Molecular epidemiological characterisation of carried *Neisseria meningitidis* isolates in Scotland, 1974 – 2004 and a comparison with an invasive meningococcal disease strain collection

Kevin J. Scott
BSc (Hons.)

Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

Institute of Infection, Immunity & Inflammation
College of Medical, Veterinary and Life Sciences
University of Glasgow

June 2011

Abstract

Neisseria meningitidis is an important cause of meningitis and septicaemia worldwide. Invasive meningococcal disease (IMD) is cyclical and varies by age group, being more common in children, especially those under 5 years. However, IMD is a rare outcome relative to asymptomatic carriage of the organism by the host and disease-causing isolates represent the tip of the iceberg in terms of the overall meningococcal population biology. The emphasis of previous research into *N. meningitidis* has rested firmly on the study of IMD isolates. In more recent times, however, there has been a more conscious effort to re-dress this balance to aid in our understanding of the relationship between carriage and disease.

The Scottish *Haemophilus*, *Legionella*, Meningococcus and Pneumococcus Reference Laboratory (SHLMPRL) have accrued an extensive isolate archive dating back to the 1960s. Only recently with the expansion of molecular techniques has the SHLMPRL begun to investigate this valuable resource. For around a decade or more the SHLMPRL have routinely used genotyping techniques to characterise all IMD isolates it receives. However, these methods have hitherto not been employed to investigate isolates of the archive not obtained from cases of IMD.

This study sought to characterise a collection of carried meningococci assembled from the isolate archive of the SHLMPRL that was obtained during the 31-year period, 1974 – 2004. Multi-locus sequence typing (MLST), *porA* Variable Region (VR) subtyping and a panel of genogrouping PCRs was used to characterise 791 carriage isolates. Temporal analyses of the data revealed how the presence of individual serogroups, clonal complexes, Sequence Types (STs), PorA subtypes and individual strain types in carried meningococci had changed in Scotland. Furthermore, these data were used in a comparison with a previously characterised collection of IMD isolates obtained during the same 31-year period to investigate the association of individual serogroups, clonal complexes, STs, PorA subtypes and individual strain types either with a carriage phenotype or with invasive disease.

Nongroupable isolates [Odds Ratio: OR 17.66; 95% Confidence Interval: CI (12.71 to 24.54)] and those of serogroups W135 [OR 2.49; 95% CI (1.72 to 3.60)], Y [OR 6.26; 95% CI (4.18 to 9.39)], X [OR 3.13; 95% CI (1.20 to 8.14)], Z [OR 131.89; 95% CI (18.00 to 960.76)] and 29E [OR 21.30; 95% CI (4.76 to 95.36)] were significantly associated with a carriage phenotype. In contrast, serogroups A [OR 3.64; 95% CI (1.96 to 6.76)], B [OR 2.65; 95% CI (2.25 to 3.12)] and C [OR 1.92; 95% CI (1.58 to 2.33)] were significantly associated with invasive disease.

The carriage strain collection reported herein was also observed to be highly diverse with the majority of STs identified only once. This diversity observed within the carriage strain collection [0.981; 95% CI (0.955, 1.006)] was significantly greater than the diversity within an IMD strain collection [0.938; 95%CI (0.934, 0.942)] from the same period. Temporal changes in the most prevalent clonal complexes (ccs) were observed throughout the 31-year period with increases in cc22, cc41/44 and cc269 and decreases in cc1, cc5, cc8, cc11, cc32, cc35, cc37, cc254, cc334 and cc364. Furthermore, for several ccs a significant association with a carriage phenotype (cc22, cc23, cc35, cc92, cc167, cc174, cc212, cc213, cc254, cc461, cc750, cc1157 and meningococci unassigned to a clonal complex) or with invasive disease (cc1, cc8, cc11, cc32, cc41-44 and cc269) was observed. Four lineages were identified amongst capsule null locus-containing meningococci, two of which, cc53 and cc1117, contained a unique allele (*cnl-8*) that was distinct from isolates of these lineages reported elsewhere.

Despite significant associations at the level of cc, distinct differences in those associations were apparent for individual STs within a given clonal complex; most notably the significant association of ST41 and both ST43 and ST44 with invasive disease and a carriage phenotype, respectively. A feature of the carriage strain collection was the concentration of cc8 isolates during the period 1984 – 1986 and the high proportion of isolates obtained from individuals resident in Lanarkshire at a time when an episode of increased disease was experienced within that region. In this study, cc8 was found to be significantly associated with invasive disease. Furthermore, whilst ST8 was also significantly associated with invasive disease the most common strain type within cc8, C:8:8:5,2,36-2, [OR 1.68; 95% CI (1.25, 2.27)] was however, significantly associated with a carriage phenotype. The strain types B:213:22,14,36 [OR 2.38

(95% CI 1.40, 4.07)] and B:43:19,15-1,36 [OR 2.88 (95% CI 1.42, 5.88)] were also significantly associated with a carriage phenotype.

Due to the heterogeneity of PorA subtypes in meningococci in Scotland the potential coverage by experimental or licensed PorA-based OMV vaccines would be limited. Therefore the introduction of monovalent or multivalent PorA-based vaccines in Scotland may be of little benefit. Improved strain coverage as a whole, not just against those of serogroup B, may require the addition of other vaccine antigens. Several other vaccine targets, including factor H-binding protein, are currently under investigation to improve coverage. Surveillance of these antigens and of the different lineages and serogroups in carriage and IMD isolates is essential to accurately monitor the effects that future vaccines will have on the meningococcus. We must remain vigilant despite a downward trend in cases of meningococcal disease in more industrialised countries.

Table of Contents

List of Tables

List of Figures

Acknowledgements

This experience has been both challenging and rewarding in equal measure and I would like to take this opportunity to convey my gratitude to my supervisors: firstly Dr. Mathew Diggle, at the SHLMPRL (now of Nottingham University Hospital NHS Trust); thank you for the opportunity to carry out this project, for the many helpful and insightful discussions and for your constructive comments throughout. Secondly, thank you to Prof. Tim Mitchell, at the University of Glasgow (and to the other members of his research group) for helpful discussion and constructive comments throughout.

Gratitude is also due to all my colleagues, both past and present, at the SHLMPRL, for their support and encouragement over, not just the course of my PhD, but the last nine years. Latterly in particular, thank you to Miss Roisin Ure for countless discussions and helpful advice. The SHLMPRL has an extensive isolate archive; to have been allowed access to such a valuable and important resource has been a huge privilege.

I would like to thank the curators of the Neisseria MLST and PorA databases, Drs. Keith Jolley and Julia Bennett, for the assignment of new MLST alleles and STs and new PorA VR variants. This publication made use of the Neisseria Multi Locus Sequence Typing website (http://pubmlst.org/ neisseria/) developed by Keith Jolley and Man-Suen Chan and sited at the University of Oxford (Jolley et al. 2004, *BMC Bioinformatics*, 5:86). The development of this site has been funded by the Wellcome Trust and European Union.

For helping to keep the work-life balance on as near an even keel as possible I am grateful to all my friends; whilst I can't name you all individually, a few deserve a special mention. To Dr. Karen Smith and Mrs Susan Sheridan thank you both for your support and for being wonderful friends; I couldn't imagine my life had our paths not crossed. To Mr Michael Pugh; thank you for dragging me out running on many an evening. It was (mostly) invigorating; one day I might actually be able to keep up with you. In advance, may I wish you congratulations on becoming Dr. Michael Pugh.

Lastly, to my parents Ian and Isabel; for shaping me into the person that I am today I am forever grateful. Thank you to both of you and also to my twin brother (and sometime flatmate) Michael; I couldn't have done it without your support, love and encouragement.

Bro, I'll have that pint now.

Author's declaration

I declare that the work presented herein embodies the results of my own special work, that it has been composed by me and that it does not include work forming part of a thesis presented successfully for a degree in this or another University.

Kevin J. Scott

List of Abbreviations

5CVMB	Novartis 5 Component Vaccine against Meningococcus B
AA	Ayrshire & Arran
ABC	Active Bacterial Core
abcZ	putative ABC transporter
AC	Argyll & Clyde
ACDP	Advisory Committee on Dangerous Pathogens
adk	adenylate kinase
AFLP	Amplified fragment length polymorphism
aroE	shikimate dehydrogenase
ATCC	American Type Culture Collection
BR	Borders
BURST	Based Upon Related Sequence Types
cc	clonal complex
ccs	clonal complexes
CDS	Communicable Diseases (Scotland)
CEACAM	carcinoembryonic antigen-related cell adhesion molecule
CI	Confidence Interval
cnl	capsule null locus

CO_2	Carbon dioxide
COSHH	Control of Substances Hazardous to Health
CPA	Clinical Pathology Accreditation (UK) Ltd
cps	capsule synthesis operon
CREE	Correia-repeat enclosed elements
CSF	cerebrospinal fluid
ctrA	capsule polysaccharide export outer membrane protein A
ctrABCD	capsule polysaccharide transport operon
ctrB	capsule polysaccharide export inner-membrane protein B
ctrC	capsule polysaccharide export inner-membrane protein C
ctrD	capsule polysaccharide export ATP-binding protein D
D	Simpson's index of diversity
DG	Dumfries & Galloway
DLV	double locus variant
DNA	Deoxyribonucleic acid
DUS	DNA uptake sequences
ECDC	European Centre for Disease Prevention and Control
ELISA	enzyme-linked immunosorbant assay
EMGM	European Monitoring Group for Meningococci

EMDS	European Meningococcal Disease Society
ESMD	Enhanced Surveillance of Meningococcal Disease
ET	Electrophoretic Type
EU	European Union
EU-IBIS	European Union Invasive Bacterial Infections Surveillance Network
FetA (FrpB)	iron-regulated outer membrane protein
FF	Fife
fH	factor H
fHBP	factor H-binding protein
fumC	fumarate hydratase
FV	Forth Valley
g	grams
GG	Greater Glasgow
GR	Grampian
galE	UDP-glucose 4-epimerase
galU	UTP-glucose-1-phosphate uridylyltransferase
gdh	glucose-6-phosphate dehydrogenase
GNA	Genome-derived neisserial antigen
HG	Highland

HPA	Health Protection Agency
HPS	Health Protection Scotland
IL-1	Interleukin-1
i.m.	intra-muscular
i.v.	intra-venous
IMD	Invasive meningococcal disease
IMPACT	Immunization Monitoring Program, Active
kg	kilograms
L	litres
LN	Lanarkshire
LO	Lothian
IcbA	capsular polysaccharide phosphotransferase
lipA (*ctrE*)	lipolyl synthase A
lipB (*ctrF*)	lipoate-protein ligase B
MAbs	monoclonal antibodies
MALDI-TOF	Matrix-assisted laser desorption/ionization – time of flight
ManNAc	N-acetyl-D-mannosamine
ManNAc-P	N-acetyl-D-mannosamine-1-phosphate
MCC	Meningococcal serogroup C polysaccharide-protein conjugate

MCP	membrane cofactor protein
MCV4	meningococcal conjugate quadrivalent ACYW135 vaccine
MeNZB	New Zealand meningococcus serogroup B vaccine
mg	milligrams
ml	millilitres
MIDAS	Meningococcal Infectious Disease Augmented Surveillance
MLEE	Multilocus enzyme electrophoresis
MLST	Multilocus sequence typing
MPSV4	meningococcal polysaccharide quadrivalent vaccine
MRLS	Meningococcus Reference Laboratory (Scotland)
mynA(*sacA*)	UDP-N-acetyl-D-glucosamine 2-epimerase
mynB(*sacB*)	capsular polysaccharide phosphotransferase
NANA	N-acetyl neuraminic acid (NeuNAc)
NadA	Neisseria adhesion A
NCTC	National Collection of Type Culture
ND	not determined
NG	nongroupable
NHBA	Neisseria heparin-binding antigen
NHS	National Health Service

NIPH	Norwegian Institute of Public Health
NK	not known
Nm	*Neisseria meningitidis*
NST	nonsubtypeable
NT	nontypeable
NTHi	nontypeable *Haemophilus influenzae*
NVI	Netherlands Vaccine Institute
NZMH	New Zealand Ministry of Health
OMP	outer membrane protein
OMV	outer membrane vesicle
Opa	opacity-associated protein
Opc	class 5 outer membrane protein
OR	Orkney
OR	Odds Ratio
PAI-1	Plasminogen-activator-inhibitor-1
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
pdhC	pyruvate dehydrogenase subunit
PFGE	Pulse-field Gel-Electrophoresis

pgm	phosphoglucomutase
PiI	Pilin subunit
PilE	type IV pilin protein
porA	Porin, class 1 major OMP PorA
porB	Porin, class 1 major OMP PorB
Rmp	reduction modifiable protein, class 4 major OMP
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
siaA (*synX*)	polysialic acid capsule biosynthesis protein A
siaB (*synB*)	polysialic acid capsule biosynthesis protein B
siaC (*synC*)	polysialic acid capsule biosynthesis protein C
siaD (*synD*)	polysialyltransferase
siaABCD	sialic acid biosynthesis pathway operon
SH	Shetland
SHLMPRL	Scottish, *Haemophilus*, *Legionella*, Meningococcus & Pneumococcus Reference Laboratory
SLV	single locus variant
SMPRL	Scottish, Meningococcus & Pneumococcus Reference Laboratory
sodC	superoxide dismutase
SOPs	Standard Operating Procedures

SSCP	Single-stranded conformation polymorphism
ST	Sequence Type
STs	Sequence Types
START	Sequence Type Analysis and Recombinational Tests
tex	toxin expression
TY	Tayside
UA	unassigned
UK	United Kingdom
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
USA	United States of America
VNTR	Variable number tandem repeat
VR1	Variable Region 1
VR2	Variable Region 2
VR3	Variable Region 3
WI	Western Isles
xcbA	serogroup X-specific capsule biosynthesis gene
µl	microlitre

1 Introduction

1.1 *Neisseria meningitidis* - the meningococcus

Neisseria meningitidis, the meningococcus, is a Gram negative diplococcus that colonises the human nasopharynx. The meningococcus can be carried asymptomatically by up to 30% of the general population and infrequently will invade the host and cause disease. Of the *Neisseria spp.* only *N. meningitidis* and *N. gonorrhoeae* are considered pathogenic and their only known host is man. Meningococci are transmitted directly by person-to-person contact or close contact via aerosols.

N. meningitidis is an important cause of meningitis and septicaemia worldwide. Invasive meningococcal disease (IMD) is cyclical and varies by age group, being more common in children, especially those under 5 years. A further peak in disease occurs in adolescence and rates decline during early adulthood and subsequently increase in older age groups (van Deuren, Brandtzaeg *et al.* 2000; Rosenstein, Perkins *et al.* 2001). The prevalence of different serogroups changes over time and can vary by geographic location. In temperate climates most cases of meningococcal disease occur during the winter or early spring, while in the "meningitis belt" in sub-Saharan Africa, the incidence of meningococcal infection rises sharply towards the end of the dry and dusty season (the harmattan) and falls with the onset of the rains (Jones 1995).

Diagnosis of IMD is problematical as initial symptoms are often non-specific and resemble other respiratory illnesses. The progression of disease is rapid, case-fatality rates exceed 10% and permanent sequelae are even more common. Isolation of the causative organism is not always possible, particularly if antibiotics have been administered prior to hospital admission, and laboratory-confirmed cases of IMD are increasingly being diagnosed by non-culture methods. Vaccination remains the best form of disease prevention. However, currently licensed vaccines do not offer broad protection against all serogroups, or are not immunogenic in those individuals most at risk of IMD (i.e. infants <2 years).

