARF patients with carditis. Reactivity of ARF sera was screened in whole heart lysates, as well as aorta and mitral valve lysates. Specific bands detected in ARF sera and not healthy control sera have been subject to mass spectrometry for identification. Secondly, high content array technology has been applied to identify potential autoantibody targets in an unbiased fashion. Arrays containing 9000 (Protoarray) and 15000 (HuProt Array) human proteins have been screened with ARF patient sera with and without carditis and matched healthy controls. Analysis has revealed a panel of proteins that have potential as biomarkers in ARF. Validation of these potential hits in immunoasays with larger patient numbers will be presented as a route to assessing the utility of the proteins in clinical diagnosis of ARF.

Defining “strain”: Development of an MLST scheme based on surface protein genes for group A streptococci
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Through contributions from >50 investigators, multi-locus sequence typing (MLST) of group A streptococci (GAS) based on 7 core housekeeping loci reveals 921 sequence types (STs) (https://pubmlst.org/spyogenes/; May 2017). ST coupled with emm type provides a rough definition for “clone.” New “clones” of clinical significance often emerge from point mutations or small indels affecting transcriptional regulatory genes. A working definition for “strain” can allow for a deeper evolutionary analysis of the GAS species. Based on genomes of 200 GAS isolates having unique combinations of emm type and genetically-distant STs, plus published data on ~60 additional genomes, sequences were analyzed for genes mapping to the emm- and FCT-regions of the chromosome and encoding proteins with cell wall sorting signals. The data provide a foundation for development of an MLST scheme based on surface protein genes (MLStsp). Phylogenetic analysis of each surface protein gene allows for assignment of major sequence clusters. The distribution among GAS of sequence clusters for multiple surface protein genes provides evidence for a history of extensive horizontal gene transfer between
Genomic sequencing analysis of emm66 group A streptococcus (GAS) isolates associated with an ongoing outbreak of invasive infection in England and Wales from January 2016
Laura Bubba1,2, Juliana Coelho2, Georgia Kapatai2, Roger Daniel2, Nick Bundle2, Sooria Balasegaram2, Charlotte Anderson2, Maya Gobin2, Colin Brown2, Vicki Chalker2, Derren Ready2
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An outbreak of an uncommon type, emm66 of group A streptococcus (GAS) infection, was investigated in England and Wales, defining a case as an infected person who injects drugs, homeless, reporting problematic alcohol use, or epidemiologically linked to cases. No common source of infection was identified for the ongoing outbreak.

Whole genome sequencing (WGS) was performed on 55 emm66 GAS isolates (n=50 emm66.0, n=5 emm66.1); including 34 cases, 3 contemporaneous (2016) patients not fitting the case definition and 18 sporadic historical isolates (2005-2015). The evolutionary rate was evaluated by BEAST analysis on emm66.0. Thirty-two emm66.0 outbreak cases merged into a single clade (single nucleotide polymorphisms, SNPs average difference: 6.8, range 0-16 SNPs); the remaining two (one emm66.1) had > 90 SNPs difference with other outbreak isolates. All emm66.0 2015 historical isolates (n=6) and the three 2016 contemporaneous cases fitted into the outbreak clade (SNPs average: 6.3). Outbreak cases had an average of 4,103 SNPs distance from the other historical isolates (emm66.0 and emm66.1) and 32 from the reference strain. Within the same town, cases had an average difference of 2.6 SNPs; six clusters with zero SNPs difference were identified in five towns. The evolutionary rate was 9 bases per year.

WGS proved relatedness within outbreak cases and to non-outbreak cases in 2015-17 suggesting the emm66.0 strain was introduced to England and Wales in 2015. Close links within geographically clustered individuals were also confirmed. WGS can confirm links in a difficult to define population, to help target services to prevent further cases.

An occurrence of ICE-emm12 element containing tetM and ermB resistance genes among Russian and Vietnamese Streptococcus pyogenes strains
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Streptococcus pyogenes (group A streptococcus, GAS) is causative agent of numerous diseases in human. Recently, the novel 64.9 kb integrative and conjugative element with tetracycline and macrolide resistance genes designated ICE-emm12, was discovered in some of Hong Kong isolates associated with scarlet fever outbreaks in South-East Asia in 2011-2012. Goal of this study was to analyze an occurrence of ICE-emm12 element among Vietnamese and Russian GAS strains.

A total of 49 Vietnamese strains and 33 Russian strains were studied. Strains were cultured in THY broth. DNA was isolated by phenol/chloroform extraction. Presence of ICE-emm12 was tested by PCR. Sequence of ICE-emm12 element of Vietnamese strain was determined by Next Generation Sequencing. Pulsed field gel electrophoresis (PFGE) was done as previously described. emm-typing was done as recommended by the Centers for Disease Control and Prevention. All strains were tested for susceptibility to erythromycin and tetracycline.

Complete sequence of ICE-emm12 element of Vietnamese GAS strain was identical to that of Hong Kong isolate. ICE-emm12 element was discovered in 11 out of 49 (22.4%) Vietnamese strains, and 1 out of 33 (3.0%) Russian strains. These 11 Vietnamese strains were isolated in different regions of Vietnam in 2013-2014 and belonged to emm12 genotype. They were resistant to erythromycin and tetracycline and characterized by similar PFGE patterns. In contrast, Russian ICE-emm12 positive strain belonged to emm88.2 genotype. Interestingly, this ICE-emm12 positive strain was isolated in Moscow in 2008 that can give new insight at evolution of ICE-emm12 and arise of epidemiologically actual emm12 GAS clones.

Genetic variants associated with Rheumatic Fever and Rheumatic Heart Disease: A HuGENet™ systematic review and meta-analysis
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Evidence from familial studies suggests that genetic predisposition may explain susceptibility to Rheumatic heart
Getting to know you: what makes an emm75 GAS human challenge strain stick?

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Background: Successful establishment of a controlled human infection ('challenge') model of GAS pharyngitis holds great promise in accelerating vaccine development and understanding of GAS infection and immunity. A well-defined challenge bacterial inoculum is central to building a model.

Methods: The aim of this work was to explore growth, adhesion, invasion, viability, and delivery characteristics of the primary emm75 GAS challenge strain (311024), and two back-up emm12 strains (611020 and 611025).

Results: All candidate strains grew well in a chemically defined medium free of animal products and adhered to pharyngeal-derived Detroit-562 cells. Optimal adherence occurred at early-logarithmic phase, and streptococci were harvested at this time point for storage. All three strains maintained their adherence to Detroit cells following immediate thaw from storage at -80°C for 7 days. Viability of the frozen stock cultures was maintained at 61 days post-freezing. The properties of different swabs for pharyngeal delivery of the inoculum were explored, with a Dacron swab found to be most suitable.

Conclusions: This work highlights important parameters relevant for safe and reliable deployment of a GAS inoculum in a human challenge model of GAS pharyngitis.
Caveolin-1 restricts group A Streptococcus invasion into non-phagocytic host cells
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Caveolae are composed of two major proteins, caveolin-1 (CAV1) and cavin1/polymerase transcript factor I (CAVIN1). Here we demonstrate that CAV1 levels modulate invasion of group A Streptococcus (GAS) into non-phagocytic mammalian cells. GAS showed enhanced internalization into CAV1 knockout mouse embryonic fibroblasts (MEFs), and CAV1 knockdown human epithelial HEp-2 cells, whereas over-expression of CAV1 in HEp-2 cells reduced GAS invasion. This effect was not dependent on the expression of the GAS fibronectin binding protein SfbI, which has previously been implicated in caveolae-mediated uptake. Nor was this effect dependent on CAVIN1, as knockout of CAVIN1 in MEFs resulted in reduced GAS internalization. While CAV1 restricted GAS invasion into host cells, we observed only minimal association of invading GAS (strain M1T15448) with CAV1 by immunofluorescence, and very low association of invading M1T15448 with caveolae by transmission electron microscopy. These observations suggest that physical interaction with caveolae is not needed for CAV1 restriction of invading GAS. An indirect mechanism of action is also consistent with the finding that changing membrane fluidity reverses the increased invasion observed in CAV1-null cells. Together, these results suggest that CAV1 protects host cells against GAS invasion by a caveolae-independent mechanism.

Relative contributions of M- and FCT-region cell surface proteins to fitness and virulence of a classical group A streptococcal skin strain
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Group A streptococci (GAS) are highly prevalent human pathogens whose primary ecological niche is the superficial epithelial layers of the throat and/or skin. Many GAS strains having a strong tendency to cause pharyngitis are distinct from strains that tend to cause impetigo; thus, genetic differences between them may confer host tissue-specific virulence. In this study, the FbaA surface protein gene is found to be present in most skin specialist strains, but largely absent from a genetically-related subset of pharyngitis isolates. Using an Δfbaa mutant constructed in the impetigo strain Alab49, loss of FbaA resulted in a slight but significant decrease in GAS fitness in a humanized mouse model for impetigo; the Δfbaa mutant also exhibited decreased survival in whole human blood due to phagocytosis. Using assays with highly sensitive outcome measures, Alab49Δfbaa was compared to other isogenic mutants lacking M- or FCT-region virulence genes known to be disproportionately associated with classical skin strains. FbaA and PAM (i.e., M53 protein) have additive effects in promoting GAS survival in whole blood. In addition to FbaA and PAM, other Mga-regulated genes make a significant contribution to virulence in both the impetigo and phagocytosis assays. The pilus adhesin tip protein Cpa promotes Alab49 survival in whole blood, and appears to fully account for the antiphagocytic effect attributable to pil. That numerous for strain-associated virulence factors make slight but significant contributions to virulence underscores the incremental contributions to fitness of individual surface protein genes and the multifactorial nature of GAS-host interactions.

