Pathogenesis of *Streptococcus pneumoniae*

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This thesis is dedicated to my mother, and sisters Janine and Suzanne.

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Abstract

Streptococcus pneumoniae is the principal bacterial cause of otitis media (OM). While OM does not have the high rates of mortality associated with invasive pneumococcal diseases, such as meningitis, it has an extremely high rate of morbidity, with most children suffering at least one episode of OM during their early childhood years. Despite being a major public health burden worldwide, certain communities are more impacted than others. In Australia, remote Aboriginal communities have the highest rates of OM in the world with absolutely no improvement over the last few decades. Therefore, understanding the pathogenesis of pneumococcal OM is of vital importance.

At the start of this project, serotype 3 middle ear and nasopharyngeal isolates were obtained from both remote Aboriginal communities and the general Australian population from the Menzies School of Health Research (MSHR) in Darwin and the Women's and Children's Hospital (WCH) in Adelaide, respectively. This serotype is an important middle ear pathogen, including in remote Aboriginal communities. In addition, serogroup 11 nasopharyngeal and sinusitis isolates were received from the same sources. Serogroup 11 is rarely reported in OM in remote Australian Aboriginal communities and is generally not as prevalent as serotype 3 in other communities. Furthermore, the serogroup 11 isolates tested were avirulent in mouse invasive disease models, while the serotype 3 isolates tested were virulent.

A serotype 11A nasopharyngeal isolate from a remote Aboriginal community was capsule switched to serotype 3. However, in a pneumonia/sepsis mouse model, it was less fit in the middle ears compared to the middle ear isolate from which the serotype 3 capsule

locus was derived. This indicated the serotype 3 isolates of this study possess genetic factors apart from serotype that influence OM.

Multilocus sequence typing identified two STs in the serotype 3 middle ear isolates (ST180 and ST458). Generally, ST180 is the dominant ST within serotype 3 worldwide, but interestingly, all the isolates typed from MSHR belonged to ST458. The genomes of ST180 and ST458 isolates were analysed using a variety of molecular biological techniques, including a comparison with serogroup 11 isolates by DNA microarray analysis. However, no gene common to one serotype/group but not the other was identified and therefore, the capsule loci remained the only distinguishing feature between serotype 3 and serogroup 11. In order to determine if there were any genes present in the genomes of ST180 and ST458 isolates which were not represented on the microarray slide, PCR-based subtractive hybridisation was employed, and a putative cellobiose phosphotransferase system (PTS) was identified. This PTS is part of a 10 kb island, which includes a sulfatase and ROK family protein. However, there is a large deletion in ST458, which includes the sulfatase and part of the putative cellobiose PTS operon. A large number of strains from a variety of serotype/groups, which included serogroup 11, were found to carry the island through PCR analysis or bioinformatic searches, with most possessing the full island. In ST180, mutagenesis of the island and subsequent virulence studies revealed that the island confers a competitive advantage in a variety of niches, including the ear. Unfortunately, mutagenesis in ST458 was not possible despite numerous attempts.

Finally, the availability of next generation sequencing and a fully sequenced genome of a serotype 3 ST180 strain (OXC141), allowed the investigation for genes which had been missed by PCR-based subtractive hybridisation due to limitations with this technique. The genome of an ST458 middle ear isolate (MSHR17) was analysed, along with an unrelated serotype 3 strain (WU2) as a comparison. Both OXC141 and MSHR17

had regions not represented on the microarray slide, but these regions had not been detected by PCR-based subtractive hybridisation due to their absence in the other ST. Therefore, the PTS island remained the only region common to both STs. Nonetheless, the repertoire of regions possessed by ST180 may explain the dominance of this ST in serotype 3 worldwide, while the repertoire possessed by ST458 may mean it is better adapted to the microenvironmental niches encountered in remote Aboriginal communities.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Lauren Joy McAllister, and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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List of Abbreviations

This thesis uses abbreviations that are acceptable to the American Society for Microbiology without definition. Listed below are additional abbreviations, which are defined when first used in the text.

$A_{520,540,570,600}$	Absorbance at 520, 540, 570, 600 nm, respectively
Amp	Ampicillin
AOM	Acute otitis media
AP	Alkaline phophatase
AR	Accessory region
BA	Blood agar
BHI	Brain heart infusion
bp	Base pair(s)
BSA	Bovine serum albumin
CBP	Choline binding protein
CFU	Colony forming unit
CSOM	Chronic supparative otitis media
CPS	Capsule
CSP	Competence stimulating peptide
C-terminus	Carboxy terminus
DIG	Digoxigenin
DOC	Sodium deoxycholate
EDTA	Ethylene diamine tetraacetic acid
Ery	Erythromycin
g	Gravity units
GAG	Glycoaminoglycan
Gent	Gentamycin
h	Hours

Hic	Factor H-binding inhibitor of complement
hRBC	Human red blood cells
IgA	Immunoglobulin A
i.n.	Intranasal
i.p.	Intraperitoneal
IPD	Invasive pneumococcal disease
JCVI	J. Craig Venter Institute, San Diego and Rockville, USA
kb	Kilobase pair (s)
KEGG	Kyoto Encyclopedia of Genes and Genomes
LB	Luria Bertani broth
LD_{50}	50% lethal dose
LPS	Lipopolysaccharide
LPxTG	Leucine-proline-x-threonine-glycine binding motif
LytA	Autolysin A
min	Minute(s)
MLST	Multilocus sequence type
MSHR	Menzies School of Health Research, Darwin, Australia
Nan(A,B,C)	Neuraminidase (A, B, C)
NCBI	National Center for Biotechnology Information at the U.S. National Library of Medicine, Bethesda, Maryland, USA
nt	Nucleotide(s)
N-terminus	Amino terminus
OM	Otitis media
OME	Otitis media with effusion
O/N	Overnight
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Ply	Pneumolysin
PP1	Pneumococcal pathogenicity island 1
PspA	Pneumococcal surface protein A
PspC	Pneumococcal surface protein C
PTS	Phosphotransferase system
PTS 1	The putative cellobiose PTS identified in Chapter 4 of this study

PTS 2	The putative cellobiose PTS described by McKessar and Hakenbeck (2007)
RD	Region of diversity
ROK	Repressor, ORF of unknown function, kinase
RT	Room temperature
RT-PCR	Reverse transcriptase polymerase chain reaction
SB	Serum broth
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
Sec	Second (s)
ST	Sequence type
TEMED	N,N,N',N'-tetramethyl-ethylene-diamine
TH	Todd-Hewitt broth
THY	Todd-Hewitt broth supplemented with yeast extract
WCH	Women's and Children's Hospital, Adelaide, Australia
WHO	World Health Organization
X-gal	5-bromo-4-chloro-3-indolyl- β-D-galactopyranoside