The meningococcus currently remains an important pathogen of man despite the best efforts of the medical and scientific communities over the preceding two centuries and longer. The potential of genomic sequencing offers exciting prospects for the future of meningococcal research and as the complexity of this bacterium is unravelled perhaps a more potent method for the eradication of meningococcal disease can be discovered. Meanwhile, current strategies to control the burden of *N. meningitidis* should not be taken for granted; vaccination and antibiotic use has been shown to be effective in high-risk populations. However, continued surveillance is required to establish the long-term effect on individual lineages should they undergo genetic changes that render a particular vaccine or antibiotic ineffective.

1.2 Historical perspectives

Although it is thought that the English physician Thomas Willis (1621-75) was probably first to report an outbreak of cerebrospinal fever, Vieusseux is usually credited with the first clear account of epidemic meningitis with his description of an outbreak during March 1805 in Geneva, Switzerland. This was soon followed by the first formal description of an outbreak in the USA. Consequently over the next decade reports of meningococcal disease spread throughout the eastern United States and Canada. Descriptions on both sides of the Atlantic soon followed. During the first half of the 19th century the clinical characteristics were clearly recognised: its contagious nature (although the cause was unknown at the time); the predilection of the disease for the military; sudden onset of meningococcal disease; extremely high mortality and the capacity to infect most members of a family within a few days. It was not until 1887 that Anton Weichselbaum first isolated an organism (which he called *diplococcus intracellularis meningitidis*) from the meningeal exudates of six out of eight cases of primary, sporadic meningitis. The first demonstration that patients with meningococcal meningitis could also carry the bacteria in the oropharynx came around the same time (Cartwright 1995a).

Formal notification systems were in place in many UK cities not long after the start of the 20th Century. At this time meningococcal disease in the UK appeared to be a particular problem in large cities, perhaps associated with poverty and overcrowding. Substantial outbreaks of cerebrospinal fever in Glasgow in 1906-7 and in Belfast in 1907-8 were documented. Compulsory notification of many infectious diseases, including cerebrospinal fever, was introduced in Britain in September 1912. During the following three years (1912-14) notified cases in England & Wales varied from 87 to 315. In contrast, the first full year of World War I (1915) saw 3496 notified cases. Numerous outbreaks in British and colonial army recruits began to occur and investigations by Captain JA Glover of the Royal Army Medical Corps (RAMC) at the Guards Depot Caterham, London found a close correlation between periods of severe overcrowding and the meningococcal carrier rate (Cartwright 1995a).

The first group of specific antimicrobial agents, the sulphonamides, became widely available in time to be used in large outbreaks of meningococcal meningitis which occurred in combatant countries during mobilisation at the outset of World War II. In 1940, for example, there were more than 12,000 notified cases in Britain. The introduction of sulphonamides had a dramatic effect on mortality in meningococcal meningitis, and they were also first used during the war to successfully treat carriage. Later in World War II, penicillin became available following Fleming's 1929 discovery and the subsequent purification of the active agent by Florey and Chain (Cartwright 1995a). During the latter part of the 20th Century, progress in reducing mortality rates has slowed somewhat despite improvements in the recognition and diagnosis of meningococcal disease and early intervention with antimicrobials.

1.3 Characterisation of Meningococci

1.3.1 Introduction

The meningococcus has on its surface several structures including outer membrane proteins (OMPs) and capsular polysaccharide that have been used as a

means of characterising strains. OMPs can be differentiated into five classes (Class 1-5) according to molecular weight. The meningococcus has several OMPs including PorA (Class 1), PorB (Class 2/3), Rmp (Class 4) (which has homology to *E. coli* OmpA) and the Class 5 Opa and Opc proteins. Other proteins involved in meningococcal pathogenesis include iron limitation-induced OMPs i.e. FetA (previously known as FrpB), the transferrin-binding proteins, Tbp-1, Tbp-2 and 37kDa Fe-binding protein, Fbp. FetA is an iron-regulated OMP that is also a vaccine target. However, its antigenic diversity may limit its usefulness or require the inclusion of multiple variants (Thompson, Feavers *et al.* 2003; Urwin, Russell *et al.* 2004). The PorA and FetA proteins are European Monitoring Group for Meningococci (EMGM)-recommended typing targets (Jolley, Brehony *et al.* 2007).

N. meningitidis can be classified serologically using antisera raised by immunising lab animals: into serogroups on the basis of structural differences in their capsular polysaccharide; into serotypes according to variation in their Class 2 or 3 OMPs (PorB) and into serosubtypes according to variation in their Class 1 OMPs (PorA) (Frasch, Zollinger *et al.* 1985; Abdillahi and Poolman 1988). Lipopolysaccharide can also be used to define immunotypes. Serological classification methods are phenotypically based and suffer from the deficiency that groupings based on them may contain strains that are genotypically dissimilar. Furthermore, the panel of monoclonal antibodies (MAbs) is not exhaustive nor do isolates always react, resulting in many nongroupable (NG), nontypeable (NT) or nonsubtypeable (NST) strains (Brooks, Fallon *et al.* 1995; van der Ende, Hopman *et al.* 1995; Feavers, Fox *et al.* 1996). Consequently, molecular typing schemes are increasingly replacing phenotypically-based techniques (Russell, Jolley *et al.* 2004). Numerous different methodologies have been published in the peer-reviewed literature; each has its own advantages and disadvantages.

Several molecular typing techniques have been used to characterise meningococcal isolates. This list is by no means complete but has included Multilocus Enzyme Electrophoresis (MLEE) (Selander, Caugant *et al.* 1986), Multilocus Sequence Typing (MLST) (Maiden, Bygraves *et al.* 1998), Pulsed-Field Gel-Electrophoresis (PFGE) (Bygraves and Maiden 1992; Yakubu, Abadi *et al.* 1994; Bevanger, Bergh *et al.* 1998), Polymerase Chain Reaction-Restriction

Fragment Length Polymorphism (PCR-RFLP) have been used to discriminate between serogroup (Borrow, Claus *et al.* 1998) or class 1 OMP (Speers and Jelfs 1997; Stefanelli, Fazio *et al.* 2001) and class 2/3 OMP PorB (Stefanelli, Fazio *et al.* 2001), Amplified Fragment Length Polymorphism (AFLP), Ribotyping (Woods, Helsel *et al.* 1992; Verdu, Coll *et al.* 1999), Variable Number Tandem Repeat (VNTR) (Yazdankhah, Kesanopoulos *et al.* 2005; Yazdankhah, Lindstedt *et al.* 2005), Single-Stranded Conformation Polymorphism (SSCP) (Newcombe, Dyer *et al.* 1997; Kesanopoulos, Tzanakaki *et al.* 2005), Matrix-Assisted Laser Desorption/Ionization – Time Of Flight (MALDI-TOF) analysis for genotyping (Lowe, Diggle *et al.* 2004; Honisch, Chen *et al.* 2007) and pyrosequencing to discriminate between strains (Diggle and Clarke 2004) or in determining reduced susceptibility to antibiotics (Thulin, Olcen *et al.* 2008). Various reviews and comparisons of the merits, or otherwise, of many of these methods have been published in the literature (Swaminathan, Matar *et al.* 1996; Yakubu, Abadi *et al.* 1999; Diggle and Clarke 2006).

1.3.2 Polysaccharide capsule: (sero)group

1.3.2.1 Introduction

Meningococci can be divided into twelve serogroups; A, B, C, H, I, K, L, W-135, X, Y, Z and Z'(29-E), on the basis of structural differences in their capsular polysaccharide (Liu, Gotschlich *et al.* 1971a; Liu, Gotschlich *et al.* 1971b; Bundle, Jennings *et al.* 1973; Bundle, Smith *et al.* 1974; Bhattacharjee, Jennings *et al.* 1975; 1976; Bhattacharjee, Jennings *et al.* 1978; Jennings, Lugowski *et al.* 1983; van der Kaaden, van Doorn-van Wakeren *et al.* 1984; van der Kaaden, Gerwig *et al.* 1985). However, the majority of disease is caused by serogroups A, B, C, W-135, Y and more recently X, although this varies geographically (Jones 1995; Rosenstein, Perkins *et al.* 1999; Djibo, Nicolas *et al.* 2003). Capsular polysaccharide of the disease-associated serogroups B, C, W-135 and Y are composed of sialic acid (Liu, Gotschlich *et al.* 1971a). Sialic acid is an N-acetylated nine-carbon acidic ketose (N-acetyl neuraminic acid; NeuNAc or NANA) that results from the condensation of pyruvic acid and N-acetyl mannosamine (ManNAc). The serogroup B and C capsules are homopolymers of

sialic acid linked α2-8 and α2-9, respectively. The α2-9 polysialyl capsule (serogroup C) is variously *O*-acetylated; the α2-8 polymer (serogroup B) is not. The serogroup Y and W-135 capsules are co-polymers of NeuNAc linked α2-6 to glucose (serogroup Y) or galactose (serogroup W-135) and both are variously *O*-acetylated. The serogroup B capsule is poorly immunogenic and is known to be structurally homologous with sialic acid in human cell lines (Neural cell adhesion molecule; NCAM) (Finne, Leinonen *et al.* 1983; Finne, Bitter-Suermann *et al.* 1987). Consequently, alternative means must be sought when developing vaccines to target serogroup B meningococcal disease. Serogroup A meningococci have a polymer of (alpha 1-6)-linked N-acetyl-D-mannosamine-1-phosphate (ManNAc-P) for a capsule (Liu, Gotschlich *et al.* 1971b).

1.3.2.2 Capsule biogenesis

The genetic basis of capsular polysaccharide synthesis, transport and expression has been studied extensively. The region of the chromosome responsible is termed the capsule locus (*cps*) and can broadly be divided into three regions; A, B, C, that contain the genes responsible for synthesis, modification and transport, respectively (Figure 1.1).

Figure 1.1 has been removed due to copyright restrictions

Figure 1.1 - Schematic of the cps complex of *Neisseria meningitidis*

Region A, capsule synthesis operon (*synXBCD*; also known as *siaABCD*); Region B, transport (*lipA/B* or *ctrE/D*); Region C, capsule transport operon (*ctrABCD*); Region D, LOS synthesis; Region E, regulation; P, divergent promoter (Swartley, Marfin *et al.* 1997)

Early studies cloning the *cps* from Group B meningococci into *Escherichia coli* revealed that a 24kb fragment was required for expression on the cell surface (Frosch, Weisgerber *et al.* 1989). This region showed homology to some or all other *N. meningitidis* capsular serogroups or *Neisseria* species depending on the region studied (Frosch, Weisgerber *et al.* 1989; Frosch, Edwards *et al.* 1991; Swartley, Ahn *et al.* 1996). Subsequently it has been shown that the capsule transport operon, *ctrABCD* (region C), and capsule synthesis operon, *siaABCD/synxBCD* (region A), are divergently transcribed from a shared 134bp promoter region (Swartley, Ahn *et al.* 1996; Tzeng, Swartley *et al.* 2001).

Enzymes encoded by *siaA-C* synthesise sialic acid (CMP-NANA) and are conserved among sialic acid-producing serogroups B, C, W-135 and Y (Ganguli, Zapata *et al.* 1994; Swartley, Marfin *et al.* 1997). Sialyltransferases, which catalyzes the serogroup-specific linkage of sialic acid homopolymers or sialic acid with glucose or galactose is encoded by the fourth gene of the *sia* operon, *siaD* and is unique for each serogroup (Claus, Vogel *et al.* 1997). Serogroup A capsular polysaccharide is composed of (alpha 1-6)-linked N-acetyl-D-mannosamine-1-phosphate (Liu, Gotschlich *et al.* 1971b) and the genes encoding this capsule operon, *mynA-D*, are unique to this serogroup. Novel capsule biosynthesis genes are also present in serogroup X meningococci (Tzeng, Noble *et al.* 2003). Some homology has been suggested between *xcbA* (serogroup X), *sacB* (serogroup A) and *lcbA* (serogroup L) (Tzeng, Noble *et al.* 2003), however the cps of the less common serogroups has not been described in the literature.

The capsule transport operon, *ctrABCD,* is highly conserved amongst serogroups B, C, W-135, Y, and A. The proteins encoded form a predicted ABC transporter that translocates capsular polysaccharide chains from the cytoplasm to the surface of the meningococcus (Frosch, Edwards *et al.* 1991; Frosch, Muller *et al.* 1992; Frosch and Muller 1993). The *ctrA* gene has been used as a non-specific target to identify meningococcal DNA in clinical specimens from individuals suspected of having meningococcal disease (Corless, Guiver *et al.* 2001) and as a serogroup-specific target to identify serogroups A, H, X, Z or 29E strains (Sadler, Fox *et al.* 2003; Bennett, Mulhall *et al.* 2004).

Flanking *ctrABCD* and *siaABCD* are *tex* (Region E) and *galE or galU* (Region D) which are located 5' and 3' respectively. Nucleotide sequences between *siaD* and *galE/U* vary depending on the strain sequenced. The nucleotide sequence of the *tex* gene is highly similar to hypothetical proteins of unknown function in *E. coli* and *Haemophilus influenzae* (Frosch, Edwards *et al.* 1991; Petering, Hammerschmidt *et al.* 1996) while in *Bordetella pertussis* the *tex* gene locus negatively affects toxin expression (Fuchs, Deppisch *et al.* 1996). Upstream of *tex* are *lipA* and *lipB* (Region B), which were originally proposed to be involved in cytoplasmic phospholipid substitution of the capsular polysaccharide chains (Frosch and Muller 1993), but more recently have been found to be involved in translocation and surface expression of the lipidated polymer (Tzeng, Datta *et al.* 2005). Furthermore, the authors proposed renaming LipA and LipB as CtrE and CtrF, respectively, to reflect their roles in capsule translocation, not lipidation (Tzeng, Datta *et al.* 2005). LipA and LipB are present in encapsulated *N. meningitidis* but not found in *N. gonorrhoeae* or *N. lactamica*. In *N. gonorrhoeae* and *N. lactamica, tex* and *galE* are adjacent to each other. These species are acapsulate and the genes responsible for synthesis and transport of capsule polysaccharide in *N. meningitidis* are absent from other members of the genus *Neisseria* (Frosch, Muller *et al.* 1992; Claus, Maiden *et al.* 2002).

1.3.2.3 Capsule switching

The exchange of DNA coding for the sialyltransferase by meningococci enables clones to express different capsular polysaccharide (Swartley, Marfin *et al.* 1997). Reports from continental Europe describing capsule switching in clinical isolates of *N. meningitidis* (Stefanelli, Fazio *et al.* 2003; Lancellotti, Guiyoule *et al.* 2006) is of particular importance to countries like the UK who have, since 1999, been using the Meningococcal serogroup C polysaccharide-protein conjugate (MCC) vaccine. Fortunately capsule switching has not been reported as yet in the UK (Maiden and Stuart 2002; Mooney, Christie *et al.* 2004; Diggle and Clarke 2005). Beddek and colleagues provided evidence that an invasive meningococcal strain may have acquired an entire capsule locus from a carriage-associated strain (Beddek, Li *et al.* 2009). Molecular surveillance data from Portugal during 2002 – 2006 indicates that C-to-B capsule switching has occurred

in some invasive lineages (Simoes, Cunha *et al.* 2009). The authors also highlighted the emergence of a number of B:2b:P1.5,2 strains (based on current nomenclature, rather than that used by the authors) in neighbouring Spain following an epidemic wave due to C:2b:P1.5,2 strains during 1996-7 (Alcala, Arreaza *et al.* 2002a; Alcala, Salcedo *et al.* 2002b).