Role of streptokinase in early stages of group A streptococcus skin infection
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Streptokinase (Ska) is a critical virulence factor of group A streptococcus (GAS) that functions as a plasminogen activator, leading to plasmin formation and dissolution of fibrin clots. The three major structural forms of Ska correlate with GAS strains associated with different diseases. The goal of this study is to test the effects of different Ska forms on superficial skin infection by GAS, using a humanized mouse model for impetigo. The impetigo strain Alab49 expresses the plasminogen-binding protein (PAM) and Ska-2b, which act together to generate cell-bound plasmin activity. Deletion of the Ska-2b gene leads to loss of both cell-bound and fluid-phase plasmin activity, and a significant decrease in virulence at the skin at 3 d post-inoculation. In contrast, replacement of Ska-2b with Ska-1 on the Alab49 strain background yields both fluid-phase and cell-bound plasmin activity, and hypervirulence during the first 48 h of infection; the mean generation time at the skin for the Ska-1 chimera is less than half the time relative to WT. Although streptokinase has an established role as a "spreading factor" that facilitates invasion of deep tissue, data point to a critical role for streptokinase during the earliest stages of superficial skin infection. Overall, the findings are consistent with the idea that streptokinase acts to delay wound healing following minor trauma, thereby allowing the bacterium to establish a better foothold within the host.

Sensor protein CovS regulates Rgg-LacD.1 system through modulating the phosphorylation level of
compared to the M’ mutant and an engineered isogenic emm1 knockout strain. Co

Results. Unexpectedly, following 24h experimental infection non

Methods. Following a 24h infection, invasive isolates were whole genome sequenced. Additional isogenic strains
to determine the mechanisms permitting this to o

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8. Department of Pediatrics, Academic Children Hospital Queen Fabiola, Brussels, Belgium

Background: Group A Streptococcus (GAS) M proteins have an exceptional ability to induc

A novel interaction between C4BP and the Enn protein of Group A streptococcus

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Selection of M protein deficient Streptococcus pyogenes: mechanisms and insights

A novel interaction between C4BP and the Enn protein of Group A streptococcus

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8. Department of Pediatrics, Academic Children Hospital Queen Fabiola, Brussels, Belgium

Background: Group A Streptococcus (GAS) M proteins have an exceptional ability to induce phagocytosis resistance and aid in establishing infections. Binding of the potent inhibitor of complement, C4BP, to M proteins has been characterized to a structural level. Binding of C4BP to whole GAS has been demonstrated for 89/100 emm-types. However, studies using purified proteins found no interaction with M proteins from 13/22 emm-types positive for whole cell binding, suggesting other C4BP binding partners exist.

Methods & Results: C4BP-binding motifs were defined based on sequence patterns in C4BP-binding M proteins. We discovered predicted binding sites in several GAS Enn proteins. The gene for Enn is present in 90% of over 1400 GAS genomes and up to two thirds contain predicted C4BP-binding motifs. 9 C4BP-binding GAS strains with M proteins negative for C4BP-binding were selected. The ability of the whole bacteria to bind C4BP was confirmed using surface plasmon resonance. Enn proteins from these C4BP-binding GAS strains with or without the predicted C4BP-binding motif were produced (n=7 & 2 respectively). Binding of C4BP was observed by pull-down assays for 2 proteins containing the motif, but was negative for another without a motif. We have mapped binding to the N-terminus of Enn for the two C4BP binding proteins and determined essential residues required for C4BP-binding using targeted mutagenesis.

Conclusions: This work suggests that Enn proteins may play a significant role in binding of C4BP at the GAS surface. The impact of this interaction on virulence and vaccination requires further investigation.

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Background: Group A Streptococcus (GAS) M proteins have an exceptional ability to induc

Conclusions: This work suggests that Enn proteins may play a significant role in binding of C4BP at the GAS surface. The impact of this interaction on virulence and vaccination requires further investigation.
protein (1 µg/ml) increased the multiplication of the emm1 negative strain to at least 20-fold greater than the WT. These results were corroborated using a panel of Lactococcus lactis strains: WT L. lactis was readily phagocytosed by neutrophils whereas L. lactis expressing cell wall-anchored or soluble M1 protein significantly reduced uptake by neutrophils.

Conclusion. The role of M1 protein is dual faceted and can rely on either cell wall-anchored or soluble forms. Our study elucidates the nuanced way in which M protein leads to the evasion of opsonophagocytosis.

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**RocA is a pseudokinase that enhances the virulence-regulating activity of the CovR/S two-component system in the group A Streptococcus**

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Regulating gene expression during infection is critical to the ability of pathogens to circumvent the immune response and cause disease. This is true for the group A Streptococcus (GAS), a pathogen that causes both invasive and non-invasive diseases. The control of virulence (CovR/S) two-component system has a major role in regulating GAS virulence factor expression, tailoring it in a disease-specific manner. We identified that the regulator of cov (RocA) protein functions through CovR/S to dramatically alter gene expression, including enhancing expression of more than a dozen immunomodulatory virulence factors. The regulatory activity of RocA reduces the virulence of GAS during invasive infection, as evident from Lancefield bactericidal assays and a murine bacteremia model of infection. While predicted to be a membrane-spanning kinase, we identified that only the membrane-spanning domains of RocA, not the dimerization or HATPase domains, are required for complementation of a rocA mutant strain. Thus, our data are consistent with RocA being a pseudokinase. How RocA functions through CovR/S remains to be fully elucidated, but we have determined that RocA enhances the ratio of phosphorylated to non-phosphorylated CovR. We propose a model in which RocA complexes with CovS and enhances CovS kinase activity. Consistent with this, Mg2+ and LL-37, which positively and negatively regulate CovS activity respectively, are attenuated in their activity in the absence of RocA. Thus, we propose that RocA, as a key accessory protein to the CovR/S system, influences the ability of GAS to modulate gene expression in response to host factors.

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**Streptococcus pyogenes nuclease A (SpnA) mediated virulence does not exclusively depend on nuclease activity**

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Streptococcus pyogenes, or Group A Streptococcus (GAS), is a human pathogen that causes a wide range of diseases, including pharyngitis, necrotizing fasciitis and toxic shock syndrome. This bacterium produces a large arsenal of virulence factors, including the cell wall-anchored Streptococcus pyogenes nuclease A (SpnA), which facilitates immune evasion by degrading the DNA backbone of neutrophil extracellular traps. SpnA consists of a C-terminal endo/exonuclease domain and a N-terminal domain of unknown function. Site-directed mutagenesis of SpnA has been carried out to further define the mechanism of the nuclease. The ability to degrade DNA by these recombinant SpnA mutants was either abolished or reduced when predicted metal-binding and catalytic site residues were mutated. To investigate the role of SpnA in virulence in vivo, Galleria mellonella (wax moth) larvae were used as an infection model. A GAS spnA deletion mutant showed reduced virulence in this model, with the spnA wt complementation completely restoring virulence. Interestingly, complementation with the spnA catalytic site mutant SpnA H716A only partially restored virulence. Our results outline the critical role of several predicted residues in enzymatic activity and demonstrate that nuclease activity is not exclusively responsible for SpnA-mediated GAS virulence in a Galleria mellonella infection model.

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**Roles of Gal-3 and Gal-8 in Group A streptococcus infection**

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Group A streptococcus (GAS) is an important human pathogen that causes a wide variety of cutaneous and systemic infections resulting in considerable morbidity and mortality worldwide. Although originally thought to be an extracellular bacterium, numerous studies have demonstrated that GAS can trigger internalization into nonimmune cells to escape from immune surveillance or antibiotic-mediated killing. Epithelial cells possess a defense mechanism involving autophagy-mediated targeting and killing of the invading GAS within lysosome-fused autophagosomes. In endothelial cells, in contrast, we previously showed that autophagy is not sufficient for GAS killing. In the present study, we show differential galectin (Gal)-3 and Gal-8 expression in endothelial cells and epithelial cells. The recruitment of Gal-3 to GAS is higher and the recruitment of Gal-8 to GAS is lower in...
endothelial cells compared to epithelial cells. We further show that Gal-3 promotes GAS replication and diminishes the recruitment of Gal-8 and ubiquitin, the latter of which is a critical protein for autophagy sequestration. After knockdown of Gal-3 in endothelial cells, the colocalization of Gal-8 and ubiquitin-decorated GAS is significantly increased. Animal studies confirmed that Gal-3-knockout mice develop less severe skin damage, and GAS replication can only be detected in the air pouch but not in organs and endothelial cells. These results demonstrate that Gal-3 inhibits ubiquitin recruitment by blocking Gal-8 resulting in GAS replication in endothelial cells.

Don't stain! show live bacteria
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Live bacteria microscopy has been utilized effectively to teach germ theory in communities affected by rheumatic fever, TB and HIV. Sessions have been conducted in three nations. These include with Aboriginal people in north Australia, with Luo people in western Kenya and with South Africans in Cape Town. This method is effective for those without previous exposure to scientific information or factual accounts of causes of the diseases that are prevalent in their communities. Success of this method is demonstrated in the interventions that people devise themselves, and in changed behaviour, resulting from participating in live microscopy sessions. Examples include construction of crude hand-washing facilities, building latrines and sterilizing drinking water.

Theory: critical awareness theory frames this ‘discovery’ education where people are given opportunity to discover new information themselves.