The epidemic strain in Spain pre-dates those reported by Simoes *et al* 2009 Rather than the Portuguese B:2b:P1.5,2 strain arising as a result of capsule switching from a Portuguese C:2b:P1.5,2 strain it may be the case that the B:2b:P1.5,2 strain that emerged in Spain may have dispersed into, and become maintained in, the Portuguese population. Furthermore, the Spanish B:2b:P1.5,2 strain was thought to have arisen due to strain displacement rather than a detectable capsule-switching event (Alcala, Arreaza *et al.* 2002a; Alcala, Salcedo *et al.* 2002b). Subsequent surveillance data in Spain observed a further serogroup C epidemic after 1999 due to the C:2a:P1.5 ST-11 strain. Following mass vaccination in 2002 with the MCC vaccine dramatic reductions in serogroup C disease in Spain were observed (Salleras, Dominguez *et al.* 2003). However, cases of meningococcal disease due to the B:2a:P1.5 strain that may have undergone a recombination event involving the cps have been reported (Castilla, Vazquez *et al.* 2009).

Reports of possible capsule switching have not been restricted to continental Europe. In Canada, it is possible that strains may have undergone a Y-to-B capsule switch (Tsang, Law *et al.* 2005). Active Bacterial Core surveillance (ABCs) data from the United States seems to indicate that transformation events leading to capsule switching may not be rare; particularly among serogroup C isolates (Harrison 2008; Harrison, Shutt *et al.* 2010). In their study of 1,180 isolates from the period 2000 – 2005, Harrison and colleagues observed that 518 (43.9%), 281 (23.8%) and 319 (27.0%) isolates were of serogroup B, C and Y, respectively. A further 62 (5.3%) were of other serogroups or NG. Isolates of serogroup B (n=8, 1.5%), C (n=32, 11.4%) and Y (n=2, 0.6%) (Harrison 2008) or, in an updated report on 1,160 isolates, eight of serogroup B, three of serogroup Y and 36 of serogroup C showed evidence of capsule switching (Harrison, Shutt *et al.* 2010). Evidence for capsule switching in an isolate was defined "as the presence of an unusual ST-serogroup combination; specifically a genetic sequence type (ST)/clonal complex in an isolate of a serogroup more generally

associated with another serogroup" (Harrison 2008). The authors presented data indicating 35 B-to-C, seven C-to-B, two W-135-to-Y and one W-135-to-B capsule switching events (Harrison 2008; Harrison, Shutt *et al.* 2010).

1.3.2.4 Capsule-deficient meningococci

Rates of carriage of meningococci in more industrialised countries are typically around 10% (Jolley, Kalmusova *et al.* 2000; Claus, Maiden *et al.* 2005). A high proportion of carriage isolates are unencapsulated and are defined as NG. In a study of carriage strains from the Czech Republic nearly half the isolates were NG (Jolley, Kalmusova *et al.* 2000). The genetic basis for this has been investigated; often unencapsulated strains possess the genes necessary for capsule synthesis and transport but one or more genes can be inactivated by a variety of mechanisms (Jolley, Kalmusova *et al.* 2000; Claus, Maiden *et al.* 2002; Dolan-Livengood, Miller *et al.* 2003; Weber, Claus *et al.* 2006). Some isolates have been shown to harbour one or two homopolymeric tracts, exhibiting length variation in the serogroup B, W-135 and Y polysialyltransferase genes, as well as in the *siaA* gene, which could be phase variable (Weber, Claus *et al.* 2006). Irreversible mutation of the *cps* complex by one-base pair deletions, insertions of 1-34bp, or single nucleotide polymorphisms leading to premature stop codons can occur in some isolates (Weber, Claus *et al.* 2006). Insertion sequence (IS) elements, including IS*1301* which is often found in the same position in the *siaA* gene, or others found at various positions of the cps complex, can also lead to loss of encapsulation (Arreaza, Alcala *et al.* 2001; Dolan-Livengood, Miller *et al.* 2003; Weber, Claus *et al.* 2006).

Alternatively, unencapsulated strains lack the genes required for capsule synthesis (*siaA-D*) and transport (*ctrA-D*) (Claus, Maiden *et al.* 2002); regions A and C in Figure 1.1, respectively. Characterisation of the meningococcal capsule-synthesis cluster in carried meningococci isolated from healthy subjects in Bavaria, Germany has shown that a significant proportion of isolates harbour a non-coding intergenic region of either 113 or 114bp in length, termed the capsule null locus (*cnl*) (Claus, Maiden *et al.* 2002). The *cnl* of a subset of the Bavarian isolates was sequenced and compared to different strains of *N.*

lactamica and *N. gonorrhoeae*, which are acapsulate. This identified six different alleles; some alleles were found in more than one of the three species (Claus, Maiden *et al.* 2002). Additionally the *cnl* was associated with particular lineages. This may suggest a reduced dependence on expression of a capsule for transmission in meningococci possessing the *cnl*, compared to other meningococci (Claus, Maiden *et al.* 2002).

Interestingly, there have been reports in the literature of *cnl*-possessing meningococci causing disease in immunocompromised and, more remarkably, immunocompetent individuals (Vogel, Claus *et al.* 2004b; Hoang, Thomas *et al.* 2005; Findlow, Vogel *et al.* 2007). It has been demonstrated that polysaccharide capsule promotes survival in extracellular fluids like the bloodstream, mediating resistance to phagocytosis and complement-mediated bacteriolysis (serum resistance) (Vogel, Weinberger *et al.* 1997; Kahler, Martin *et al.* 1998) and also aids in intracellular survival in vitro (Spinosa, Progida *et al.* 2007). However, our understanding has been challenged by these reports of capsule-deficient strains causing IMD.

Other normally encapsulated bacterial species, such as *Streptococcus pneumoniae* and *H. influenzae*, are also frequently characterised as nontypeable (NT) or unencapsulated. Unencapsulated *S. pneumoniae* can be isolated from disease and carriage, particularly in association with epidemics of conjunctivitis among healthy young people. Characterisation of a collection of genuine NT pneumococci isolated from carriage and acute otitis media among children in Finland identified a lineage of NT pneumococci that had lost its capsular locus (Hanage, Kaijalainen *et al.* 2006). Moreover, *H. influenzae* that lack capsular polysaccharides are referred to as nontypeable (NTHi). Although NTHi are most commonly associated with asymptomatic colonisation, they are also pathogenic. *H. influenzae* is frequently associated with otitis media, chronic bronchitis, and community-acquired pneumoniae, and the strains associated with these mucosal infections are NTHi (Erwin, Nelson *et al.* 2005; Erwin and Smith 2007).

1.3.3 Neisserial Porins: PorB (sero)type & PorA (sero)subtype

Neisserial porins function as pores and are essential for bacterial survival as they modulate the exchange of ions between the bacterium and its surroundings. *N. meningitidis* expresses two porin proteins, PorA and PorB, on its outer membrane. Topology models of neisserial porins predicts PorA to be composed of highly conserved transmembrane sequences that form a ß-sheet structure with eight surface exposed loops (Loops I-VIII) (Figure 1.2) (van der Ley, Heckels *et al.* 1991).

Figure 1.2 - Schematic of the *N. meningitidis* class 1 OMP PorA
VR, variable region; (Derrick, Urwin *et al.* 1999)

Three dimensionally, PorA forms a trimer (Figure 1.3) and all but two of the loops vary extensively in both length and sequence (Derrick, Urwin *et al.* 1999). One loop, Loop II, is important in monomer-monomer interactions, while Loop III is proposed to lie inside the pore of each monomer, potentially influencing pore function (Derrick, Urwin *et al.* 1999).

Figure 1.3 - Three-dimensional structure of monomeric and trimeric PorA
A, cross-sectional view of monomeric PorA; B, aerial view of trimeric PorA; roman numerals correspond to the individual loops as indicated in Figure 1.2 (Derrick, Urwin *et al.* 1999)

Traditionally, serotyping (PorB) and serosubtyping (PorA) were performed using a panel of monoclonal antibodies (MAbs) (Abdillahi and Poolman 1988), however meningococci are often only partially serosubtyped or increasingly, are classified as non-serosubtypable either because a variant is not recognised by MAbs or because PorA is not expressed (Jelfs, Munro *et al.* 2000; Devoy, Dyet *et al.* 2005). To overcome these problems nucleotide sequencing of *porB* (Abad, Alcala *et al.* 2006) and *porA* VR1 (Loop I) and VR2 (Loop IV) is now becoming the method of choice. This allows VR families and variants to be assigned on the basis of amino acid sequence relationships rather than their reactivity with MAbs. The nomenclature was revised and family groupings were determined with ≥ 80% amino acid identity as a guide, or where amino acid identity was slightly below 80%, the presence of a particular motif representative of the family (Russell, Jolley *et al.* 2004). Furthermore, sequencing of VR3 (Loop V) has shown that strains possessing identical VR1 and VR2 can have a different VR3, although variation is less diverse (Molling, Unemo *et al.* 2000; Molling, Backman *et al.* 2001; Clarke, Diggle *et al.* 2003b).

PorA is also a target for meningococcal vaccines (Devoy, Dyet *et al.* 2005; Oster, Lennon *et al.* 2005; Martin, Ruijne *et al.* 2006); therefore it is important to monitor the evolution of variation within this protein in the meningococcal population to inform vaccine policy and monitor the effects of strain specific

vaccines or multivalent vaccines based on PorA subtypes, should they be introduced. This is also particularly relevant following the reports of a PorA-deficient invasive meningococcal strain isolated during an outbreak (van der Ende, Hopman *et al.* 2003) and a *porA*-negative strain isolated from a sporadic case (van der Ende, Hopman *et al.* 1999) given that the stable expression of PorA would be required for a PorA-based vaccine to be fully efficacious.

Variable expression of PorA can be caused by guanidine residue insertion and/or deletion due to slipped-strand mispairing on the polyguanidine stretch within the intervening sequence of the -35 and -10 regions of the promoter (van der Ende, Hopman *et al.* 1995; van der Ende, Hopman *et al.* 2000; Devoy, Dyet *et al.* 2005). Additionally, it seems variation due to mutations in the VR2 epitope that compromises recognition by the serosubtypes-specific MAbs is more common (Jelfs, Munro *et al.* 2000; Devoy, Dyet *et al.* 2005). The insertion element IS*1301* has been found to lead to insertional inactivation of the *porA* gene (Newcombe, Cartwright *et al.* 1998). As mentioned previously, IS*1301* has been shown to mediate capsule phase variation in *N. meningitidis* by reversible insertional inactivation of the *siaA* gene (Arreaza, Alcala *et al.* 2001; Dolan-Livengood, Miller *et al.* 2003; Weber, Claus *et al.* 2006) and is widely distributed in meningococci, but varies with serotype in the isolates studied (Hilse, Hammerschmidt *et al.* 1996; Hilse, Stoevesandt *et al.* 2000). Given that IS*1301* was found in multiple copies in *N. meningitidis* of different serogroups (Hilse, Hammerschmidt *et al.* 1996; Hilse, Stoevesandt *et al.* 2000) it would be of interest to discover the location of the other insertion sites in the genome and what effects they may have on meningococcal pathogenesis.

1.3.4 Multilocus Enzyme Electrophoresis (MLEE) and Multilocus Sequence Typing (MLST): Electrophoretic Type (ET) and Sequence Type (ST)

MLEE determines the mobility of a selection of constitutive metabolic enzymes in a starch gel or polyacrylamide gel matrix. Changes in the mobility of enzymes are caused by charge differences due to amino acid substitutions in the

polypeptide sequence (Selander, Caugant *et al.* 1986). MLEE has been widely used to study genetic relationships among strains of meningococci for over 20 years (Caugant, Bovre *et al.* 1986; Caugant, Froholm *et al.* 1986; Caugant, Mocca *et al.* 1987; Caugant, Hoiby *et al.* 1994). However, it is a labour-intensive and time-consuming technique. Furthermore, MLEE detects only those alleles with differences in coding sequences that lead to replacement of a differently charged amino acid and hence a change in electrophoretic mobility.

Multilocus sequence typing (MLST) was developed and validated on *N. meningitidis* in 1998 (Maiden, Bygraves *et al.* 1998) and since then it has been applied to many other organisms, including other encapsulated bacteria such as *S. pneumoniae* (Enright and Spratt 1998) and *H. influenzae* (Meats, Feil *et al.* 2003). Typically this procedure involves sequencing internal fragments of around 500 base pairs of seven housekeeping genes; in the meningococcus the housekeeping genes are *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate dehydrogenase), *fumC* (fumarate hydratase), *gdh* (glucose-6-phosphate dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit) and *pgm* (phosphoglucomutase). MLST was adapted from MLEE and identifies alleles directly from nucleotide sequence rather than comparing the electrophoretic mobility of the enzymes they encode. This is advantageous as it allows more allelic variation to be detected and therefore distinguishes more alleles per locus, thus permitting a higher level of discrimination between isolates. Strain associations shown by MLST were consistent with clonal groups previously established by MLEE (Maiden, Bygraves *et al.* 1998).

Nucleotide sequence data is unambiguous and electronically portable thus comparison between laboratories is easy. The exchange of molecular typing data is centrally located on a curator-controlled global database accessed via the internet (http://pubmlst.org/neisseria/). MLST can be used for strain surveillance, for monitoring individual clones as they spread or for the identification of unique lineages. This can be applied to short term or local epidemiology in the case of disease outbreaks (Feavers, Gray *et al.* 1999; Birtles, Hardy *et al.* 2005) and even when isolates are not available MLST can be performed directly on clinical material e.g. blood or CSF (Diggle, Bell *et al.* 2003; Birtles, Hardy *et al.* 2005). MLST can also be useful for long term or global

epidemiology to allow geographical or temporal comparisons to be made (Yazdankhah, Kriz *et al.* 2004; Zhang, Shao *et al.* 2008).

Typically, bacterial pathogens consist of large heterogeneous collections of isolates that rarely cause disease and a small number of groups of closely related strains (clones or lineages) that are particularly associated with outbreaks of disease. So called "Hypervirulent" lineages or clones which have the same allele at all seven housekeeping loci are frequently isolated and have an increased capacity to cause disease. Strains can be grouped into a clonal complex on the basis that they share five out of the seven alleles. Each complex is then designated STn-complex (n being the founder ST). MLST has the potential to identify existing and newly emerging hypervirulent lineages and to monitor their global spread. This is of particular importance if a clone acquires the ability to be antibiotic non-susceptible (McGee, McDougal *et al.* 2001). Recently recommendations by the European Meningococcal Disease Society (EMGM) for the typing of *N. meningitidis* isolates were proposed to include serogroup, PorA VRs 1 & 2, FetA VR, sequence type (clonal complex); i.e. C:P1.5,2-1:F5-4:ST-11(cc11) (Jolley, Brehony *et al.* 2007).

1.4 Interactions of *N. meningitidis* with the human host

1.4.1 Exposure

Exposure to the meningococcus requires the inhalation of infected aerosols from a previously colonised individual. This person-to-person transmission requires frequent or close contact. Colonisation itself is not dangerous; between 25% and 40% of young adults in temperate climates carry the meningococcus in their throat in the absence of disease. The non-carrier is potentially at risk since their ability to establish a commensal relationship with a newly acquired strain is not known. Additionally, certain circumstances, in which populations coming together from diverse geographic locations and where overcrowding may be a problem, are known to predispose to meningococcal carriage, infection and disease outbreaks. These include new military recruits, pilgrims, boarding

school, new university students, prisoners and even close contact within a discotheque (Jones and Sutcliffe 1990; Cartwright 1995a; 1995b; Cookson, Corrales *et al.* 1998; Gilmore, Jones *et al.* 1999; Neal, Nguyen-Van-Tam *et al.* 1999; Ala'Aldeen, Neal *et al.* 2000; Neal, Nguyen-Van-Tam *et al.* 2000; Nelson, Charlett *et al.* 2001; Aguilera, Perrocheau *et al.* 2002; Hahne, Gray *et al.* 2002; Grecki and Bienias 2006; Tully, Viner *et al.* 2006).

1.4.2 Adherence and Invasion

Following exposure to the bacterium, adhesion to host cells is the critical first stage in the sequence of events necessary for colonisation of the nasopharynx (and the possible subsequent dissemination throughout the body). Damage to the ciliated epithelial layer is believed to be a contributing risk factor towards meningococcal infection. This may be due to preceding viral infection or active or passive smoking (Cartwright 1995b; Fischer, Hedberg *et al.* 1997; Yusuf, Rochat *et al.* 1999; Kriz, Bobak *et al.* 2000; MacLennan, Kafatos *et al.* 2006). At 6am on the 26th March 2006 Scotland became the first country in the United Kingdom to implement a smoking ban in enclosed public spaces. The Smoking, Health and Social Care (Scotland) Bill came into force making it illegal to smoke in restaurants, bars, cafes, hotels, theatres, bingo halls, church halls, sports centres, shopping centres, public transport, schools, hospitals, clubs and workplaces, including lorries and vans (Anonymous 2005). Acts in Wales, Northern Ireland and England (Health Act 2006) banning smoking in public spaces came into force as of 2nd April, 30th April and 1st July 2007 respectively (Department of health www.dh.gov.uk). It is difficult to speculate how this new legislation may impact on the transmission and carriage of *N. meningitidis* and the incidence of meningococcal disease in Scotland. Would a change in smoking habits affect exposure to cigarette smoke in a household setting and, in turn, impact on transmission amongst the household?

Initial attachment to epithelial cells in the nasopharyngeal mucosa is mediated by Type IV pili. Neisserial pili are composed of repeating polypeptide subunits called pilin (Pil) and are subject to phase variation (on/off) and antigenic variation. Fifteen Pil proteins (including the major subunit PilE) are responsible

for Pilin biogenesis in *N. meningitidis* and mutational analysis of their corresponding genes has attempted to define at which step of the process each acts (Carbonnelle, Helaine *et al.* 2006). Contact by pili with the host cell is via the membrane cofactor protein (MCP or CD46) (Kallstrom, Liszewski *et al.* 1997). Type IV pili induce signal transduction pathways in host cells (Kallstrom, Islam *et al.* 1998). Type IV pilus appears to be absolutely required for adhesion to host cells by encapsulated meningococci (Meyer, Pohlner *et al.* 1994). Capsulate Nm lacking assembled pili did not adhere efficiently and expression of Opa or Opc did not alter the adherence of these bacteria (Virji, Kayhty *et al.* 1991). Pilus-facilitated adherence in capsule-deficient bacteria, but not capsulate bacteria, may result in internalisation.

A secondary, more intimate interaction with host cells is achieved via class 5 OMPs, such as Opa and Opc. In the absence of pili and capsule, opa or opc variants are sufficient to confer adhesive and, under some conditions, invasive phenotypes. Opa protein binds to the N-terminal domain of CEACAM1/CD66a adhesion molecule (Virji, Watt *et al.* 1996) and Opc binds to heparan-sulphate proteoglycans (Gray-Owen 2003). In vitro, meningococci and gonococci have been shown to induce localised rearrangement of the cortical actin cytoskeleton at the sites of bacterial adhesion (Merz and So 1997). Signal transduction events induced by pili, Opa, and Opc culminate in cytoskeletal rearrangements and engulfment of meningococci by epithelial cells and transcellular traversal (Merz and So 1997; Gray-Owen 2003; Plant and Jonsson 2003). Data from Virji et al 1992 suggests that bacteria expressing opc might colonise and invade the mucosal epithelium more efficiently than other bacteria if the local conditions down-regulate capsule production (Virji, Makepeace *et al.* 1992).

1.4.3 Carriage of N. meningitidis

The association of *N. meningitidis* with the human nasopharynx has been recognised for over a century. In recent decades investigations into meningococcal carriage has focused on closed or semi-closed populations i.e. military recruits (Caugant, Hoiby *et al.* 1992; Andersen, Berthelsen *et al.* 1998; Jones, Christodoulides *et al.* 1998; Riordan, Cartwright *et al.* 1998; Block,

Gdalevich *et al.* 1999) or in higher-risk groups such as university students (Gilmore, Jones *et al.* 1999; Ala'Aldeen, Neal *et al.* 2000; Neal, Nguyen-Van-Tam *et al.* 2000) or religious pilgrims (Balkhy, Memish *et al.* 2003; Wilder-Smith 2003; Wilder-Smith, Barkham *et al.* 2003a; Wilder-Smith, Barkham *et al.* 2003b; Wilder-Smith, Paton *et al.* 2003; Balkhy, Memish *et al.* 2004; Nicolas, Ait M'barek *et al.* 2005) or in household contacts of patients with meningococcal disease (Marks, Frasch *et al.* 1979; Olcen, Kjellander *et al.* 1981; Frasch and Mocca 1982; Cartwright, Stuart *et al.* 1991; Cardenosa, Dominguez *et al.* 2001) or in communities where a recent outbreak has occurred (Cartwright, Stuart *et al.* 1987; Stuart, Cartwright *et al.* 1987; Davies, O'Flanagan *et al.* 1996; Conyn-van Spaendonck, Reintjes *et al.* 1999; Gilmore, Jones *et al.* 1999; Fitzpatrick, Salmon *et al.* 2000). A few studies have focused on randomly sampled groups to determine genetic lineages (Caugant, Kristiansen *et al.* 1988; Caugant, Hoiby *et al.* 1994; Jolley, Kalmusova *et al.* 2000; Claus, Maiden *et al.* 2005; Caugant, Fogg *et al.* 2006) or to identify risks associated with carriage (Davies, O'Flanagan *et al.* 1996; Tayal, Rashid *et al.* 1997; Kremastinou, Tzanakaki *et al.* 1999; Fitzpatrick, Salmon *et al.* 2000; Bakir, Yagci *et al.* 2001; Dominguez, Cardenosa *et al.* 2001; Pavlopoulou, Daikos *et al.* 2004; Bogaert, Hermans *et al.* 2005; MacLennan, Kafatos *et al.* 2006). Carriage has also been assessed to evaluate the effects of conjugate vaccination in teenagers in the UK (Maiden and Stuart 2002).

Carriage of the meningococcus is known to vary in relation to the age-group of the population sampled; rates of carriage are low in young children, particularly under 2 years where disease rates are highest, and peak in teenagers (Gold, Goldschneider *et al.* 1978; Cartwright, Stuart *et al.* 1987; Caugant, Kristiansen *et al.* 1988; Caugant, Hoiby *et al.* 1994; Pavlopoulou, Daikos *et al.* 2004; Bogaert, Hermans *et al.* 2005; Claus, Maiden *et al.* 2005; Maiden, Ibarz-Pavon *et al.* 2008) and these isolates are frequently NG (Young, Harris *et al.* 1983; Cartwright, Stuart *et al.* 1987; Caugant, Kristiansen *et al.* 1988; Caugant, Hoiby *et al.* 1994; Ala'Aldeen, Neal *et al.* 2000; Jolley, Kalmusova *et al.* 2000; Maiden, Ibarz-Pavon *et al.* 2008). In the three years of the UK carriage study conducted to monitor the effects of the MCC vaccination campaign carriage prevalence among 15 – 19 year olds were 16.7%, 17.7% and 18.7% respectively (Maiden, Ibarz-Pavon *et al.* 2008). MacLennan and colleagues suggest that it is the social

behaviour that greatly influences the risk of carriage rather than age *per se*. Attendance at pubs or clubs, intimate kissing and cigarette smoking were all independently and strongly associated with increased risk of carriage; carriage in those with none of the risk factors was 7.8% compared to 32.8% in those with all three. Passive smoking was also linked to higher risk for carriage (MacLennan, Kafatos *et al.* 2006).

In multi-centre studies, carriage has been found to vary across different locations at the regional, city or institutional level (Maiden and Stuart 2002; Glitza, Ehrhard *et al.* 2008). Carriage is higher among family members or caregivers of individuals with meningococcal disease and often the strains they carry are phenotypically (Frasch and Mocca 1982) or genotypically (Cardenosa, Dominguez *et al.* 2001; Tzanakaki, Urwin *et al.* 2001) indistinguishable from the patient isolates. However, carriage of disease-associated or outbreak-associated strains in the wider community does not appear to be high (Kellerman, McCombs *et al.* 2002). Increased rates of carriage were observed in situations of overcrowding or the recent coming together of individuals i.e. military recruits (Caugant, Hoiby *et al.* 1992), religious pilgrimage or university students (Neal, Nguyen-Van-Tam *et al.* 2000).

The majority of carriage studies can be termed “point-prevalence” or “cross-sectional” studies as they provide information on the rate of carriage at a given time in a defined place/population i.e. in a population of n = x individuals n = y individuals will carry *N. meningitidis*. This contrasts with “longitudinal” studies which investigate the duration of carriage of *N. meningitidis* strains in individuals by repeated swabbing at timed intervals (Andersen, Berthelsen *et al.* 1998; Ala'Aldeen, Neal *et al.* 2000; Glitza, Ehrhard *et al.* 2008).

Uncertainty remains regarding the optimal sampling method to identify meningococcal carriage. Different carriage studies often use different methods and, as such, are difficult to compare. The choice of sampling site, transport medium versus direct plating of swabs, single versus multiple swabs and length of time between swabbing and plating can influence the level of carriage. The sensitivity of swabbing in detecting carriage is variable, 60-83% (Trotter and Gay 2003) and must influence the interpretation of the results of carriage studies. A recent review by Roberts and colleagues (Roberts, Greenwood *et al.* 2009) of the

published literature concluded that meningococcal carriage would be best assessed by swabbing the posterior pharyngeal wall through the mouth with direct plating or keeping transport time to below 5 hours. Additionally the use of charcoal swabs was recommended. Furthermore; it is recommended to use selective antibiotics to inhibit the growth of non-meningococcal bacteria and thus improve the yield of meningococci in the sample (Caugant, Hoiby *et al.* 1992).

1.4.4 Symptoms and the spectrum of meningococcal disease

The diagnosis of meningococcal disease is problematic due to the spectrum of clinical manifestations involved, some of which are difficult to differentiate from those due to less life-threatening but more common illnesses. One or more of the following may be present in an individual person; meningococcal meningitis, meningococcal septicaemia, meningococcemia (purpura fulminans and the Waterhouse-Friderichsen syndrome), respiratory tract infections (pneumonia, epiglottitis, and otitis media), focal infections (conjunctivitis, septic arthritis, urethritis, purulent pericarditis) and chronic meningococcemia (van Deuren, Brandtzaeg *et al.* 2000; Rosenstein, Perkins *et al.* 2001). In children, the most common form of disease is meningococcal meningitis; symptoms more commonly associated with older children are high fever, vomiting, drowsiness and headache, stiff neck and photophobia. In infants and younger children symptoms may be more subtle and more non-specific; fever, vomiting, irritability, drowsiness and confusion, or in more severe cases, declining consciousness and convulsions leading to coma. The presence of a rash is more indicative of meningitis caused by *N. meningitidis* however this is more common in patients with meningococcal septicaemia. This form of meningococcal infection is associated with greater mortality and presenting features include high fever, vomiting, muscle aches, shivering, and confusion. A petechial or purpuric rash is usually present; however the severity of rash does not correlate with the degree of circulatory failure (Brandtzaeg 1995; Nadel, Levin *et al.* 1995; Steven and Wood 1995). Interestingly, in meningococcal

disease, bacterial load is associated with likelihood of death, permanent sequelae and prolonged hospitalisation (Darton, Guiver *et al.* 2009).

1.5 Global epidemiology of meningococcal disease

1.5.1 Introduction

Historically, serogroup A, B, C, W-135 and Y have been responsible for most endemic and epidemic meningococcal disease, although this varies with geographic location (Figure 1.4) and seasonality. Serogroup A was responsible for outbreaks of disease in the more industrialised countries in the early 1900s, but cases with this serogroup have been rare since World War II. Serogroup B and C are responsible for most cases of meningococcal disease in the more industrialised countries (Clarke, Reid *et al.* 2002a; Gray, Trotter *et al.* 2006). However serogroups W-135 and Y have been emerging as the cause of disease outbreaks in Africa, serogroup W-135 has emerged in the middle east (WHO 2006) and serogroup Y in North America (Rosenstein, Perkins *et al.* 2001; Pollard 2004).

Figure 1.4 has been removed due to copyright restrictions

Figure 1.4 - Geographic distribution of IMD due to individual meningococcal serogroups (Stephens 2007)

1.5.2 Meningococcal disease in Scotland & the Meningococcus Reference Laboratory

Laboratory isolations of meningococci were first notified to the Communicable Diseases (Scotland) (CDS) Unit in February 1967 and meningococcal isolates were sent to the Meningococcus Reference Laboratory (Scotland) (MRLS), as it was known at the time, at Ruchill Hospital, Glasgow for determination of serogroup, serotype and sulphadiazine sensitivity. Five years later in 1972, details of age, sex, nature of disease and outcome were sought for all patients to determine mortality and morbidity associated with infection (Fallon, Brown *et al.* 1984).

Isolates of *N. meningitidis* were obtained from patients with meningitis or septicaemia without meningitis in Scotland during 1972 – 1982. In that eleven year period, 1185 strains of *N. meningitidis* were isolated; of these 927 were examined at the MRLS. 19.3%, 63%, 9.6% and 6% were of serogroup A, B, C and W-135, respectively. A further 1.6% were of other serogroups and NG strains were rare (Fallon, Brown *et al.* 1984)**.** Group B meningococci predominated through the 1970s but, by 1985, were virtually equalled in number by serogroup C; the trend was maintained in the first half of 1986. Throughout most of the 1970s serogroup A meningococci outnumbered serogroup C however by the mid-1980s serogroup A had fallen to a very low level (Fallon 1986a; 1986b; 1986c). During the late 1970s and early 1980s the proportion of two predominant serotypes, types 2 and 15, of serogroup B meningococci fluctuated. The proportion of these two types displayed an inverse relationship so that when type 15 formed a small proportion of serotypes type 2 dominated and vice versa (Fallon and Morris 1986).

During the eleven year period, 1972 - 82, 1156 strains of meningococci isolated from carriers were also forwarded to the reference laboratory; many from a study of meningococcal carriage in patients attending a department for sexually transmitted diseases. Most strains were isolated from the respiratory tract, and many were isolated from genital sources. Serogroup distribution differed from that in disease; 2.9% were of serogroup A and 40% were of serogroup B. 32%

were NG as opposed to 0.4% in cases. Meningococci of serogroup Z' (29E) were only seen in carriers (Young, Harris *et al.* 1983). This study concluded that among patients attending a clinic for sexually transmitted diseases, women without gonorrhoea were significantly less likely to be colonised with meningococci than were women with gonorrhoea and men with or without gonorrhoea. Women without gonorrhoea were also significantly less likely to be colonised with groupable meningococci but there were no significant differences in carriage rates of NG meningococci.

The authors discussed the effect of behavioural differences and therefore relative-risk on the different groups based on their results. Many of the women from which throat swabs were taken were asymptomatic contacts (of men with gonorrhoea) while the vast majority of males from whom throat swabs were taken attended the clinic with symptomatic urethritis. It was suggested that a higher incidence of meningococcal colonisation of the pharynx appears to be associated with the greater rate of recent mouth-to-mouth contact in the more sexually active and hence 'high-risk' patients rather than directly correlated with gonococcal infection. Furthermore, the authors suggested that these results may be explained if predominantly different transmission routes for groupable and NG isolates were to exist. If NG isolates were primarily spread via droplet spray then most individuals would be exposed to a similar extent. In contrast, if groupable meningococci were primarily spread from direct salivary spread then they would be expected to be of higher incidence in their 'high risk' patients by virtue of their greater degree of mouth-to-mouth contact.