Process: familiar objects such as ants are used to orientate learners to functions of the microscope. Magnification is steadily increased and a ‘hands on’ approach is encouraged. A saliva specimen is provided by the operator and live bacteria, leucocytes and skin cells are visualised as they move across the slide. Movement observed in cytoplasm and motion of leucocytes confirms the existence of microscopic beings and germ theory.

A narrative of germ theory is provided in people’s languages during microscopy. Behaviour of leucocytes and bacteria is expounded thus providing intellectually convincing reasons for disease causation that is accessible to people with low literacy. Knowledge is also gained about the function of specific medicines used to treat or manage diseases.

EGFR-mediated post-translational modification of group A Streptococcus (GAS) serine/threonine phosphatase (SP-PTP) and protein tyrosine phosphatase (SP-PTP)
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Co-expressing membrane-bound SP-STK and secreted SP-STP, and the low-molecular-weight SP-PTP play an important role in the complex multifactorial mechanism of GAS pathogenesis. However, the nature of early interactions of the secreted SP-STP and SP-PTP proteins with host cells is not fully understood. In the present study, the interaction of SP-STP and SP-PTP with the EGFR, a major surface tyrosine kinase receptor of human lung epithelial cells was examined to test the hypothesis that during early interaction SP-PTP, if not SP-STP dephosphorylates EGF-stimulated EGFR because of its ability to dephosphorylate phospho-Tyr residues. For this, the tyrosine kinase domain of EGFR was first subjected to dephosphorylation in the presence of increasing concentrations of SP-PTP and SP-PTP using in vitro phosphorylation assays. None of these phosphatases dephosphorylated EGFR even at a high concentration. Instead, these phosphatases were, in fact, differentially phosphorylated in the presence of EGFR in a dose-dependent manner. Intriguingly, the innate phosphatase activities of both SP-STP and SP-PTP also increased substantially upon phosphorylation. LC-MS/MS-based proteomic analysis of EGFR-phosphorylated SP-STP and SP-PTP revealed several phosphosites. These sites included ten residues (5 Thr, 3 Ser, and 2 Tyr residues) within the SP-STP sequence and two Tyr residues within the SP-PTP sequence. The enzymatically-active secreted SP-STP enters the host cell nucleus by crossing two membrane barriers and causes host-cell apoptosis/pyroptosis. Thus, the enhanced de novo phosphatase activity of the secreted SP-STP and/or SP-PTP by the EGFR-mediated phosphorylation, occurring soon after the protein/GAS entry within the host cell provides a novel mechanism of GAS pathogenesis.

Eukaryote-like serine/threonine kinase (StkP)- and phosphatase (PhpP)-mediated modulation of the S. pneumoniae virulence
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Pneumococcal Ser/Thr kinase (StkP) and its cognate phosphatase (PhpP) play a crucial role in bacterial cytokinesis. Unlike homologs of PhpP in other gram-positive pathogens, the precise role of PhpP in pneumococcal virulence is presently unknown since most available studies on these proteins are carried out using unencapsulated pneumococcal strains. Here, we demonstrate that the encapsulated pneumococcal strain D39-derived ∆PhpP and ∆StkP mutants grow differentially in the presence of different non-glucose carbohydrate
Context-specific effects of the chemokine cleaving enzyme SpyCEP on neutrophil clearance of S. pyogenes

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Background The Streptococcus pyogenes cell envelope protease (SpyCEP) cleaves the chemokine CXCL8 (IL-8) and all ELR+ CXC chemokines. Reduced neutrophil recruitment is linked with increased bacterial burden and tissue necrosis. SpyCEP over-expression can be associated with an absence of neutrophils or with neutrophil death at sites of infection; we set out to examine the effects of SpyCEP on neutrophils and S. pyogenes clearance.

Methods Recombinant S. pyogenes strains lacking the SpyCEP cell wall anchoring domain were used to express soluble SpyCEP. SpyCEP was purified by affinity chromatography using an anti-SpyCEP antibody loaded sepharose column, and by His-FLAG modification and nickel affinity chromatography. Activity of SpyCEP and inhibitors was assessed by IL-8 cleavage assay. The gene encoding SpyCEP, cepA, was disrupted by allelic replacement in contemporary emm1 clinical isolates; a parent strain, an isogenic covR mutant, and a covS mutant. The impact of SpyCEP on bacterial and neutrophil survival was assessed.

Results The yield of purified SpyCEP was approximately 200 μg/litre and activity was 0.1ng CXCL8/h/ng SpyCEP. SpyCEP had complex effects on human neutrophils dependent on context; necrosis was observed only for purified SpyCEP. Although uptake of Lactococcus lactis by neutrophils was impaired by SpyCEP expression, expression of SpyCEP by emm1 S. pyogenes did not affect uptake, except in the setting of covR mutation.

Conclusion A spectrum of compensatory virulence mechanisms is employed by emm1 S. pyogenes to resist opsonophagocytic killing. The impact of SpyCEP on neutrophil uptake, survival, and bacterial clearance depends on context.

Early Extracellular Lymphatic Dissemination of Streptococcus pyogenes: Prevalence and Implications for Pathogenesis

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Background We recently described lymphatic tropism in hyper-encapsulated emm18 S. pyogenes that requires interaction of capsular hyaluronan with the receptor LYVE-1. The utilisation of this lymphatic infection route among more clinically-prevalent S. pyogenes strains, as well as its importance in other manifestations of streptococcal disease are unexplored. We determined the frequency of lymphatic dissemination of a range of clinically relevant S. pyogenes strains and assessed host cell involvement. Furthermore, we characterised the kinetics and dynamics of the process to explore the interplay with systemic infection.

Methods Lymphatic dissemination of S. pyogenes isolates was measured by plating CFU from organs following i.m infection of BALB/c and FVB/n mice. LYVE-1 blocking antibodies were used to obstruct lymphatic tropism.

Results Emm1, Emm3, and Emm89 S. pyogenes reached the draining lymph node within 30 minutes following deep tissue infection. Gentamicin protection assays demonstrated that the majority (>99%) of S. pyogenes in the lymph node were extracellular. Flow cytometric analysis showed that bacteria at the infection site were not associated with host leukocytes. Isogenic emm1 isolates revealed that both covR mutation and upregulated capsule expression increased bacterial load in the draining lymph node and systemic organs, whereas capsule deletion reduced this. Blocking LYVE-1 prior to infection reduced bacterial load in the draining lymph node but increased systemic bacterial load.

Conclusion Lymphatic dissemination of S. pyogenes is a phenomenon with pertinence beyond hyper-encapsulated strains. A link between uptake and retention of S. pyogenes in the lymphatics and systemic infection spread warrants
Lactobacilli can be used as oral probiotics for upper respiratory tract infections and can together with commensal viridans streptococci eradicate Group A Streptococci

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The viridans group streptococci (VGS) have been used as probiotic bacteria in upper respiratory tract infections due to their good interfering activity against Group A streptococci (GAS) and S.pneumoniae. Lactobacilli have also been discussed in this regard.

The aim of the study was to see if the interfering activity of selected clinical and commercial strains of Lactobacilli could inhibit the growth of GAS strains in vitro.

Different commercial lactobacilli strains were tested. As commensal bacteria, clinical isolates of VGS (S. oralis and S. sanguinis) were used. Both the agar overlay method and the PBS supernatant method were employed under different pH conditions

Results: Of the tested commercial strains, Lactobacillus plantarum LB931 and Lactobacillus rhamnosus LB21 most effectively inhibited GAS. However, they were not as competent as S.oralis and S.sanguinis, both being very good inhibitors. Together, lactobacilli and commensal VGA strains produced the fastest and most effective killing of the GAS.

Conclusion: Some lactobacilli strains had an interfering effect on GAS. Together with commensal bacteria as VGA, an improved growth inhibitory effect could be seen. We also found that the pH is important when testing bacterial interference in vitro.

We suggest that the efficacy of probiotic strains of Lactobacilli in eradicating GAS should be further investigated in clinical studies.

The Group A Streptococcus serotype M2 pilus plays a role in host cell adhesion and immune evasion.

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Group A Streptococcus (GAS), or Streptococcus pyogenes, is a human pathogen that causes diseases ranging from skin and soft tissue infections to severe invasive diseases, such as toxic shock syndrome. Each GAS strain carries a particular pilus type encoded in the variable fibronectin-binding, collagen-binding, T antigen (FCT) genomic region, of which 9 types have been identified but only a few have been studied. In this study we describe the functional analysis of the serotype M2 pilus encoded in the FCT-6 region, which shows low sequence homology to other better studied FCT regions. We found that, in contrast to other investigated GAS pili, the ancillary pilin 1 lacks adhesive properties. Instead, the backbone pilin is important for host cell adhesion and binds several host factors, including fibronectin and fibrinogen. Using a panel of recombinant pilus proteins, GAS gene deletion mutants and Lactococcus lactis gain-of-function mutants we show that, unlike other GAS pili, the FCT-6 pilus also contributes to immune evasion. This was demonstrated by a delay in blood clotting, increased intracellular survival of the bacteria in macrophages, higher bacterial survival rates in human whole blood and greater virulence in a Galleria mellonella infection model in the presence of fully assembled FCT-6 pili.

Central inflame induced by group A streptococcal infection is attenuated by blocking peripheral TNF

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Group A streptococcus (GAS) infection causes a strong inflammatory response associated with cytokine storm which leads to multi-organ failure characterized as streptococcal toxic shock syndrome (STSS). However, little is known about the systemic inflammatory effects of GAS infection on neurodegeneration in the brain. Therefore, we utilized a bioluminescent GAS strain and the reporter mice carrying firefly luciferase under transcriptional control of the NF-κB promoter to concurrently monitor the host immune response and bacterial burden in a single mouse. Interestingly, in addition to the inoculation locus, we additionally detected strong luminescence signals from NF-κB activation in the brain without detectable bacterial signal, suggesting the central inflammation is not mediated by disseminated bacteria. The inflamed brain showed increased expression of GFAP and NADPH oxidase components, displayed greater microglial activation and blood brain-barrier (BBB) disruption. Furthermore, Fluoro-Jade C positive cells were increased in the brain indicating that neurons were undergoing degeneration. Peripheral TNF, which contributes to pathology in brain injury, was elevated in the circulation and
the expression of its receptor was also increased in the inflamed brain. Blockage of peripheral TNF effectively decreased brain inflammation and injury, preventing BBB disruption and improving survival. Our studies provide new insight into GAS-induced central inflammation, such as encephalopathy, which can be attenuated by circulating TNF blocking.

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**Structural and functional studies of a transcriptional regulator Rgg in group A Streptococcus**

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The ability of group A Streptococcus (GAS) to survive and replicate intracellularly for deep tissue infection requires the coordination of gene expression that relies on the activity of transcriptional factors in a regulatory network. NAD-glycohydrolase (NADase; Nga) has been identified to involve in GAS replication inside endothelial cells. Expression of Nga is transcriptionally repressed by regulator Rgg, however, the molecular details remain unclear. Our study demonstrated that Rgg acts as a negative regulator of NADase. In addition, we applied small-angle X-ray scattering to construct the full atomic model of Rgg, including the DNA-binding helix-turn-helix motif of Rgg which is not visible in the currently available homologous structures. Furthermore, we identified the key residues of Rgg involved in regulating the NADase activity by structure-based mutagenesis studies. Our results provide the structural information of Rgg to understand the mode of action of Rgg associated with GAS replication in endothelium.

### References


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**Alteration of polymorphonuclear leukocyte regulated cell death by Group A Streptococcus contributes towards inflammation**

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Invasive infections caused by Group A Streptococcus (GAS) are characterised by an unregulated host inflammatory response. Of the >663,000 invasive cases annually, 25% result in mortality, with mortality rising over 50% when associated with the development of toxic shock syndrome. Strains isolated from invasive infections often contain mutations in the control of virulence regulatory system (covRS). Non-M1 serotype GAS with mutations in covRS have previously shown increased resistance to polymorphonuclear leukocyte (PMN) mediated phagocytosis and killing in vitro and in vivo. Additionally non-M1 GAS with mutations in covRS have been associated with dysregulation of apoptotic PMN cell death pathways, promoting an inflammatory mode of cell death and increased cytokine release. Recent research shows that many forms of “necrotic-like” cell death are mechanistically executed by the cell, redefining regulated cell death pathways. The alternative pro-inflammatory regulated cell death pathway necroptosis has been identified as a mode of cell death during infection of human PMNs with GAS, resulting in cell lysis. PMN lysis and subsequent release of cytotoxic contents can damage host tissue. Suppression of PMN apoptosis or induction of pro-inflammatory cell death by GAS with mutations to covRS may be a factor during the development of invasive infections.

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**The NAD-glycohydrolase mediates the multiplication of Group A Streptococcus in endothelial cells**

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Group A Streptococcus (GAS) is a human pathogen causing wide spectrum of diseases from mid pharyngitis to life-threatening necrotizing fasciitis. Although GAS is considered as an extracellular pathogen, increased evidences indicated that GAS could efficiently invade into host cells to evade the host immune defense and antibiotics-mediated killing. GAS secretes several virulence factors to enhance intracellular survival, but the exact role is still unclear. In present study, we found significantly greater intracellular multiplication of invasive strains NZ131 and A20 than non-invasive strain SF370 in human endothelial cells (HMEC-1). Using genetic manipulation, we demonstrated that nga-knockout and ifs (an endogenous inhibitor of NADase) overexpression strains dramatically decrease NADase activity and growth capacity in HMEC-1 cells, indicating that enzymatic activity of nga is required for GAS multiplication in endothelial cells. NADase hydrolyzed NAD+ to nicotinamide and ADP-ribose. The wild type NZ131-infected HMEC-1 cells showed lower intracellular NAD+ level than in nga mutant-infected cells, implying that intracellular energy imbalance may cause impaired intracellular bacterial clearance of endothelial cells. Confocal microscopic images further demonstrated that intracellular GAS was encapsulated into LC3-positive vacuoles, but prevented co-localization with lysosome and acidification in wild type NZ131 compared with its nga mutant. Moreover, immunoblotting images also revealed that a signal decrease in LC3-II conversion and autophagic adaptor protein p62/SQSTM1 in the nga mutant relative to wild-
type strain at the late stage of infection. These results indicated that intracellular GAS secretes NADase to alter intracellular NAD⁺ content, leading to escape autophagic killing and efficiently multiply in infected cells.

Whole metagenome profiling confirms the molecular link between Sarcopes scabiei and impetigo
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Epidemiological studies indicated a link between the epidermal infestation with Sarcopes scabiei and impetigo, particularly in tropical settings. Scabies mites are known to promote Group A Streptococcal (GAS) and S. aureus infections by breaching the skin barrier and excreting molecules that inhibit host innate immune responses. However, little is known about the composition and the function of the scabies-associated microbiota.

Here, high-throughput whole-metagenome sequencing was used to explore the scabies-associated microbiome. Scabies mites and their micro-environments were isolated from two patients (A, B) in northern Australia. Two ~80M paired-end reads Illumina libraries were generated of which ~2M (2.54%) and 0.4M (0.62%) microbial sequences were filtered out by mapping to human genome (hg19) and the recently available draft mite genomes. Taxonomic profiling revealed a microbial community dominated by the phylum Firmicutes (A: 79% and B: 59%). Actinobacteria (A: 11% and B: 1.8%) - which are most prevalent in normal skin samples - were underrepresented. The most abundant genera comprise Streptococcus (mostly A and G), Staphylococcus (mostly S. aureus and S. argenteus), Acinetobacter (mostly A. baumannii) and Corynebacterium. The microbiome community associated with scabies was enriched in metabolic functions such as carbohydrates and fatty acid metabolisms. Major streptococcal virulence factors were identified.

This study confirmed the association of GAS and S. aureus with scabies, identified further associated pathogens such as non-group A streptococci or A. Baumannii. Based on these crucial fundamental findings a larger amplicon-based study including patients from five different countries has commenced.

Differences in SpeB protease activity among group A streptococci associated with superficial, invasive, and autoimmune disease
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The secreted cysteine proteinase SpeB is an important virulence factor of group A streptococci (GAS), whereby SpeB activity varies widely among strains. To establish the degree to which SpeB activity correlates with disease, GAS organisms were recovered from patients with pharyngitis, impetigo, invasive disease or acute rheumatic fever (ARF), and selected for analysis using rigorous sampling criteria; >300 GAS isolates were tested for SpeB activity by casein digestion assays, and each GAS isolate was scored as a SpeB-producer or non-producer. Highly significant statistical differences (p < 0.01) in SpeB production are observed between GAS recovered from ARF (41.5% SpeB-producers) compared to pharyngitis (20.5%), invasive disease (16.7%), and impetigo (5.5%). SpeB activity differences between pharyngitis and impetigo isolates are also significant, whereas pharyngitis versus invasive isolates show no significant difference. The disproportionally greater number of SpeB-non-producers among ARF-associated isolates may indicate an altered transcriptional program for many rheumatogenic strains and/or a protective role for SpeB in GAS-triggered autoimmunity. Comparative transcriptomics and qRT-PCR are used to evaluate the relative RNA transcript levels of virulence genes between different GAS isolates in accordance with disease.

GAS and scabies - New insights into the pathogenic mechanisms between scabies and associated secondary streptococcal skin infections
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Skin infection by parasitic scabies mites is one of the most common dermatological conditions globally, resulting in considerable morbidity, disability, stigma and exacerbation of poverty. Tropical regions, including Fiji, are particularly affected. The initial mite infection causing the alteration of the skin barrier often leads to secondary infections with opportunistic bacteria. Group A streptococcus (GAS) is a common pathogen associated with scabies in the tropics. Notably, these infections can cause severe post-infective complications, such as post-streptococcal glomerulonephritis, acute rheumatic fever and rheumatic heart disease. Our recent research revealed a fascinating synergy between parasite, associated opportunistic pathogens and their human host. Two projects will be discussed:

Investigating the tripartite interactions between host, parasite and microbial pathogens could serve as a basis to develop novel intervention strategies targeting both, mites and bacteria. We proposed that scabies mites play a role in the establishment, proliferation and transmission of GAS. Scabies mites secrete several classes of complement inhibiting proteins into the mite gut and excrete them into the epidermal mite burrows. These inhibitors promoted the growth various GAS clinical isolates in whole blood bactericidal assays and reduced the opsonisation of the bacteria surface as well as the phagocytosis of bacteria by neutrophils.

We commenced scabies microbiome studies to elucidate the impact of scabies on the healthy skin microbiota and to identify the pathogenic and symbiotic bacteria carried by scabies mites. Shotgun metagenomic and amplicon-based approaches are underway to understand the microbiomes associated with scabies mites in patients from Northern Australia, India, France and Mexico.

Is dried blood spot (DBS) a real-world alternative to serum sampling for the collection of blood penicillin levels?