The years 1984 – 1986 saw an upward trend in meningococcal isolations and disease cases. This was first noted in Lanarkshire in 1984 (Thomson and Jackson 1987) and was also noted in both east and west Scotland. However, unlike in England and Wales there were no reported local outbreaks of disease. Furthermore; around this time notification of serogroup C disease, and strain isolations, increased and subsequently predominated for the first time in Scotland since laboratory surveillance of infections commenced.

For a short period, serogroup C was dominant but then declined over the next 10 years to the mid 1990s (Mooney and McMenamin 2004). During the 1990s it was evident that the most common serotype was changing whereby C:2b declined

and C:2a increased (Smart 1997; 1999; Clarke 1999b; 2001). The most common serogroup B strains were also undergoing a change; the decline of B:2b:- ,P1.10,- and B:15:1.7,P1.16, - began around 1992 and by '98-'99 none had been received and characterised by the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) (as the reference laboratory had come to be known since its move to Stobhill Hospital in 1992) (Smart 1997; 1999; Clarke 1999b; 2001). This coincided with the emergence of B:4:-, P1.4,- strains from around 1994 becoming the most dominant through to the year 2000 and beyond (Smart 1997; 1999; Clarke 1999b; 2001; 2002c; 2003a; 2003c; Mooney and McMenamin 2004; Clarke 2005; Hopkins and McMenamin 2005).

During the late 1990s, serogroup C disease began increasing again, prompting the introduction of the MCC vaccine in November 1999, initially targeting those less than 18 years for vaccination. The following year, a decrease in serogroup C disease was observed in those age groups targeted. In 2002, vaccination was extended to include 20-24 year olds. Year-on-year from 1999 there has been a decrease in the percentage of disease caused by serogroup C meningococci (Clarke 2001; 2002c; 2003a; 2003c; 2005; Hopkins and McMenamin 2005); in 2002 the majority of serogroup C cases occurred in those over 25 years (Clarke 2003c). By 2003 90% of all isolates were serogroup B (Clarke 2005).

Overall, disease incidence increased from 4.1 cases per 100,000 population in 1996 to 6.7 cases per 100,000 population in 2000 (Smart 1997; 1999; Clarke 1999b; 2001; 2002c), and since then has decreased yearly to 3 cases per 100,000 population (Clarke 2003a; 2003c; Mooney and McMenamin 2004; Clarke 2005; Hopkins and McMenamin 2005). This can be partly attributed to the dramatic reduction of serogroup C disease following the introduction of the MCC vaccine in the age groups targeted (Mooney, Christie *et al.* 2004; Diggle and Clarke 2005). Only three cases of serogroup C IMD were reported in each of 2003, 2004, 2006 and 2007; with one case in 2005 (Ure 2008). In 2002 the annual incidence was 3.5 cases per 100,000 population (n=178), falling to 3.07 cases per 100,000 population (n=155) in 2007. The annual incidence of disease has remained fairly stable since then (Figure 1.5) and the latest surveillance data in Scotland puts the rate for 2009 at 2.69 cases per 100,000 population (McDonald 2010a). For the last two calendar years, 2008-2009, no cases of serogroup C disease have been reported to HPS (McDonald 2010a).

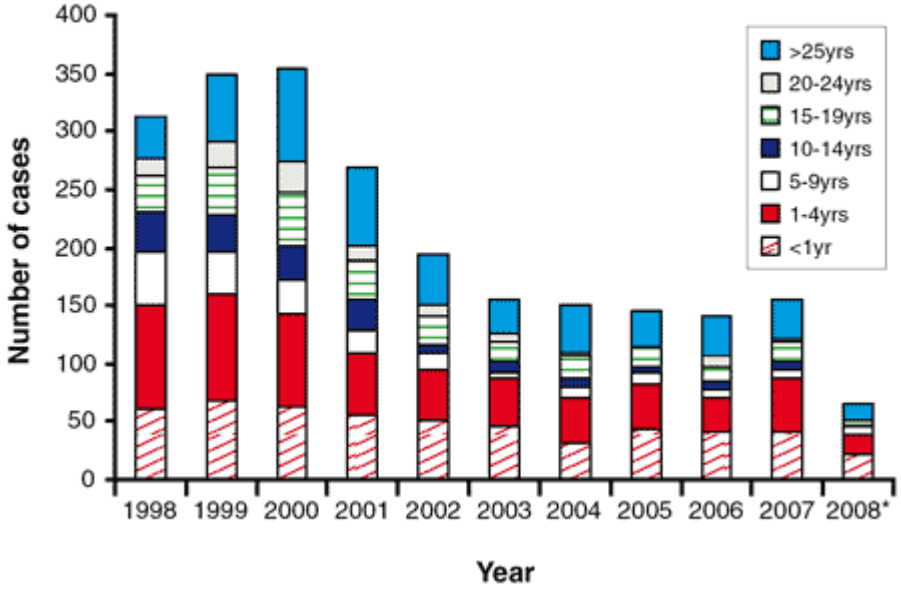

Figure 1.5 - Cases of meningococcal disease reported to HPS, 1998 - 2008*

*, weeks 1-26 only; (Shakir, Cameron *et al.* 2008)

Enhanced surveillance of meningococcal disease (MD) in Scotland, through the Meningococcal Infectious Disease Augmented Surveillance (MIDAS) scheme, was implemented in November 1999 (Clarke 1999a) to coincide with the MCC immunisation campaign and the SHLMPRL[1] maintains a record of all patients notified as having MD. In 1999 the Enhanced Surveillance of Meningococcal Disease (ESMD) was established in England, Wales and Northern Ireland to obtain accurate incidence data and develop a robust surveillance system (Shigematsu, Davison *et al.* 2002). ESMD was established to monitor the impact of the MCC vaccine during and following its introduction in the UK from November 1999. The scheme included improved case ascertainment data for meningococcal infection directly from clinical specimens.

[1] In late 2009 the SMPRL merged with the Scottish *Legionella* Reference Laboratory (SLRL) and is currently known as the Scottish *Haemophilus*, *Legionella*, Meningococcus & Pneumococcus Reference Laboratory (SHLMPRL)

1.5.3 Epidemiology of meningococcal disease in England, Wales & Northern Ireland

Laboratory-confirmed cases of meningococcal disease rose from 1,448 in 1995 to peak at 2,804 in 1999 falling to 1,233 in 2008. An increase in serogroup C cases from 1995-9 prompted the introduction of the serogroup C conjugate MCC vaccine into the UK population. The proportion of disease attributed to serogroups B, C, Y or W-135 altered markedly following the MCC introduction. In 2008, 90% of all confirmed cases were serogroup B, 3% were serogroup Y, and serogroup C and W-135 comprised just 2% each. Transient changes in W-135 infections in 2000-1 were associated with Hajj pilgrims and their contacts in the UK. Phenotypic and genotypic shifts have been observed; specifically the relative proportions of MLST clonal complexes ST-41/44, ST-269, ST-32, ST-213 and ST-11 (Gray, Campbell *et al.* 2009). Disease incidence during the epidemiological year July 2002 – June 2003 based on laboratory-confirmed cases only was reported as being 2.8 per 100,000 (Anonymous 2003).

1.5.4 Continental Europe

In recent years, a downward trend in cases of IMD has been occurring across the continent, irrespective of serogroup and with or without the introduction of serogroup C conjugate vaccination campaigns. Undoubtedly MCC vaccination campaigns have reduced the burden of disease due to this serogroup in the countries where the vaccine has been implemented. In countries using the MCC vaccine around 80% or more of disease is now due to serogroup B. Several countries not using the MCC vaccine are doing so because the actual numbers of disease cases due to this serogroup are low.

Surveillance of bacterial infection is vital so that the epidemiology can be monitored accurately and the impact of immunisation campaigns can be measured. Since IMD is relatively uncommon (particularly after vaccination has been introduced), pooling data across European countries should increase the power of any epidemiological analysis. With this in mind, the European Union

Invasive Bacterial Infections Surveillance Network (EU-IBIS) was established in 1999 with the following aims; to improve the epidemiological information on invasive disease caused by *N. meningitidis* and *H. influenzae* within the EU; to improve the laboratory capacity to accurately characterise the isolates of *N. meningitidis* and *H. influenzae* using standardised methods; to evaluate the impact of vaccination with conjugate vaccines on the epidemiology of *N. meningitidis* and *H. influenzae*; to compare the impact of vaccination with conjugate vaccines produced by different manufacturers and according to different schedules; to form a focus for wider collaboration with non-EU countries and candidate EU countries.

In 2007 the project was transferred to the European Centre for Disease Prevention and Control (ECDC). In a recent ECDC report on data from 2007 of 5,586 cases (5,180 confirmed) the highest notification rates with 3.8 and 2.5 per 100,000 were observed in Ireland and the UK, respectively. Contrastingly, the lowest notification rate was observed in Italy (0.3 per 100,000). Of 4,993 cases with known age data, 44% were reported in children under 5 years old. This age group had the highest notification rate at 8.6 per 100,000, followed by the 15 – 24 year olds (1.6 per 100,000). The notification rate peaked in winter and fell during spring to a low during the summer period (June to September). Serogroup B (77%) and C (16%) remain the major cause of IMD in Europe. Serogroup B predominates in all age groups, but is especially dominant in the under 10 year olds, while serogroup C was more evident in the age groups over 10 years old, especially the groups 10-14 and 25-44 years. The case fatality ratio was highest in serogroup C (13%) and Y (13%), the latter is most likely because it mainly affects the older age group 45 – 64 years (ECDC 2009). It should be noted that disease incidence rates may be affected by any differences between the surveillance systems in place in each country.

The recent epidemiology of IMD in Europe and North America is summarised in Table 1.1. Most countries are experiencing a reduction in disease incidence, irrespective of conjugate vaccination campaigns. In Austria during 2008 there were 95 cases of meningococcal disease; an incidence of 1.14 per 100,000. Of the 84 laboratory-confirmed cases 69.1%, 28.6%, 1.2% and 1.2% were serogroup B, C, Y and W-135 respectively. A reported rise in incidence, solely due to serogroup B cases, has followed the previous two years where the incidence was

<1.0 per 100,000 (Heuberger, Mikula *et al.* 2009). In the Czech Republic the incidence of IMD has been stable since 2005 at around 0.8 per 100,000. Serogroups B, C and Y accounted for 67.4%, 20.9% and 9.3% respectively. Predominant clonal complexes were cc11, cc18, cc41/44 and cc32; 18.6%, 13.9%, 9.3% and 9.3%, respectively (Kriz, Klamusova *et al.* 2009). In Denmark during the past 20 years the incidence of IMD has continued to decline from around 5 per 100,000 to 1.4 per 100,000 in 2007 and 1.2 per 100,000 in 2008 (St-Martin, Christensen *et al.* 2009).

France has experienced local outbreaks of both serogroup B and C recently which has prompted intervention with targeted vaccine campaigns (Parent du Chatelet, Taha *et al.* 2009). In Greece serogroup B is responsible for around 80% of cases of meningococcal disease during 2007-8, followed by serogroup C; 8.1% in 2007 and 7.8% in 2008 (Tzanakaki and Kremastinou 2009). In Finland, following a period of higher incidence in 1995-6 caused by serogroup B and C, the incidence of IMD has fluctuated at low levels between 0.5 – 1.1 per 100,000. Most cases were due to serogroup B (73%) and C (16.4%). Since 1996 the incidence of serogroup C has remained low, 0.02 – 0.17 per 100,000. There are currently no plans to introduce the MCC vaccine (Toropainen, Kayhty *et al.* 2009).

In Ireland the incidence rate of IMD notification continues to decline from the peak in 1999 (14.8 per 100,000) down to 179 cases (4.8 per 100,000) and 169 cases (4.0 per 100,000) in 2007 and 2008 respectively. Since introduction of the MCC in 2000 the incidence of serogroup C disease has declined from 3.9 per 100,000 (1999) to 0.07 per 100,000 (2008); a 98% reduction. During the same period meningococcal B disease had declined from 8.1 per 100,000 to 3.3 per 100,000, a 59% reduction. In 2008, 82% of IMD cases were due to serogroup B, 3 cases serogroup C IMD (1.8%) and other serogroups accounted for 3.6% of cases (Cotter, O'Lorcain *et al.* 2009). In Italy, despite the recent introduction of the MCC vaccine, serogroup C is still responsible for a considerable proportion of disease; with clonal outbreaks of disease still being experienced (Stefanelli, Fazio *et al.* 2009).

Table 1.1 – Epidemiological summary of Invasive Meningococcal Disease in Europe & North America

Country	Disease Incidence per 100,000/Year														MCC[2] Y/N (Year)	Common serogroups
	≤1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009		
Austria											<1.0	<1.0	1.14		N	B = 69% C = 28.6%
Belgium						3.7							1.0		Y (2002)	B (C)
Croatia													0.6		N	B = 85.7%
Czech Rep										0.8 since 2005					N	B = 67%, C = 21%, Y = 9.3%
Denmark	5.0 (~1980)											1.4	1.2		N	B = 69% C = 29%
E + W[1]							2.8								Y (1999)	B = 90%
Estonia							0.4 – 1.0								N	B
Finland						Higher in 1995-96; 0.5 – 1.1 since then									N	B = 73% C = 16%
France						1.0 – 2.0 for the past 20 years						1.3	1.2		Y*	B = 67% C = 24%
Germany							0.9			0.7		0.5	0.5		Y (2006)	B (C)
Greece												0.95	0.67		Y	B = >80% C <8%
Ireland				14.8								4.8	4.0		Y (2000)	B = 82%
Italy												0.3	0.3		Y	B = 55.7% C = 36.45
Poland		0.37										1.03			N	B = 51.6% C = 44%
Russia										1.92	1.7	1.56			N	A = 36% B = 24% C = 18.5%
Scotland	4.1 (1996)				6.7		3.5					3.07		2.69	Y (1999)	B >85%
Sweden													0.54		N	B, C, Y
USA			0.93		0.78					0.33		0.33			MCV4[3] 2006	Y = 37%, C = 30% B = 25%
Canada							0.62				0.42				Y (2002-2005)	B (children) C & Y(adults)

[1] E+W, England and Wales; [2] MCC, meningococcal conjugate serogroup C vaccine; [3] MCV4, meningococcal conjugate quadrivalent vaccine; *, Local outbreaks only

Furthermore, in Poland disease incidence has increased overall from 0.37 per 100,000 in 1997 to 1.03 per 100,000 in 2007. Since 2002 serogroup C has been increasingly isolated from cases of sporadic infections and in outbreaks of disease (Kadlubowski, Skoczynska *et al.* 2008). The epidemiology of IMD in Russia is particularly variable according to geographic area. The national mean incidence declined from 1.92 per 100,000 in 2005 to 1.56 per 100,000 in 2007. Regionally the incidence varied from 0 – 6.0 per 100, 000 with most regions within 0 – 3 per 100,000. Nationally the serogroup prevalence was 36.1%, 24.1% and 18.5% for serogroups A, B and C, respectively. However, in Central and Southern regions of Russia serogroup A largely predominated whereas in North-Western and Eastern regions serogroup B predominated (Koroleva, Beloshitskij *et al.* 2009).