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Background

DBS are well established for testing several different drug levels. DBS for penicillin has been developed to assist with pharmacokinetic studies in children, however the stability of DBS has not been validated. DBS, like other methods still require freezing for long term storage.

Aims

To assess the reliability of DBS as an alternative for collecting blood penicillin levels in real world scenarios.

Methods

Fresh heparinised blood was collected and spiked with penicillin (1mg/L) and dispensed on to standard protein saver cards (40μL/spot). All samples were then placed in an air tight container with desiccant. Samples were exposed to a range of temperatures to mimic real collection scenarios and degradation was assessed; refrigeration (4°C), room temperature (22°C), hot room (35°C), and hot car (45°C). An esky (with ice bricks) and portable fridge (Waeco) sampling was also conducted with exposure to 45°C ambient temperature.

Results

At 4°C and the portable fridge (10°C), there was no significant penicillin degradation at 8 hours. Room temperature showed minimal degradation (1.5%) at 8 hours. Eskys with ice-bricks expose to 45°C for 8 hours showed a 2.5% degradation of penicillin. When dried in hot environments DBS showed accelerated degradation of penicillin (>5%) at 2.5 hours (35°C) and less than 1 hour (45°C).

Conclusion

When using DBS to assess penicillin levels, keeping temperatures <22°C or <10°C allow approximately 6 hours and 8 hours of sample stability respectively. These results validate the use of the DBS penicillin assay as a viable alternative to traditional collection methods in clinical practice.

Global quality of benzathine penicillin G (BPG) – is potency an issue?

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Background

BPG is used widely for the treatment many infections. It is recommended as the first line therapy for acute streptococcal glomerulonephritis, acute rheumatic fever and rheumatic heart disease. Our recent research revealed a fascinating synergy between parasite, associated opportunistic pathogens and their human host. Two projects will be discussed:

Investigating the tripartite interactions between host, parasite and microbial pathogens could serve as a basis to develop novel intervention strategies targeting both, mites and bacteria. We proposed that scabies mites play a role in the establishment, proliferation and transmission of GAS. Scabies mites secrete several classes of complement inhibiting proteins into the mite gut and excrete them into the epidermal mite burrows. These inhibitors promoted the growth various GAS clinical isolates in whole blood bactericidal assays and reduced the opsonisation of the bacteria surface as well as the phagocytosis of bacteria by neutrophils.

We commenced scabies microbiome studies to elucidate the impact of scabies on the healthy skin microbiota and to identify the pathogenic and symbiotic bacteria carried by scabies mites. Shotgun metagenomic and amplicon-based approaches are underway to understand the microbiomes associated with scabies mites in patients from Northern Australia, India, France and Mexico.
rheumatic fever prophylaxis as well as syphilis treatment in most cases. Clinicians have often used newer medications owing to a number misconceptions around BPG, including questioning the potency of supply and effectiveness of BPG. As such, newer broad spectrum agents are often used. Clinician confidence in BPG is a cornerstone of rheumatic heart disease control programs as Streptococcus pyogenes remains universally sensitive to penicillin.

Aim
To reassure clinicians that commercially available BPG preparations are stable and are manufactured to good manufacturing practice.

Methods
BPG donated from 10 countries were used for high performance liquid chromatography (HPLC) analysis. Two milligrams of powdered BPG was extracted from each vial, diluted in 1ml of dimethylformamide, then analysed using HPLC for potency and degradation products (penicilloic acid). Inter-batch variability was also tested when supplied. Thermal stability of Bicillin® was assessed over six months at 35°C.

Results
Samples provided contained a nominal range of between 96.6% (95% CI 93.3-99.7) and 106.7% (95% CI 102.0-111.3) of BPG. No evidence of degradation products was detected. Thermal stress of Bicillin® showed no significant degradation products at six months.

Conclusion
Preliminary data suggest that supply of BPG is of sufficient quality, as per manufacturing specifications. Supplied samples showed no evidence of degradation in tropical temperatures. Simulated stability testing suggests that unmixed Bicillin® can be kept outside the fridge for short periods at room temperature without premature degradation as per manufacturer guidelines.

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Streptococcus is still a common cause of diabetic foot infection, a retrospective observational study in a multidisciplinary diabetic foot unit (MDFU) inpatient cohort
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Background
Empiric antimicrobial regimes for diabetic foot infections (DFI) vary according to local epidemiology and prior colonisation/infection with resistant pathogens. The utility of using results from culture of superficial swabs to guide empiric antibiotic therapy is not well established in Australia, particularly in the context of rising prevalence of drug resistant organisms.

Aims
To assess the utility of DFI microbiology results and their relationship to empiric antibiotic prescribing.

Methods
Retrospective observational study of 151 admissions in 128 patients admitted to Fiona Stanley Hospital MDFU from 1st February 2015 to January 30th 2016.

Results
The mean age of patients was 60.4±14.9 years, 11.7% were Indigenous Australians. Ninety-two percent of admissions were moderate or severe DFI. The DFI's culturing Streptococcus, 97% were moderate or severe. Of those with positive cultures, 85% were polymicrobial. Dominant pathogens included: S. aureus (48%), beta-hemolytic streptococci (24%), Enterobacteriaceae (15%), enterococci (8%) and Pseudomonas (6%). Excluding cultures of known skin commensals, the proportion of superficial cultures concordant with deep tissue or blood cultures for Streptococcal species ranged from 0-41% compared with 21% for S. Aureus. Streptococcal bacteraemia occurred on 3 admissions. Despite following antibiotic prescribing guidelines, overprescribing with anti-MRSA and/or anti-pseudomonal agents occurred commonly (76%), however empiric prescribing provided insufficient antimicrobial activity in 12%.

Conclusion
Streptococcus remain a common cause of diabetic foot infections. Superficial swabs results correlated poorly with deep tissue or blood cultures. Local knowledge regarding causative organisms may allow for reduction in broad spectrum empiric antibiotic therapy, minimizing the development of resistant organisms.

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Role of angiopoietin-1 and angiopoietin-2 during group A streptococcal infections: clinical and human endothelial cell studies
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Group A streptococcus (GAS) is an important human pathogen that causes life-threatening invasive diseases, including necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). Angiopoietin-1 (Ang-1) promotes stabilization and maturation of new blood vessels, whereas angiopoietin-2 (Ang-2) can either promote VEGF-induced angiogenesis or destabilize blood vessels, causing endothelial apoptosis and leakiness in a context-dependent fashion. We hypothesized that Ang-1 and Ang-2 may regulate endothelial cell activation of GAS infection. Our data showed that in patients with invasive GAS infections, NF and STSS, the Ang-2 was increased at acute phase in contrast to Ang-1 which was decrease. These clinical results suggested that the differential regulation of Ang-1 and Ang-2 during severe GAS infection. To further elucidate the role of angiopoietins in the pathogenesis of severe GAS infection. First, angiopoietin dysregulations detected in GAS-infected cells were explored by RT-PCR and western blotting. In addition, a significant amount of Ang-2 is presynthesized and stored in the endothelial cell-specific organelles Weibel-Palade bodies (WPBs) under normal conditions and is rapidly released upon stimulation with various factors, such as thrombin and histamine. We next stimulated HUVECs with GAS and measured the levels of released Ang-2 by western blotting. These results suggested that GAS induced rapid Ang-2 release from endothelial cells. By transwell permeability assay, we observed that GAS induces endothelial hyperpermeability in HUVEC. Our studies implied that the vascular leakage was specifically induced by the differential regulation of Ang-1 and Ang-2 in severe GAS infections.

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**Group A Streptococcus pharyngitis and pharyngeal carriage: A systematic review**

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Antibiotic treatment of Group A Streptococcus (GAS) pharyngitis is important in acute rheumatic fever (ARF) prevention, however clinical guidelines for prescription vary. A proportion of patients with GAS culture-positive pharyngitis are GAS carriers with acute viral infections. These patients may receive antibiotics unnecessarily. This review aimed to assess the prevalence of GAS pharyngitis and carriage in different settings. A systematic literature review and meta-analysis were performed using Medline and EMBASE databases. Pooled prevalence estimates for GAS culture-positive pharyngitis, serologically-confirmed (‘true’) GAS pharyngitis and asymptomatic pharyngeal carriage were generated. Findings were stratified by age group, recruitment method and country income level. 284 eligible studies were identified. The prevalence of GAS culture-positive pharyngitis was 24.1% (95% CI: 22.6-25.6%) in clinical settings, but significantly less in active sore throat management programmes 10.0% (8.1-12.4%). The prevalence of serologically-confirmed GAS pharyngitis was 10.3% (6.6-15.7%) in children from OECD countries and their asymptomatic GAS carriage prevalence was 10.5% (8.4-12.9%). GAS culture-positive pharyngitis was more prevalent in OECD countries (24.3%, 22.6-26.1%) than non-OECD countries 17.6% (14.9-20.7%). These findings have important implications for clinical sore throat management and ARF prevention. In clinical settings, approximately 10% of children with throat swabs have true GAS pharyngitis, but this increases to around 55% when the child is GAS culture-positive. In active sore throat management programmes, the prevalence of GAS detection is lower, and if it declines towards the asymptomatic carriage rate, there may be little benefit from

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**Long-Term Effect of Mass Drug Administration For Scabies Control In Fiji: Experience From The SHIFT trial**

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Control of scabies based on treatment of individual cases is difficult because of frequent re-infestation. We implemented a community intervention trial of mass drug administration (MDA) for scabies to ascertain the efficacy and safety of two alternative regimens (topical permethrin and oral ivermectin MDA), compared with standard care. We identified three isolated island communities in Fiji and randomly assigned one of the three treatment regimens: ivermectin MDA, permethrin MDA or standard care with permethrin. All participants were sought for re-examination at 12 months, and at 24 months via a 20% sample. The study enrolled 2051 people. At baseline, scabies prevalence was high in all arms (32.1%, 41.7%, 36.6% in the three arms respectively). After one year the prevalence of scabies, previously reported, fell to 1.9% in the ivermectin arm corresponding to a reduction in prevalence of 94%. Scabies prevalence was also reduced to a lesser extent in the two other arms. At two years, scabies prevalence in the ivermectin arm was 3.7%, compared to 13.4% and 15.4% in the other two arms. In conclusion, the effect of MDA, particularly with ivermectin, was long lasting, with very low prevalence
Group A streptococcal vaccine diplomacy – opportunities for Oceanic leadership
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A shared interest in communicable disease control spurs international collaboration for vaccine development. The oral polio vaccine engendered collaboration between Soviet Union and United States scientists. In the Middle East and North Africa region neglected tropical disease vaccines research addresses shared interests. During Zika vaccine trials, vaccine candidate developers called for technical and regulatory harmonisation between Brazil, Russia, India, China and South Africa.
In Oceania, development of a group A streptococcal (GAS) vaccine, with rheumatic fever endpoint, is particularly important. The burden of GAS diseases is high and addressing disparities in rheumatic fever is a political issue. The Pacific Island Countries and Territories have twice declared rheumatic fever a public health priority. New Zealand has made major investments in rheumatic fever targets and Australia is developing an endgame for rheumatic heart disease.
The governments of New Zealand and Australia have already worked together to support the Support the Coalition to Advance New Vaccines Against Group A Streptococcus (CANSVAX) initiative. This kind of collaboration could be amplified by framing support for a GAS vaccine as a contribution to global development and strategic focus of foreign policy for Oceania. Vaccines are highly palatable political investments and with significant positive externalities. The return on investment in vaccine development is considerable; potentially greater than existing regional efforts in health and development investment. This review explores how clinicians, researchers, people living with GAS disease and civil society can make a compelling case for regional investment in the economic, scientific and political domains of GAS vaccine development.

Addressing shortcomings in International Classification of Diseases (ICD) codes for monitoring the burden of rheumatic heart disease (RHD): flogging a dead horse?
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Introduction
Administrative hospital and mortality data can potentially address the paucity of high-quality RHD data globally. We identified systematic problems with RHD codes (ICD-10-AM I05-I09) where some cardiac valve conditions are coded as RHD even when medical charts do not specify rheumatic origin. We aim to develop approaches to improve identification of RHD in administrative data.
Methods
Phase 1 involved a chart review validating RHD-coded hospital admissions. Phase 2 included national consultation with government managers, coders, clinicians and epidemiologists to develop an algorithm to increase the specificity of codes used for identifying RHD. Phase 3 reviewed the impact of this algorithm on identification of RHD admissions from three tertiary hospitals.
Results
A third of RHD-coded patients were identified as having RHD in the chart review, varying by age and high-risk status. Particular ICD-codes (I07/I08) were more likely to be false positives. The validation data, coding directives and clinical knowledge informed the consultation process, resulting in categorisation of codes as likely, possible or not RHD. When the new algorithm was tested on unlinked hospital data, RHD-coded admissions were 26.7% lower than using traditional RHD codes in 15-59year olds, 84.7% lower in ≥60yrs, and 21.9% lower in Aboriginal people. However, a review of linked hospital data showed that false positives were still likely.
Conclusion
Based on the findings, a more quantitative approach is being developed to improve case ascertainment of RHD using linked hospital/death data. Reliable RHD data are essential for monitoring progress towards goals to eliminate RHD in Australia.

Rheumatic heart disease in the Western Pacific region
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Background
Some of the highest recorded rheumatic heart disease (RHD) prevalence and mortality rates are from the Western Pacific Region (WPR) of the World Health Organization (WHO). This region comprises approximately 1.8 billion people, across 37 developmentally diverse countries and areas.

While RHD burden is well documented in much of the WPR sub-region of Oceania, RHD in the WPR nations outside Oceania has received relatively less attention. Understanding contemporary disease burden and identifying data gaps is crucial to guide policy and programmatic action and inform research priorities.

Methods
This review narratively synthesises existing RHD burden information for the 13 WHO WPR Member States and areas outside Oceania, and Taiwan. Multiple data sources are used: English-language peer-reviewed literature, official government health statistics, modelled estimates from the Global Burden of Disease study and WHO’s Global Health Estimates database.

Results
Over 40 publications documenting ARF/RHD burden and fulfilling inclusion criteria were identified; the majority were from China. Methods and estimates of disease burden varied considerably, between countries and over time. Some countries have no contemporary primary data publically available, and modelled estimates are used to approximate disease burden.

Conclusions
RHD morbidity and mortality appear to have fallen in association with economic development in some countries. In others, particularly poorer countries of the WPR, the impact of RHD appears to continue unabated. This review highlights considerable data gaps – in some countries, insufficient contemporary data makes it difficult to gauge the current status of RHD burden and control.

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Morbidity and mortality following valve replacement surgery for rheumatic heart disease in Fiji
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Fiji has one of the highest recorded prevalences of Rheumatic Heart Disease (RHD) in the world. If RHD is not effectively controlled, many children and young adults identified with early stages of the disease will eventually require cardiac surgery and life-long medical care. No surgical capacity for undertaking open heart surgery including valve replacement is available in the Pacific Islands region; instead Fiji (and other other near by countries) are visited annually by fly-in-fly out cardiac surgical teams who undertake mechanical valve replacement surgery. Anecdotal reports from clinicians in Fiji, and one prior research study, have shown that RHD patients have poor outcomes and much associated morbidity and mortality following valve replacement.

We reviewed patient medical records of all RHD patients who had cardiac surgery with artificial replacement of heart valves in Fiji during a 5 year period. Patient medical records were extensively examined to identify the treatment, drug therapy, clinical management, complications and outcomes. 123 patients were identified; 109 medical records were located and reviewed. Mortality and complication rates were high: 27% of patients found to be deceased and post-surgical complications, particularly related to thrombosis common. The majority of complications were recorded in the period of 1 month – 1 year post surgery. A survival analysis will be undertaken. These data provide a greater understanding of clinical management issues that can potentially be targeted by the Fiji Ministry of Health RHD control programme to improve patient care and outcomes to reduce the rates of post-surgical RHD complications and mortality.

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Secondary antibiotic prophylaxis for latent rheumatic heart disease: current state and opportunities for improvement
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Background
Delivery of secondary antibiotic prophylaxis (SAP) for rheumatic heart disease (RHD) is challenging in many settings. We aimed to investigate adherence to SAP among young people with screening-detected RHD in Fiji,
and to understand current practice and evaluate opportunities to improve adherence.

Methods
We collected SAP injection data from 76 health clinics across Fiji. Adherence was measured using the proportion of days covered (PDC) from 2012 to 2014. Multivariate logistic regression analysis was used to identify characteristics associated with adherence. A structured interview used the WHO adherence framework to evaluate attitudes, practice, barriers and improvement strategies.

Results
494 patients were included in the adherence study (median age 14 years). 203 (41%) had no injections recorded and just 33 (7%) had adequate adherence (PDC>0.8). Non-iTaukei ethnicity (OR 2.6) and urban residence (OR 3.4) were associated with adequate adherence, whereas time since diagnosis ≥1.5 years (OR 0.4) was inversely associated.

101 young people were interviewed. Reasons for missing injections included lack of awareness, feeling well, transport factors and medication unavailability. Inclusion of adolescents in decision making and improving educational materials were among the opportunities identified. Reminder strategies, particularly phone-based, were considered helpful by 94%.

Conclusions
This is the first study to assess adherence following screening, and the first to utilise the PDC measure for RHD adherence. Adherence in Fiji is currently inadequate for individual patient protection or population disease control. We characterised several factors influencing adherence, from which interventions can be developed. These interventions should be prioritised over further screening.

The natural history of screening-detected rheumatic heart disease: clinical and echocardiographic outcomes in Fiji

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Background:
Policy for rheumatic heart disease (RHD) screening is limited by the paucity of data on outcomes after screening. We aimed to compare the clinical outcomes and echocardiographic progression for young people with screening-detected RHD to a screen-negative population and those with clinically-diagnosed RHD.

Methods:
This was a cohort study in Fiji, with a primary cohort of 70 young people with screening-detected, definite RHD. Screen-negative and clinically-diagnosed comparison groups were matched 1:1 for demographic characteristics. Retrospective data were collected on clinical outcomes and healthcare episodes. Screening-detected participants with any reported RHD then underwent follow-up echocardiography. The diagnosis and severity of re-reported baseline and follow-up echocardiograms were compared.

Results:
There was one (1%) RHD-related death and 14 (20%) complications in the screening-detected group. There were nine (13%) RHD-deaths in the clinically-diagnosed group and 39 (56%) developed complications, and only 1 complication in the screen-negative group. The differences in mortality and complications were statistically significant by Kaplan-Meier analysis. Admission and surgery were more frequent in the screening-detected than screen-negative group. Ninety-eight participants were recruited for echocardiography (median follow-up, 7.5 years). 70% of definite RHD cases persisted or progressed, including four requiring surgery. 24% of borderline cases progressed to moderate-severe, definite RHD.