1.5.5 North America

In the United States, since World War II, the annual incidence of meningococcal disease has varied from 0.5 to 1.5 cases per 100,000 population. During the past three decades, the incidence has increased and decreased in multi-year cycles. The most recent peak in incidence occurred during the mid 1990s. As is the case in many European countries, a downward trend in IMD cases has also been observed in the United States; decreasing to 0.33 cases per 100,000 in 2007 (Table 1.1). While undoubtedly due in part to the introduction of conjugate vaccines (MCV4 in 2004 and MCC in 1999 in the US and UK, respectively), surveillance data suggest the downward trend had begun already; caused by decreases in the three most common serogroups in the United States: B, C and Y (Harrison 2010). Furthermore, disease cases due to serogroup B meningococci have been in decline despite not being included in either of the conjugate vaccines (Cohn, MacNeil *et al.* 2010; McDonald 2010a). Serogroup Y accounted for only 2% of meningococcal infections during 1989-1991 (Jackson and Wenger 1993). However, by the mid-1990s, the incidence increased and serogroup Y strains accounted for one-third of meningococcal infections (Rosenstein, Perkins *et al.* 1999).

Active Bacterial Core surveillance (ABCs) is a core component of CDC's Emerging Infections Programs network (EIP), involving a collaboration between CDC, state health departments, and universities. ABCs is an active laboratory- and population-based surveillance system for invasive bacterial pathogens of public health importance. ABCs was initially established in four states in 1995. It currently operates among 10 EIP sites across the United States, representing a population of approximately 41 million persons. However, most areas restrict the population further for certain pathogens to ensure complete reporting and good audit data. At this time, ABCs conducts surveillance for six pathogens: group A and group B Streptococcus (GAS, GBS), *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, and methicillin-resistant *Staphylococcus aureus* (MRSA).

In 2007, data from the ABCs indicated that 25%, 30% and 37% of cases were due to serogroups B, C and Y, respectively, with a further 9% of cases being caused by serogroup W-135, other serogroups and NG strains (CDC 2008). County-wide surveillance of bacterial infectious disease is not practical due to the size of the USA. Subsequently, surveillance data may be skewed by any geographical or demographical change in disease epidemiology; evidence for this has occurred in recent years in the form of a prolonged serogroup B outbreak in the state of Oregon which has warranted separate data analyses by the CDC, or its collaborators, in recent publications (CDC 2008; 2010b).

In Canada, surveillance data from 2002-2007 (IMPACT; Immunization Monitoring Program, Active) revealed that disease incidence has also been declining; from 0.62/100,000 in 2002 to 0.42/100,000 in 2006 (Table 1.1). Serogroups B, C and Y caused the majority of IMD in Canada but the distribution of serogroups varied among adults and children and by province. Serogroup B occurred more frequently in children while serogroups C and Y occurred more frequently among adults. The incidence of serogroup C disease declined over the 5 year period; mostly attributable in provinces (British Columbia, Alberta and Quebec) that had started earlier (2002-2003 versus 2005) MCC vaccination campaigns. The incidence of serogroups B, Y and W-135 remained stable (Bettinger, Le Saux *et al.* 2008).

1.5.6 Africa and the meningitis belt

In the meningitis belt in Africa, most disease is caused by serogroup A meningococci, predominantly of ST-5 and ST-7 (Nicolas, Norheim *et al.* 2005), which differ only at the *pgm* locus. It is thought that ST-5 strains were introduced prior to 1988. In one study characterising meningococcal isolates from cases of meningitis between 1988 and 2003 in 13 countries of the African meningitis belt, most cases between 1988 and 2001 were due to ST-5 (Nicolas, Norheim *et al.* 2005). Sequence Type 7 is reported to have been identified for the first time in Algeria in 1995 (Nicolas, Decousset *et al.* 2001) and has gone on to replace ST-5 strains in many countries of Africa (Nicolas, Decousset *et al.* 2001; Nicolas, Norheim *et al.* 2005).

Recently, a cause for concern has been the serogroup W-135 strains of the ST-11/ET-37 complex. Sporadic cases have been increasing since 2000 and the first epidemic in Africa was seen in Burkina Faso in 2002. The majority of W-135 cases in Africa are due to ST-11 complex strains and, more recently, a new ST-2881 clone has emerged in several countries (Nicolas, Djibo *et al.* 2005; Nicolas, Norheim *et al.* 2005). Any possible effect that this new W-135 clone may have on the whole ST-11 complex will need to be monitored. During the first 10 weeks of the 2006 meningitis season, two main outbreaks were observed; in the west caused predominantly by serogroup A meningococci, and in the east caused predominantly by serogroup W-135 meningococci (WHO 2006). Historically, cases in two provinces have accounted for the majority of IMD in South Africa predominantly caused by serogroup B and serogroup A in the Western Cape and Gauteng, respectively. In recent years, the W-135 ST-11 complex has become more prevalent, particularly in Gauteng province (von Gottberg, du Plessis *et al.* 2008).

1.5.7 Asia

In China, serogroup A is responsible for around 95% of cases of meningococcal disease, whereas serogroups B and C only cause sporadic cases (Zhu, Hu *et al.* 1995). However, recently in one province there has been an increase in

outbreaks due to serogroup C meningococci. Molecular characterisation of selected isolates revealed a dominant clone that was unique to China (Shao, Li *et al.* 2006). Furthermore this clone did not belong to any clonal complex, including the hypervirulent ST-11/ET37 or ST-8 lineages, responsible for most serogroup C disease in North America, Europe and Africa (Achtman 1995). In Taiwan, molecular characterisation of meningococcal disease isolates revealed the recent emergence of serogroups A, C and Y belonging to the major worldwide epidemic clones currently circulating. This partly contributed to an increase in cases of meningococcal disease in 2001-2002 (Chiou, Liao *et al.* 2006).

1.5.8 Latin & South America

Since 2001, serogroup B had been isolated most commonly in cases of IMD in Argentina, representing 72% of all isolates in 2006 (Sorhouet, Regueira *et al.* 2008). An epidemic wave at the end of the 1990s due to serogroup C accounted for around 20% of cases until 2005. Serogroup W-135 isolates began increasing to 13% in 2007 and 27.7% during the first 5 months of 2008. A selected number of strains genotyped as ST-11 and had the porA 5,2 (Efron, Sorhouet *et al.* 2008). Characterisation of 46 serogroup B isolates from 2006 by MLST revealed the majority of isolates grouped into four complexes. PorA VR sequencing identified 17 subtypes, three of which accounted for over 60% of all isolates. Isolates with the subtypes P1.21,16-36 and P1.22-1,14 all belonged to the ST-865 complex and ST-35 complex, respectively. The ST-41-44/Lineage 3 complex was identified ten times with heterogeneous *porA* subtypes (Sorhouet, Regueira *et al.* 2008).

1.5.9 New Zealand and Australia

Historical trends in meningococcal disease in Australia have followed a similar pattern to other industrialised countries; characteristic epidemics during the two World Wars, a rise in disease during the 1950s and a subsequent period of endemicity. During the second half of the 20th Century, serogroup A has been

replaced by serogroups B and C, particularly as a result of the spread of hypervirulent lineages of meningococci across the globe. Following successful campaigns in Europe the serogroup C conjugate vaccine was introduced in Australia in 2003 (Patel 2007).

From 1991, New Zealand experienced an epidemic of IMD, which peaked in 2001, predominantly caused by the strain type B:4:P1.7-2,4 (Martin, Walker *et al.* 1998; Baker, Martin *et al.* 2001; Devoy, Dyet *et al.* 2005). This prompted the mass vaccination with a strain-specific outer membrane vesicle (OMV) vaccine in 2004. A large reduction in disease due this strain was observed. However, the success of this vaccine campaign has been debated; disease-burden may already have been in decline and the suitability of methods used to assess vaccine efficacy have been disputed. Moreover, as of July 2008, the vaccination campaign was controversially terminated (Martin and Lopez 2008) and data on any long-term impact is awaited.

1.6 Treatment & prevention of meningococcal disease

1.6.1 Antibiotic therapy

Rapid admission to hospital is of highest priority when meningococcal disease is suspected. In the UK in suspected cases of IMD (meningitis or septicaemia) general practitioners are recommended to give intra-muscular (i.m.) or intra-venous (i.v.) benzylpenicillin (Adult, 1.2g; Infant <1yr, 300mg; Child 1-9yr, 600mg; 10 years or over, as adult) before urgent transfer to hospital. If there is penicillin allergy, cefotaxime may be used as an alternative or chloramphenicol may be used if there is a history of anaphylaxis to penicillins or to cephalosporins. For meningitis caused by meningococci, it is recommended to treat with benzylpenicillin or cefotaxime for 5 days, or alternatives as above (BNF 2010). Additionally, it is advised to use rifampicin treatment for two days to eliminate nasopharyngeal carriage. For the prevention of secondary cases it is recommended to give rifampicin 600mg every 12 hours for 2 days; child – 10mg/kg (under 1 yr, 5 mg/kg) every 12 hours for 2 days (BNF 2010).

1.6.2 Vaccines for the prevention of meningococcal disease

1.6.2.1 Introduction

The first meningococcal vaccines were available during the 1970s and 1980s. These were bivalent and quadrivalent polysaccharide vaccines targeting serogroup A & C and A, C, Y and W-135, respectively. They were proven to be safe, with good efficacy in older children and adults. However, the level of protection was variable for the different polysaccharides (particularly serogroup C) and was not long-term. In the last decade, polysaccharide-conjugate vaccines have been licensed in Europe (serogroup C) and the US (serogroups A, C, Y, W-135). Vaccines for serogroup B strains have, since the 1980s, focused on OMV and have been employed successfully in Norway, Cuba and New Zealand to curb strain-specific outbreaks of disease in those countries. Genome-sequencing projects have facilitated the identification of potential vaccine antigen targets, irrespective of serogroup (Pizza, Scarlato *et al.* 2000; Rappuoli 2001; 2008).

1.6.2.2 Plain polysaccharide vaccines

In the UK, the quadrivalent (ACYW-135) polysaccharide vaccine (manufactured by GlaxoSmithKline) is recommended for use in children over three months and less than 2 years of age and the primary course is given as two doses 3 months apart. In children over 2 years and in adults the primary course consists of one dose. A booster dose should be given every 5 years to those at continued risk, or every 2-3 years in children receiving their first dose under the age of five (DoH 2006a; 2006b). The quadrivalent polysaccharide vaccine is also recommended for individuals who are travelling or going to reside abroad. Large epidemics due to serogroup A, and more recently, serogroup W-135 occur throughout tropical Africa, particularly during the dry season in sub-Saharan Africa. Large epidemics of serogroup A and serogroup W-135 have been associated with Hajj pilgrimages to Saudi Arabia. Immunisation with A & C polysaccharide vaccines was a visa requirement before Hajj 2000 and 2001 when subsequently the quadrivalent vaccine was required of visiting pilgrims by the Saudi authorities (DoH 2006b; K.S.A. 2010). In the United States, a quadrivalent meningococcal polysaccharide

vaccine MPSV4 has been licensed for use since 1981. The vaccine is recommended for use in persons aged 2 – 55 years if MCV4 is unavailable, in children aged 2 - 10 years with a history of Guillain-Barré syndrome and in persons over 55 years (Poland 2010).

1.6.2.3 Polysaccharide-protein conjugate vaccines

1.6.2.3.1 Serogroups A, C, Y & W-135

In 1999, the UK was the first country to implement the use of the MCC (Miller, Salisbury *et al.* 2001). Initial observations have seen a considerable decrease in serogroup C disease in the age groups targeted (Balmer, Borrow *et al.* 2002; Maiden and Stuart 2002; Mooney, Christie *et al.* 2004) and a decade of surveillance data from MIDAS (Scotland) and from England & Wales (ESMD) has demonstrated the longevity of this successful vaccination campaign (McDonald 2010a). Herd immunity has also been observed (Ramsay, Andrews *et al.* 2003). In Scotland, a substantial decline of serogroup C ET-37/ST11 strains and no corresponding increase in serogroup B ET-37/ST-11 strains was observed, as yet, providing no evidence for the occurrence of capsule switching (Diggle and Clarke 2005). This was also the case in England and Wales (Trotter, Ramsay *et al.* 2006).

In the UK, since September 2006, the childhood immunisation schedule for prevention of meningococcal disease recommends vaccination with 3 doses of the meningococcal serogroup C conjugate vaccine at 3, 4 and 12 months of age. The MCC is recommended for use in persons under the age of 25 years and in older adults at elevated risk from meningococcal C disease (Health 2006b; a). The vaccines in use are conjugated with either CRM197 (non-toxic variant of diphtheria toxin) or tetanus toxoid. A number of other (western) European countries including Ireland, Spain, the Netherlands, Germany and Luxembourg, as well as Canada and Australia, have followed the UK in implementing conjugate meningococcal C vaccination programmes (Cohen 2003; de Wals, Deceuninck *et al.* 2004; Welte, van den Dobbelsteen *et al.* 2004; Larrauri, Cano

et al. 2005; de Greeff, de Melker *et al.* 2006; Wiese-Posselt, Hellenbrand *et al.* 2006; DoH 2006b; Booy, Jelfs *et al.* 2007).

Several manufacturers produce the MCC vaccine for use in the UK: Meningitec® (Wyeth Pharmaceuticals); Menjugate® (Novartis Vaccines); NeisVac-C® (Baxter Healthcare; and Menitorix (Hib/MenC) (GlaxoSmithKline). In the US, the FDA licensed the Meningococcal (Groups A, C, Y and W-135) Conjugate Vaccine (MCV-4) marketed as Menactra (Sanofi Pasteur) in January 2005 for use in all adolescents 11-18 years of age and persons aged 2-55 years who are at increased risk of IMD (Poland 2010). Unfortunately, vaccine coverage targets in adolescents have yet to be met; only a third of adolescents had received one dose of the MCV4 during 2007 (Poland 2010).

The Novartis Vaccines' Investigational MenACWY-CRM quadrivalent conjugate vaccine has undergone Phase II and III studies and has equivalent safety and tolerability as well as equivalent or superior immune responses compared with currently available vaccines (Dull, Gill *et al.* 2009; Jackson, Baxter *et al.* 2009; Reisinger, Baxter *et al.* 2009) and was licensed for use in the US in 2010 (CDC 2010a). Meanwhile a conjugate serogroup A vaccine is currently being developed and trialled for use in Africa (Jodar, LaForce *et al.* 2003; Kshirsagar, Mur *et al.* 2007; Hodgson, Forgor *et al.* 2008; Lee, Kuo *et al.* 2009; Okoko, Idoko *et al.* 2009).

1.6.2.3.2 Serogroup B

Currently there is no vaccine effective against serogroup B meningococci licensed in the UK or in the US. Strategies to develop vaccines against serogroup B disease have focused on sub-capsular antigens using OMV preparations (native or detergent-treated), recombinant proteins alone or in combination (Granoff 2010).

1.6.2.3.2.1 PorA-based OMV vaccines

In New Zealand, where an epidemic due to serogroup B meningococci has been ongoing since 1991 (Martin, Walker *et al.* 1998; Baker, Martin *et al.* 2001; Devoy, Dyet *et al.* 2005), a strain specific OMV vaccine against B:4:P1.7-2,4

meningococci has been evaluated for safety and immunogenicity in clinical trials (Oster, Lennon *et al.* 2005). The VR2 P1.4 epitope was subsequently demonstrated to be immunodominant (Martin, Ruijne *et al.* 2006). Similarly, clonal epidemics in Norway and Cuba have been targeted by the use of monovalent PorA-based OMV vaccines (Bjune, Hoiby *et al.* 1991; Rodriguez, Dickinson *et al.* 1999).