Conclusions:
Young people with screening-detected RHD have worse health outcomes than the screen-negative population, whilst the prognosis of clinically-diagnosed RHD in Fiji remains very poor. Most definite RHD cases persist on echocardiography, and others may require surgery or succumb. Close follow-up and individualised consideration of prophylaxis is need for borderline cases.

Obesity and subsequent weight gain in children hospitalised with acute rheumatic fever

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Background / Aims
Childhood obesity and acute rheumatic fever (ARF) are significant problems in New Zealand and disproportionately affect children and adolescents of Pacific Island and Māori ethnicity. Obesity is likely to contribute adversely to long-term cardiac outcomes in this population. Currently, there is no published data on the prevalence of obesity in New Zealand children diagnosed with ARF. Additionally, there is limited published data about benzathine penicillin pharmacokinetics and ARF recurrence rates in obese individuals.
This study aims to describe the prevalence of obesity amongst children hospitalised with ARF and to document their subsequent BMI trajectory and clinical outcomes.

**Methods**

Retrospective observational study of children < 15 years admitted to Kidz First Hospital, South Auckland, diagnosed with ARF between 1 January 2007 – 1 January 2012. Cases were identified by ICD codes and included if they met criteria for obesity (BMI > 97th percentile when corrected for age and gender) and 16/79 (20%) were overweight (BMI 85-95th percentile). Further details of the study and clinical outcomes will be presented.

**Results**

180 ARF cases were identified over the 5-year study period. Mean age at diagnosis was 9 years 10 months. 60% were male. Two thirds identified as Pacific peoples. Preliminary analysis of the first 79 cases found that at the time of ARF diagnosis, 37/79 (47%) met criteria for obesity (BMI > 97th percentile when corrected for age and gender) and 16/79 (20%) were overweight (BMI 85-95th percentile). Further detailed analysis of clinical outcomes, BMI trajectory, and risk factors for obesity in this population will be presented.

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### Cost of tertiary care treatment for Rheumatic Heart Disease in India

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Several initiatives to provide affordable and accessible cardiac care services, including creation of a network of AIIMS like institutions under Pradhan Mantri Swasthya Suraksha Yojana, Rashtriya Bal Swasthya Karyakram for screening of school children for heart related ailments like Rheumatic Heart Disease, are currently being implemented in India. However, lack of data on the cost of cardiac care is an impediment to the evidence based planning for such initiatives. So, we undertook an economic costing of cardiac care using bottom-up costing methodology from both patient and health system perspective. Data on all resources (capital & recurrent) and out-of-pocket expenditures for one year was collected. The results of our study showed per day-care consultation at cardiology and Cardio-thoracic and vascular surgery OPD costs INR 311 (USD 4.8) and INR 547 (USD 8.5) respectively. Per test costs for ECHO, ECG, TMT and Holter were INR 358 (USD 5.6), INR 18 (USD 0.3), INR 962 (USD 15) respectively. The per bed-day hospitalization in Cardiology & CTVS wards and I.C.U was INR 1,040 (USD 16) and INR 3,853 (USD 60) and INR 12,635 (USD 197) respectively. Per unit cost for MVR, AVR, TVR, DVR and Triple valve surgery were INR 43,311 (USD 677), INR 43,739 (683), INR 36,969 (USD 578), INR 43,208 (USD 675) and INR 57,771 (USD 90) respectively. Per unit cost of any valve replacement on average was INR 40,178 (USD 628). The mean Out-of-pocket expenditure for valve replacement was INR 1,76,391 (USD 2756). The estimates generated can be used for estimating the cost effectiveness of cardiac care services.

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### Innovations In Delivery Of Secondary Prophylaxis Treatment For People Living With RHD In Fiji Islands

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Rheumatic Heart Disease (RHD) is a significant public health problem in Fiji. Episodes of acute rheumatic fever (ARF) are often unrecognised and cases of RHD often present in severe stages. The Fiji RHD Prevention and Control Project is delivering a four year activity led by Fiji Ministry of Health and Medical Services. The Project has piloted models of care at eleven health facilities across Fiji. Patient/carer interviews were conducted in the design and interim phases. The effectiveness of interventions were evaluated via the rheumatic fever information system which records Benzathine penicillin adherence data. Data were gathered and analysed for training, media campaign and support activities. Patient support groups were led by nurses using educational flip charts followed by open discussion. The Project has reached over 700 patients and carers through support group activities. Approximately 40% of the nurse workforce were trained in ARF/RHD. Preliminary data is showing an average 20% improvement in adherence among patients at pilot sites over an 18 month period and a multi-media campaign promoting Benzathine adherence was implemented in 2017. The Project, has potential to contribute substantially to the development of a model of chronic disease care in Fiji with experience that can be applied in other high RHD prevalence (and high NCD) countries.

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### Reducing pain associated with intramuscular (IM) Benzathine Penicillin in the rheumatic fever population

This study aims to describe the prevalence of obesity amongst children hospitalised with ARF and to document their subsequent BMI trajectory and clinical outcomes.
The natural & unnatural history of acute rheumatic fever in the modern era.
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Outcome following acute rheumatic fever (ARF) is determined by the presence, severity and progression of rheumatic heart disease (RHD). The focus of our study was the long-term outcome of mitral (MV) and aortic (AV) valves, and left ventricular size and function. 144 children and adults were admitted to Middlemore Hospital 1998-2002 with their first known episode of ARF. 86 of the patients had a follow up echocardiogram completed 10 or more years after diagnosis. The mean age at diagnosis was 12.8 years. 69% were Pacific, 26% Maori, 5% other.
After a first episode of ARF only 1 in 8 children & young people develop severe mitral or aortic regurgitation. Half of these undergo valve repair or replacement. The left ventricle is not dilated at diagnosis unless there is at least one valve with severe regurgitation. 2/3 of children requiring valve surgery have a good outcome 10 or more years from diagnosis and intervention. Aortic and mitral valves that escape surgery improve in the majority if adherent to benzathine penicillin prophylaxis.

The BMI of the cohort increased markedly during follow up with 8/10 being obese at end of the study. By the end of the study, patients with a markedly increased BMI were developing structural cardiac disease attributable to obesity rather than RHD which was mostly mild. This has potential implications to the longer term cardiovascular well being of young people who have been successfully treated for their index episode of rheumatic fever.

Rheumatic fever assessment of adherence to secondary prophylaxis and factors associated with poor adherence in Tavua hospital Fiji
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Background: Rheumatic Fever (RF) and Rheumatic Heart Disease (RHD) are major public health problems in Fiji. This audit was conducted to determine adherence to secondary prophylaxis and identify factors associated with poor adherence among RF/RHD patients attending clinics in Tavua sub divisional hospital, Western Fiji

Methods: A mixed quantitative and qualitative methods were used. Twenty eight RF/RHD patients receiving prophylaxis during the period of 2014-2016 were included in the retrospective study. Demographic and medical information was extracted from patients’ folder and RHD clinic register book. Sixteen patients and care givers were interviewed to assess barriers to prophylaxis.

Results: Most patients were male (54%), 57% were iTaukei and the mean age was 26 years. Majority of patients (86%) were from the rural areas. Proportion of patients with good adherence (>80%) was 10%, 14% and 32% in 2014, 2015 and 2016, respectively. The overall adherence rate of >80% for the three years period was 14%. Poor adherence (<80%) was frequently reported among males, iTaukei ethnic groups, children <15 years of age and patients living in the rural areas. The common reasons for missing appointment were family emergency (31%), school/work commitments (16%), financial problems (16%), side effects (9%) and unfriendly medical staff (6%).

Conclusion: The study revealed poor adherence to secondary prophylaxis. Social and financial issues were cited as main barriers. Integrated and community based approach to address barriers and improve adherence should be considered. Furthermore, the development of national guidelines for secondary prophylaxis can improve and standardize treatment and care.

The benzathine penicillin G (BPG) reformulation preferences study - towards a new penicillin for rheumatic fever and rheumatic heart disease
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Acute rheumatic fever (ARF) is an autoimmune condition caused by untreated Group A Streptococcal (GAS) infection of the upper respiratory tract (and possibly skin). Multiple or severe attacks of ARF can cause cardiac damage known as rheumatic heart disease (RHD). The most effective recommended treatment of ARF requires monthly intramuscular injections of Benzathine Penicillin G (BPG) known as secondary prophylaxis. The goal of secondary prophylaxis is to prevent GAS infections that may lead to the recurrence of ARF. Rates of adherence to secondary prophylaxis schedules are usually low due to the frequency, duration, pain of injection, and access to proper and timely healthcare services. A less painful and longer acting BPG formulation would ideally help prevent ARF recurrence and improve compliance rates to this schedule. The purpose of this work is to explore the BPG reformulation preferences of children and teens currently receiving monthly BPG intramuscular injections, in addition to their families and healthcare providers. A software application has been developed that will explore the experiences of the groups who will then choose their ideal BPG formulation from a range of plausible formulations and associated dosing regimens. The software application has been optimized for use to ensure age and cultural appropriateness, and also efficient data collection. Pretesting of the software has been undertaken and interviewing of children, teens and family members has commenced. This is the first time a software application has been successfully developed to collect qualitative and quantitative data on individual
preferences for BPG formulations and dosing regimens.