The Netherlands Vaccine Institute (NVI) has produced a hexavalent OMV vaccine containing six different PorA subtypes. This vaccine, HexaMen, contains the subtypes P1.7,16; P1.5-1,2-2 and P1.19,15-1 on one OMV preparation and the subtypes P1.5-2,10; P1.12-1,13 and P1.7-2,4 on another OMV preparation. The NVI has also recently conducted Phase I trials to investigate the safety of a nonavalent outer membrane vesicle (OMV) vaccine containing nine different PorA subtypes. This vaccine, NonaMen, was based on the six subtypes of the HexaMen OMV vaccine with the addition of the subtypes P1.22,14, P1.7-1,1 and P1.18-1,3 to improve the potential immunisation coverage of serogroup B disease. Results indicated the vaccine was well tolerated with no major safety concerns (Rots and Kleijne 2008).

1.6.2.3.2.2 Alternative antigens for serogroup B vaccines

Currently, the Novartis 5 Component Vaccine against Meningococcus B (5CVMB) is being examined in clinical trials. This vaccine is composed of the New Zealand OMV (Oster, Lennon *et al.* 2005) plus five other antigens: Factor H-binding protein (fHbp; Genome-derived Neisserial antigen; GNA 1870: LP2086) variant 1; Neisserial adhesion A (NadA, GNA 1994); GNA 2091; GNA 2132; and GNA 1030. Factor H-binding protein (fHbp) is a ~ 28kDa surface lipoprotein that was originally described by Fletcher *et al.* as LP2086 (Fletcher, Bernfield *et al.* 2004) and by Masignani *et al.* as GNA 1870 (Masignani, Comanducci *et al.* 2003). Following the discovery that the protein GNA 1870 was able to bind complement factor H (Madico, Welsch *et al.* 2006) it was re-named. A number of prevalence and sequence analyses of fHbp from a variety of serogroup B *N. meningitidis* lineages indicates that all isolates have a fHbp gene and that variants so far identified can be classified into two or three distinct groups; subfamilies A and B (Fletcher, Bernfield *et al.* 2004; Murphy, Andrew *et al.* 2009) or groups 1, 2 and

3 (Masignani, Comanducci *et al.* 2003; Brehony, Wilson *et al.* 2009), respectively, in all countries surveyed.

Neisseria adhesion A (NadA) or GNA 1994 is a novel surface antigen that is thought to be involved in adhesion to, and invasion of, epithelial cells (Comanducci, Bambini *et al.* 2002; Comanducci, Bambini *et al.* 2004; Capecchi, Adu-Bobie *et al.* 2005). Four variants have been described; variants 1-3 are predominantly present in invasive isolates (Comanducci, Bambini *et al.* 2002), whereas variant 4 is only found in carrier isolates (Comanducci, Bambini *et al.* 2004). Only around 50% of meningococcal isolates harbour the *nadA* gene (Comanducci, Bambini *et al.* 2004). It is almost always present in three clonal complexes (cc); ST-32 (cc32), cc11 and cc8. Disappointingly however it has not been identified in cc41/44 (Comanducci, Bambini *et al.* 2002) and is seldom found in cc269 (Lucidarme, Comanducci *et al.* 2009); two ccs which account for the majority of IMD due to serogroup B strains in the UK (Gray, Trotter *et al.* 2006; Ure 2008; Gray, Campbell *et al.* 2009).

GNA 2132, also known as Neisseria Heparin-binding antigen (NHBA), is a surface-exposed lipoprotein that is able to bind heparin, is found in all strains studied and was also present in strains of *N. gonorrhoeae* and *N. lactamica* (Pizza, Scarlato *et al.* 2000; Jacobsson, Thulin *et al.* 2006; Jacobsson, Hedberg *et al.* 2009; Lucidarme, Comanducci *et al.* 2009). GNA 2132, along with GNA 1030 (a periplasmic protein involved in quinine metabolism) and GNA 2091 (a predicted periplasmic and or secreted lipoprotein and a putative haemolysin) are other components of the Novartis vaccine composed of the MeNZB OMV plus recombinant antigens (Jacobsson, Hedberg *et al.* 2009).

Wyeth is also currently investigating a bivalent fHbp vaccine (based on one family A and one family B variant) in clinical trials. Early results indicate that the vaccine is able to elicit antibodies that are broadly bactericidal against epidemiologically diverse invasive meningococcal serogroup B disease isolates (Jansen, McNeil *et al.* 2008; Marshall, Nissen *et al.* 2008; Richmond, Marshall *et al.* 2008; Anderson, Jones *et al.* 2009).

1.7 Neisserial genome sequences and their application

A number of strains of *N. meningitidis* have been fully sequenced: the serogroup B strain MC58; the serogroup A strain Z2491; the serogroup C strain FAM18; and the serogroup C strain 053442 (Parkhill, Achtman *et al.* 2000; Tettelin, Saunders *et al.* 2000; Bentley, Vernikos *et al.* 2007; Peng, Yang *et al.* 2008). Analyses of these genome sequences has highlighted the abundance and variety of repetitive or mobile DNA elements that contribute towards genome variability such as DNA uptake sequences (DUS), Correia-repeat enclosed elements (CREE), dRS3, IS elements, prophages and islands of horizontal transfer (Claus, Vogel *et al.* 2007). Furthermore, whole-genome sequences of three isolates obtained during a carriage study; α 14: *cnl* ST-53(cc53), α 153: 29E ST-60(cc60) and α 275: W-135 ST-22(cc22), have recently been completed (Schoen, Blom *et al.* 2008). In turn, whole-genome comparisons between these three carriage isolates and three disease-associated strains showed that there is no consistent difference in the distribution of candidate virulence genes between carriage and disease isolates. The majority of candidate virulence genes found in *N. meningitidis* can also be found in the carriage isolates studied (Schoen, Blom *et al.* 2008).

Attempts to identify virulence gene candidates that were restricted to invasive isolates from Neisserial genome comparisons has been performed using microarray hybridisation experiments. Differences in the pathogenic potential of different meningococci (carried or invasive), gonococci or other *Neisseria spp.* were measured by the mere presence/absence of a particular genetic determinant. Conflicting results regarding the association of a filamentous phage Nf1 (MDA) with invasive isolates suggests this phage is not a characteristic of all invasive meningococcal strains (Bille, Zahar *et al.* 2005; Hotopp, Grifantini *et al.* 2006).

1.8 Project background and rationale

In Scotland, a reference laboratory for *N. meningitidis* has existed, in one guise or another, for around half a century. Over the decades the laboratory has accrued a considerable isolate collection and only in recent years with the expansion of molecular biology has it been possible to take advantage of this untapped resource. The reference laboratory has, for the best part of a decade, utilised molecular typing methods to characterise meningococcal isolates routinely isolated from cases of IMD. Furthermore, investigations have previously been conducted to characterise invasive meningococci from the reference laboratory isolate archive. However, to date sequence typing methods have not been applied to large-scale studies of carried meningococci from Scotland. The present study sought to characterise further a panel of carried meningococci from the isolate archive of the SHLMPRL with the purpose of gaining further understanding on the genetic structure of the *N. meningitidis* population in Scotland over a 31-year period.

What is the level of genetic diversity in carried meningococcal isolates in Scotland?

Has the diversity of carried meningococcal isolates in Scotland changed during the study period?

How does this level of genetic diversity compare with that of invasive meningococcal isolates in Scotland?

2 Materials and Methods

2.1 Handling of bacterial isolates, COSHH and Health & Safety

The Scottish *Haemophilus*, *Legionella*, Meningococcus and Pneumococcus Reference Laboratory (SHLMPRL) is a fully accredited laboratory via the Clinical Pathology Accreditation (UK) Ltd. (CPA). All work undertaken in the laboratory was performed following the designated Standard Operating Procedures (SOPs) in place at the SHLMPRL and in compliance with the Control of Substances Hazardous to Health Regulations 2002 (COSHH) and the appropriate Procedure Risk Assessment. *Neisseria meningitidis* is classified as a Hazard Group 2 Pathogen by the Advisory Committee on Dangerous Pathogens (ACDP) and as such work with *N. meningitidis* isolates was carried out in a class 1 exhaust protective cabinet (model 20270, serial no. 111944). Live suspensions were kept in racks to reduce the likelihood of spillage and protective gloves were worn at all times.

2.2 Chemicals, Reagents and Buffers

2.2.1 0.85% Saline (2ml)

0.85% saline was used as a suspension medium for serogrouping isolates of *N. meningitidis*. 8.5g of sodium chloride (VWR International Ltd., Poole, Dorset, UK) was added to 1 litre of de-ionised water (SHLMPRL). This was distributed into glass bijoux bottles in 2ml amounts and sterilised by autoclaving at 121°C for 15 minutes.

2.2.2 ELFO Buffer x50 & ELFO Buffer x1

ELFO buffer was used in the preparation of agarose gels and in the gel electrophoresis tanks. ELFO buffer x50 was made by adding 242g of Tris (Trizma Base) (Sigma-Aldrich Co. Ltd., Dorset, UK) and 18.61g of EDTA (Ethylenediaminetetraacetic acid disodium salt dihydrate) (Sigma-Aldrich Co. Ltd., Dorset, UK) to 900ml of de-ionised water (SHLMPRL). The pH was adjusted to 7.7 with approximately 50ml of glacial acetic acid (McQuilkin & Co. Ltd., Glasgow, UK) and made up to a final volume of 1 litre with de-ionised water (SHLMPRL). For use, ELFO buffer x50 was diluted 1 in 50 with de-ionised water (SHLMPRL).

2.2.3 Ethidium bromide 1mg/ml

Ethidium bromide was used to visualise DNA products in agarose gels following amplification by PCR. In a sterile glass universal, 1ml of ethidium bromide aqueous solution 10mg/ml (Sigma-Aldrich Co. Ltd., Dorset, UK) was diluted 1 in 10 with 9ml of de-ionised water (SHLMPRL).

2.2.4 Hydrochloric acid 15%

Hydrochloric acid 15% was used in conjunction with sodium bicarbonate to produce an atmosphere of carbon dioxide (CO_2) suitable for the growth of *N. meningitidis*. 1,190ml of de-ionised water (SHLMPRL) was added to a 2 litre conical flask. 820ml of a 37% solution of Hydrochloric acid (McQuilkin & Co. Ltd., Glasgow, UK) was added slowly to the water while gently mixing. This was allowed to cool before transferring to a 5 litre container.

2.2.5 Meningococcal co-agglutinating reagents

Meningococcal co-agglutinating reagents were used to serogroup isolates of *N. meningitidis* into the 6 serogroups; A, C, Y, W135, X and Z. A 1:5 or a 1:10 dilution was prepared by adding 20µl or 10µl of each *N. meningitidis* agglutinating serum (Remel Europe Ltd., Dartford, Kent, UK) to 80µl or 90µl of Pansorbin® Cells (Merck Chemicals Ltd., Nottingham, UK) respectively in a 2ml micro tube (Sarstedt Ltd., Leicester, UK). Each new batch of co-agglutination reagents was tested against the 7 *N. meningitidis* serogroup A, B, C, W135, X, Y and Z control organisms (See Table 2.1).

In the class 1 safety cabinet, using a sterile cotton swab (McQuilkin & Co. Ltd., Glasgow, UK) for each isolate, a few colonies were picked from an overnight culture plate to make a suspension in 2ml of 0.85% saline (SHLMPRL) to give a McFarland standard of approximately 2. On a glass microscope slide (VWR International Ltd., Poole, Dorset, UK) using 1µl disposable loops (VWR International Ltd., Poole, Dorset, UK) a loop-full of control suspension was mixed with a loop-full of corresponding co-agglutination reagent. This was mixed with a wooden cocktail stick and the slide was gently tilted. The slide was observed for evidence of clumping indicative of a positive agglutination reaction. If the mixed suspensions remained milky in appearance this was indicative of a negative reaction. Each co-agglutinating reagent was tested against all the control isolates to ensure no cross reaction. Any reagents that did cross react were discarded. The two dilutions of co-agglutinating reagent was compared and the highest dilution giving good agglutination results with no cross reaction was used in subsequent testing of *N. meningitidis* isolates.

2.2.6 Mueller-Hinton Broth

Mueller-Hinton broth was used as a suspension medium when re-suspending lyophilised freeze-dried *N. meningitidis* isolates. 21g of powdered Mueller-Hinton broth (Oxoid, Basingstoke, Hamps., UK) was added to 1 Litre of de-ionised water (SHLMPRL) and mixed to dissolve. This was distributed in 2ml

amounts into glass bijou bottles and sterilised at 121°C for 15 minutes in a Priorclave Autoclave (Model No. PS/LAC/EV100).

2.3 *Neisseria meningitidis* isolates

All strains included in this study were received by the SHLMPRL between 1972 and 2004. Isolates were identified following consultation of the SHLMPRL *N. meningitidis* isolate database using the criteria defined below. Carried meningococci were defined as *N. meningitidis* strains isolated from throat or nasopharyngeal samples from asymptomatic individuals with no epidemiological link to an invasive meningococcal disease case. Table 2.1 lists the control strains used in this study.

Table 2.1 – *N. meningitidis* control strains

Organism	*Serogroup designation*	*Source*
ATCC 13077	A	NCTC, HPA, Colindale, UK
ATCC 13090	B	NCTC, HPA, Colindale, UK
ATCC 13102	C	NCTC, HPA, Colindale, UK
ATCC 35561	Y	NCTC, HPA, Colindale, UK
NCTC 11203	W135	NCTC, HPA, Colindale, UK
NCTC 10790	X	NCTC, HPA, Colindale, UK
NCTC 10792	Z	NCTC, HPA, Colindale, UK
NCTC 11202	29E	NCTC, HPA, Colindale, UK
GL0101366	NG (non-serogroupable) Capsule null locus-positive	Dr M. A. Diggle, UK Carriage Study, SHLMPRL, Glasgow, UK
GL0103161	NG (non-serogroupable) Capsule null locus-positive	Dr M. A. Diggle, UK Carriage Study, SHLMPRL, Glasgow, UK

2.3.1 Resuscitation and recovery of bacterial strains

N. meningitidis isolates were recovered from either Protect™ beads (isolates from 1996 – 2004) stored at -80°C or from freeze-dried ampoules (isolates from 1972 – 1995) stored at room temperature at the SHLMPRL. For isolates recovered from Protect™ beads; one or two beads were used to inoculate a single plate of Columbia agar with horse blood (Oxoid, Basingstoke, Hamps., UK). Isolates were incubated overnight at 37°C in an atmosphere of 5.0% v/v CO_2.

For isolates resuscitated from freeze-dried ampoules; 500µl of either Meuller-Hinton broth (SHLMPRL) or 18MΩ H_2O (ELGA Process Water, High Wycombe, Bucks., UK) was used to re-suspend the culture. Using a sterile graduated pipette (Alpha Laboratories Ltd, Eastleigh, UK) 2 or 3 drops of the re-suspended isolate was used to inoculate a single plate of Columbia agar with horse blood (Oxoid, Basingstoke, Hamps., UK). Isolates were incubated for either 24 or 48 hours at 37°C in an atmosphere of 5.0% v/v CO_2.

2.3.2 DNA extraction of N. meningitidis isolates

From an overnight pure culture plate meningococcal DNA was extracted by inoculating 500µl of 18MΩ H_2O (ELGA Process Water, High Wycombe, Bucks., UK) in a 2ml microfuge tube (Sarstedt Ltd., Leicester, UK) and heating at 80°C for 15 minutes in a water bath (Grant Instruments Ltd., Sherpeth, Cambs., UK). Following a five minute centrifugation at 15000 rpm (Biofuge Primo, Heraeus Instruments), 200µl of the supernatant containing the DNA was added to a fresh 2ml microfuge tube (Sarstedt Ltd., Leicester, UK) and stored at -80°C until required for all further PCR and sequencing reactions.