Invasive group A Streptococcal disease surveillance
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Background: Group A Streptococcus (GAS) is a ubiquitous human pathogen responsible for an unrivalled range of clinical disease. The spectrum of GAS disease includes invasive infections such as bacteraemia, necrotising fasciitis and meningitis. There are few data on the impact of invasive GAS in Australia in children.
Methods: Between 2014 and 2016, we established surveillance for invasive GAS disease at the Royal Children’s Hospital in Melbourne. This project comprises an Australia-wide roll out of surveillance to six Australian paediatric hospitals in 2017. The aims of the project are:
1 To describe the epidemiology of invasive GAS disease in children in Australia, with special emphasis on severity, hospital care, and short and long-term outcomes.
2 To describe the clinical features of invasive GAS disease in hospitalised Australian children.
3 To establish an ongoing surveillance system for hospitalised Australian children with invasive GAS disease, including the collection of bacterial isolates to advance knowledge of local GAS molecular epidemiology (including emm typing).
Results: In this presentation we outline the methods in establishing national surveillance for paediatric invasive GAS disease in Australia, including case definitions, laboratory techniques and coordination, as well as describing the surveillance network.
Discussion: Understanding the incidence, impact, and molecular epidemiology of invasive GAS disease in Australian children is an important step in raising awareness and advancing the GAS research agenda to promote development of improved management and preventive strategies, including vaccination.

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An increase in invasive Group A streptococcal (iGAS) infections concurrent to the 2015/2016 influenza season was observed. We retrospectively assessed the impact of influenza on development of iGAS infection and fatal outcome, to inform future prevention strategies, by merging the laboratory confirmed influenza and iGAS infection databases using the unique patient identifier from 2008/2009 to 2015/2016 seasons (37th- 36th week). Influenza and iGAS coinfection was defined as the concurrent diagnosis within 15 days. All-cause case fatality rate (CFR) was calculated (within one week).
Overall, 44,429 influenza and 11,124 iGAS single infections and 111/55,553 (0.2%) co-infections were identified. Among coinfections, an increase of cases was observed 2015/2016 season (1.8%; 40/2,237) compared to other seasons (0.2%; p=0.001 to 0.8%; p=0.04), and in 2010/2011 season (2.0%; 24/1,193); both dominated by influenza A(H1N1)pdm09. Irrespective to season emm1 was the predominant iGAS emm-type, following by emm89 and emm12. Influenza A/iGAS were most common coinfections (62.3%; 66/106), followed by influenza B (36.8%; 39/106).
Overall, CFR was significantly higher among co-infections (22.6% vs 13.5%; p=0.006); particularly in the 2015/2016 season (27.5% vs 11.31; p=0.001). As shown in figure 1, CFR varied by age-group; among co-infections group CFR resulted higher in children ≤5 years.
This study identified an increase of influenza/iGAS co-infection in the 2015/2016 season, which was a late influenza season dominated by influenza A(H1N1)pdm09. Given the high CFR associated with co-infection especially in children, additional analysis aimed to clarify key factors in risk and severity of iGAS co-infection (such as influenza strain-type) are needed.
A mathematical framework for understanding the role of asymptomatic carriers in the transmission of infectious diseases

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For group A streptococcus (GAS), an unknown fraction of infected hosts are asymptomatic carriers who may be able to spread disease whilst remaining symptom free. Such hosts tend to evade clinical attention making the true extent of asymptomatic carriage and the natural history of disease difficult to assess. However, mathematical modelling studies of other infectious diseases have provided some insights into when we should expect carriers to play a significant role in transmission. But how universal are these insights? Are any of them likely to apply to GAS?

This presentation will outline how we are addressing these questions. We undertook a literature review to determine the range of different approaches being used to incorporate carriers in models of infectious disease transmission. Four general modeling approaches were identified which, in their simplest formulations, are special cases of a unifying generic model of disease transmission with carriers. Our analysis of this unifying model provides a more complete understanding of the possible role of carriers in pathogen transmission, and has the potential to help guide model choice for future mathematical studies of GAS.

Our analysis underscores the importance of uncovering the fundamental nature of asymptomatic carriage for particular pathogens like GAS if future decisions on public health policy are to be based on the outputs of predictive mathematical models. An improved understanding of the role carriers play in the persistence of GAS infections will allow us to assess the likely impact of interventions, including vaccination, to reduce the burden of disease.

Low prevalence rates for group A β-Haemolytic Streptococcal Carriage in Africa: A Cross-Sectional Study and Systematic Review

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Background: Asymptomatic children can be a major reservoir of pharyngeal Group A Streptococcus (GAS). There is a need to document GAS carriage, so as to inform the development of putative GAS vaccines. We, therefore, undertook to perform a cross-sectional study to determine the prevalence of GAS carriage in school children in Cape Town; we considered our results in the context of a meta-analysis of pooled data of GAS carriage in school children in African countries.

Methods: Pharyngeal swabs were obtained from learners in Cape Town and processed at the microbiology facility at Groote Schuur Hospital. Thereafter, we conducted a systematic review through a comprehensive literature search among several sources. Prevalence estimates with 95% CIs were determined using a random-effects meta-analysis.

Results: GAS was isolated from 31/950 healthy learners (3%; 95%CI, 2%-4 %) enrolled from 2009-2011. Together
with 18 studies meeting our systematic review inclusion criteria, the pooled prevalence was 9% (95% CI, 6% to 11%; 19 studies). Sub-analyses revealed similar pooled rates across Southern, Eastern and Northern Africa. Countries within Central Africa and West Africa had notably lower estimates of <8%.

Discussion: Our cross-sectional study reports a low prevalence of GAS carriage in South African school children. Across Africa, pooled results reveal a GAS carriage estimate of 9%, lower than the 12% reported in an earlier systematic review. Given that, studies of pharyngitis report GAS prevalence of >20%, our findings emphasize the association between GAS and pharyngitis.

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Molecular epidemiology of group A streptococcus (GAS) in north India
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Group A Streptococcal infections are neglected public health problem. Therefore, molecular epidemiology of GAS was explored in Chandigarh, India where surveillance for GAS infections was conducted in the schools. A total 7004 school children (3903 boys and 3101 girls), in the age group of 5-15 years, were clinically examined from the eight government schools of Chandigarh (India). Pharyngeal swabs were taken from all the students whereas skin swabs were taken only from children having skin lesions. All the swabs were streaked on blood agar plates. GAS strains were identified and emm typed by PCR and sequencing. Out of the 7004 throat swabs, 86 GAS were isolated. The prevalence of GAS was found to be 1.4% (22/1572) among children having pharyngitis and 1.2% (64/5432) among those who did not have pharyngitis. Among 124 children with skin lesions, 24 GAS were identified. The prevalence of GAS in skin lesions was 19.4%. Out of 110 GAS identified from 7128 swabs during the study, 35 different emm types were identified; emm12 was most prevalent. High heterogeneity observed in emm type pattern of GAS in North India which is a big challenge for future vaccine development studies.

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Quantifying the age of first infection with skin sores in five remote Australian Aboriginal communities
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Prevalence of skin sores in remote Australian Aboriginal communities remains unacceptably high, with Group A Streptococcus (GAS) the dominant pathogen. We aim to better understand the drivers of GAS transmission using mathematical models. The age of first skin sores infection—the inverse of the force of infection—is quantified by synthesizing historical data across five remote Aboriginal communities.

Methods
We extracted the observed age of first skin sores infection for children under five, from three pooled studies from 2001-2007. We estimated the age of first infection using the Kaplan-Meier estimator; parametric exponential mixture model; and Cox proportional hazards. We quantified disease burden, age of first infection, proportion escaping infection and influence of cofactors.

Results
Our study included 378 children, who presented 21 (IQR 13-27) times in their first year of life. Skin sore prevalence reached 36% in 2006. Mean age of first infection was 7.8 months (95%CI 6.9-8.8), ranging from 7-10 months across communities. Up to 7% (95%CI 6.3-12.4) escaped infection. Birth year 2006 and birth quarter October-December had significantly younger ages of first infection, at 3.9 months (95%CI 2.0-7.6) and 5.6 months (95%CI 4.1-7.5) respectively, representing an increased force of infection.

Conclusion
The young age of first infection with skin sores reflects the high disease burden in these communities. Quantification of the age of first infection with skin sores, and the influence of cofactors, will inform development of GAS transmission models. Surveillance of the age of first infection can be used to measure the impact of intervention strategies.

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Epidemiology and antibiotic resistance of group A streptococci isolated from healthy schoolchildren in Rawalpindi, Pakistan.
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Objectives: To assess the epidemiology, antibiotic resistance and genotypic characterization of group A streptococci (GAS) in Rawalpindi, Pakistan.

Methods: Isolates were characterized in terms of their antibiotic resistance, the phenotypes of erythromycin
resistance, the frequencies of erm(B), erm(A) and mef(A) genes, and by emm genotyping or M typing.

Results: A total of 198 (30%) of 650 healthy school children yielded GAS from throat swab culture during 2016. The most frequent emm types were emm12 (38.3%), followed by emm75 (13.2%), emm18 (10.4%), emm22 (9.3%), emm1 (8.3%). The resistance rates to erythromycin and clindamycin were 61.0% and 47.7%, respectively. Among the erythromycin-resistant strains, constitutive resistance, inducible resistance, and the M phenotype were observed in 61.2%, 2.0% and 36.7%, respectively, which correlated with the presence of resistance genes. Most of the emm12 strains showed constitutive resistance, whereas emm18 and emm75 showed the M phenotype. The organisms with other emm genotypes were susceptible to both erythromycin and clindamycin. Conclusions: Erythromycin and clindamycin resistance is quite high in Rawalpindi, Pakistan. Constitutive resistance is more common than the M phenotype, with inducible resistance occurring rarely.