2.3.3 Non-culture DNA extraction of re-suspended freeze-dried isolates prior to incubation

Re-suspended freeze-dried isolates underwent a non-culture DNA extraction using the remaining fluid by addition to a further 500µl of 18MΩ H_2O (ELGA Process Water, High Wycombe, Bucks., UK) in a 2ml microfuge tube (Sarstedt Ltd., Leicester, UK) and used as for extraction of isolates that were successfully sub-cultured. In some instances isolates failed to grow on sub-culture and the non-culture extract was used as template for all further PCR and sequencing reactions.

2.3.4 DNA extraction from reconstituted freeze-dried specimens of N. meningitidis using FTA® Elute Cards

FTA Elute Cards (Whatman International Ltd., Maidstone, Kent, UK) are designed for room temperature collection, shipment, archiving and purification of nucleic acids from biological samples for PCR analysis. FTA Elute Cards are impregnated with a patented chemical formula that lyses cells and denatures proteins upon contact. For each sample an individual FTA Elute Card was labelled with the appropriate sample identification number. Using a P200 Gilson pipette (Anachem Ltd., Luton, Beds., UK) and 1-200µl barrier tips (Starlab UK Ltd., Milton Keynes, UK) 40µl of each reconstituted freeze-dried specimen was added to its corresponding FTA Elute Card within the printed circle area.

The samples were either left to dry completely at room temperature for at least 3 hours or, to reduce drying time, the spotted FTA Elute Card was placed in a heated drier (Thermo Fisher Scientific, Epsom, Surrey, UK) set to 50°C for 1 hour. Once the samples had completely dried, a 3mm disk was cut from the centre of the spotted circle using a 3mm Harris Uni-Core device (Whatman International Ltd., Maidstone, Kent, UK) and transferred to a 1.5ml microfuge tube (McQuilkin & Co. Ltd., Glasgow, UK). Next 500µl of 18MΩ H_2O (ELGA Process Water, High Wycombe, Bucks., UK) was added to the tube and immediately pulse-vortexed three times for a total of 15 seconds using a WhirliMixer™ (Fisons Scientific Equipment).

The disk was immediately transferred to a second 1.5ml microfuge tube (McQuilkin & Co. Ltd., Glasgow, UK) containing 100µl of 18MΩ H_2O (ELGA Process Water, High Wycombe, Bucks., UK) ensuring the disk was completely immersed in the 18MΩ H_2O. The tube was then incubated in a water bath (Type JB2, Grant Instruments Ltd., Sherpeth, Cambs., UK) at 90°C for 30 minutes. At the end of the incubation period the tube was briefly pulse-vortexed and placed in the centrifuge for 30 seconds to separate the matrix from the eluate and the disk was removed using a sterile pipette tip and discarded. The eluate contains the purified DNA. Eluted DNA was stored at -20°C until required.

2.4 Phenotypic procedures

2.4.1 Production of a 5% v/v CO_2 environment

To produce an atmosphere of 5% v/v carbon dioxide (CO_2) suitable for the growth of *N. meningitidis* approximately 10ml of hydrochloric acid 15% w/v (SHLMPRL) was dispensed into a glass universal and placed in a polypropylene jar with a suitable universal holder. Inoculated Columbia agar plates with horse blood were placed inside the jar, one sodium bicarbonate 600mg tablet (Norton Healthcare, London, UK) was added to the universal of hydrochloric acid 15% w/v and the lid of the jar was promptly closed. The jar was placed into an LTE Scientific, Raven 2 Incubator set to 37°C for at least 12 hours or overnight.

2.4.2 Serogrouping of N. meningitidis isolates by latex agglutination

The Wellcogen *N. meningitidis* B/E. coli K1 (Remel Europe Ltd., Dartford, Kent, UK) and Wellcogen *N. meningitidis* ACYW135 (Remel Europe Ltd., Dartford, Kent, UK) kits were used for the qualitative detection of specific antigen in a live suspension from an overnight culture plate. To initially serogroup encapsulated meningococci, Wellcogen latex particles coated with monoclonal antibodies specific to serogroup B or coated with polyclonal antibodies specific to serogroup A, C, Y, W135 (Remel Europe Ltd., Dartford, Kent, UK) are used in a slide agglutination technique. The following was performed in a class 1 safety cabinet. Using a sterile cotton tipped swab (McQuilkin & Co. Ltd., Glasgow, UK) a few colonies of the *N. meningitidis* isolate to be tested were picked from an overnight culture plate and used to make a suspension in 2ml of 0.85% saline (SHLMPRL) to give a McFarland standard of approximately 2. On a glass microscope slide (VWR International Ltd., Poole, Dorset, UK) using 1µl disposable loops (VWR International Ltd., Poole, Dorset, UK) a loop-full of the suspension was mixed with a drop of polyvalent Wellcogen *N. meningitidis* A, C, Y, W135 test latex reagent and a second loop-full of the suspension was mixed with a drop of Wellcogen *N. meningitidis* Group B/*E. coli* K1 test latex reagent. The

glass slide was gently tilted to and fro for up to 3 minutes and observed for an agglutination reaction; clumping of the latex particles was indicative of an agglutinated pattern and therefore a positive result.

In a negative reaction the latex does not agglutinate and a milky appearance remains. If a positive reaction was observed with the Group B/*E. coli* K1 latex suspension then the isolates is serogroup B positive. If a positive reaction was observed with the *N. meningitidis* A, C, Y, W135 polyclonal latex reagent the individual meningococcal co-agglutinating reagents A, C, Y and W135 were used to test the isolate further (see below). If a negative reaction was observed with both Wellcogen latex reagents then the meningococcal co-agglutinating reagents X and Z were used to test the isolate further. A negative reaction with all the latex and co-agglutination reagents indicates that either the *N. meningitidis* isolate is phenotypically capsule negative or the organism may lack the genes required for synthesis and transport of capsular polysaccharide. When this occurred isolates were tested further by genotypic PCR to amplify DNA specific for individual capsular polysaccharides or the capsule null locus (see genotypic procedures section).

2.4.3 Serogrouping of N. meningitidis isolates by co-agglutination

Following a positive reaction with the polyclonal Wellcogen *N. meningitidis* ACYW135 latex reagent (Remel Europe Ltd., Dartford, Kent, UK) the serogroup was determined using the panel of monoclonal co-agglutination reagents for serogroup A, C, Y and W135. Using the previously prepared suspension of the test strain that gave a positive reaction with the polyclonal latex reagent, with a sterile 1µl disposable loop (McQuilkin & Co. Ltd., Glasgow, UK) 6 loop-fulls of suspension were spotted onto a glass slide, or slides (VWR International Ltd., Poole, Dorset, UK). With a sterile 1µl disposable loop (McQuilken & Co. Ltd., Glasgow, UK) a loop-full of each individual monoclonal co-agglutination reagent was also spotted to the glass microscope slide(s) (VWR International Ltd., Poole, Dorset, UK) adjacent to a separate spot of the live suspension of the test strain. The adjacent spots of suspended organism and co-agglutination reagent were mixed gently and the glass slide was tilted to and fro and examined for

agglutination. The serogroup was then determined by a positive agglutination with only one corresponding co-agglutination reagent.

2.4.4 Storage of N. meningitidis isolates

Isolates of *N. meningitidis* that were grown successfully (from the archived isolate on Protect™ bacterial preservation beads or freeze-dried ampoules) were stored on a new vial of Protect™ bacterial preservation beads (Technical Service Consultants Ltd., Heywood, Lancs., UK). From a pure overnight culture plate *N. meningitidis* was recovered using a sterile cotton swab (Medical Wire Equipment Co., Corsham, Wilts., UK) and this was used to inoculate a vial of Protect™ bacterial preservation beads (Technical Service Consultants Ltd., Heywood, Lancs., UK). The inoculated vial was mixed and the excess fluid was removed using a sterile plastic pipette (McQuilkin & Co. Ltd., Glasgow, UK). The vial was stored in the freezer at -80°C until required.

2.5 Genotypic procedures

2.5.1 Genotypic serogrouping PCR

Oligonucleotide primers were obtained by ordering online from www.vhbio.com (VH Bio Ltd., Gateshead, UK); or www.eurogentec.com (Eurogentec Ltd., Southampton, Hamps., UK); or www.eurofinsdna.com/home (Eurofins MWG Operon, Ebersberg, Germany). Unless stated, PCR was carried out to a final volume of 25µl using the following; 1µl of each oligonucleotide primer at 5µM/ml, 20µl of 1.1x Reddymix™ PCR Master Mix (1.5mM $MgCl_2$) (Thermo Fisher Scientific, Epsom, Surrey, UK) and 3µl template DNA. Primers used for genotypic serogrouping PCR are listed in Table 2.2. PCR products were confirmed by agarose gel electrophoresis as described later; using the appropriate control organism (Table 2.1) as a positive control. Negative controls were performed using control organisms of a serogroup different to the serogroup being tested. A

negative control was also performed using 18MΩ H_2O (ELGA Process Water, High Wycombe, Bucks., UK) instead of bacterial DNA.

Table 2.2 – Oligonucleotides used in the capsular characterisation of NG meningococci

Primer Name	*Primer Sequence 5' – 3'*	*Reference*
siaD B-F	TGC ATG TCC CCT TTC CTG A	(Guiver, Borrow *et al.* 2000)
siaD B-R	AAT GGG GTA GCG TTG ACT AAC AA	(Guiver, Borrow *et al.* 2000)
siaD C-F	GAT AAA TTT GAT ATT TTG CAT GTA GCT TTC	(Guiver, Borrow *et al.* 2000)
siaD C-R	TGA GAT ATG CGG TAT TTG TCT TGA AT	(Guiver, Borrow *et al.* 2000)
siaDY/W135-F	CAA ACG GTA TCT GAT GAA ATG CTG GAA G	(Borrow, Claus *et al.* 1998)
siaDY/W135-R	TTA AAG CTG CGC GGA AGA ATA GTG AAA T	(Borrow, Claus *et al.* 1998)
ctrA 29E-F	ATT ACG CTG ACG GCA TGT GGA	(Bennett, Mulhall *et al.* 2004)
ctrA Z-F	TAT GCG GTG CTG TTC GCT ATG	(Bennett, Mulhall *et al.* 2004)
ctrA X-F	GTC TTT GTA TAA GGC CCA AG	(Bennett, Mulhall *et al.* 2004)
ctrA U-R	TTG TCG CGG ATT TGC AAC TA	(Corless, Guiver *et al.* 2001)
ctrA F	GCT GCG GTA GGT GGT TCA A	(Corless, Guiver *et al.* 2001)
cnl-F	GGA TTG GAC GAG CGA GAC	(Claus, Maiden *et al.* 2002)
cnl-R	GGT CGT GTG AAA GCT TGC CTT GCT C	(Claus, Maiden *et al.* 2002)
HC287	CGC GCC ATT TCT TCC GCC	(Claus, Maiden *et al.* 2002)
mynA-F	GTC TTA ACC GTC TTT GGC ACT	(Diggle, Smith *et al.* 2003)
mynA-R	TGA GGC ATT ACC TAT CCC GTA	(Diggle, Smith *et al.* 2003)

2.5.1.1 Serogroup B (*siaD* B) and Serogroup C (*siaD* C)

Amplification of the *siaD* gene specific for the production of serogroup B or serogroup C capsular polysaccharide was performed using primers *siaD B-F* and *siaD B-R* or *siaD C-F* and *siaD C-R,* respectively (Table 2.2). Both were amplified under the same thermal conditions; firstly, denaturation at 95°C for 10 minutes was followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 60.4°C for 1 minute and extension at 72°C for 30 seconds. This was followed by an extension step at 72°C for 5 minutes.

2.5.1.2 Serogroup Y/W135 (*siaD* Y/W135)

The high degree of similarity between the DNA sequence of the *siaD* gene encoding for the serogroup Y and serogroup W135 polysaccharide requires that detection of these serogroups is performed in two steps. Firstly, PCR using common primers that amplify both sequences, followed by a restriction digest of the amplified product to distinguish between the two serogroups. This PCR was

carried out at the same thermal conditions as the serogroup B (*siaD* B) and serogroup C (*siaD* C) PCR above using primers *siaD Y/W135-F* and *siaD Y/W135-R* (Table 2) to a final volume of 50µl (i.e. double volumes).

To distinguish between serogroups Y and W135 the following restriction digest was performed in either a 96-well plate (Web Scientific Ltd., Crewe, UK) or a 0.2ml microfuge tube (Thermo Fisher Scientific, Epsom, Surrey, UK). 15µl of amplified product was combined with 1µl of the restriction enzyme *XbaI* (Promega UK Ltd., Southampton, Hamps., UK), 3µl of 10X restriction digest buffer (Promega UK Ltd., Southampton, Hamps., UK) and 11µl 18MΩH_2O (ELGA Process Water, High Wycombe, Bucks., UK) and incubated for 6 hours at 37°C in a water bath (Type JB2; Grant Instruments Ltd., Sherpeth, Cambs., UK) or thermal cycler (Aviso, Germany). Digests were then analysed by agarose gel electrophoresis (see section 2.5.2). An *XbaI* site is unique to the serogroup Y *siaD* gene and the digest reaction produces DNA fragments of 438bp and 92bp, whilst the serogroup W135 amplicon remains intact (Borrow, Claus *et al.* 1998).

2.5.1.3 Serogroup A (*mynA*)

Amplification of the *mynA* gene specific for the production of the serogroup A polysaccharide was performed using primers *mynA-F* and *mynA-R* (Table 2.2). Firstly, denaturation at 95°C for 15 minutes was followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 53°C for 30 seconds and extension at 72°C for 30 seconds. This was followed by an extension step at 72°C for 3 minutes.

2.5.1.4 Serogroup X (*ctrA X*), serogroup Z (*ctrA Z*) and serogroup 29E (*ctrA 29E*)

A slightly different approach was used to identify serogroup X, Z or 29E; the *ctrA* gene was used to differentiate between these serogroups. This PCR used a universal upstream primer *ctrA U-R* and a downstream primer specific to each

serogroup; *ctrA X-F*, *ctrA Z-F* and *ctrA Z-F* for serogroup X, Z and 29E respectively (Table 2.2). Firstly, denaturation at 95°C for 5 minutes was followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62.5°C for 1 minute and extension at 72°C for 30 seconds. This was followed by an extension step at 72°C for 5 minutes.

2.5.1.5 Capsule null locus (*cnl*)

Some *N. meningitidis* isolates lack the genes required for capsule synthesis (*siaABCD*) and transport (*ctrABCD*) and as such the serogrouping by latex or co-agglutination and the genotypic serogrouping PCR tests outlined above will yield a negative result. The intergenic region between the region E and the region D of the *cps* cluster containing the capsule null locus was amplified by PCR using primers *cnl-F* and *cnl-R* or *cnl-R* and *HC287* (Table 2.2). Firstly denaturation at 95°C for 2 minutes was followed by 40 cycles of denaturation at 95°C for 25 seconds, annealing at 53°C for 20 seconds and extension at 72°C for 30 seconds. This was followed by an extension step at 72°C for 5 minutes.

2.5.1.6 Serogroup non-specific *ctrA* PCR

Where NG *N. meningitidis* isolates yielded a negative result with the serogroup-specific PCRs and the *cnl* PCR, the serogroup non-specific *ctrA* PCR assay used in the SHLMPRL for the detection of meningococcal DNA in clinical specimens in suspected cases of meningococcal disease (Corless, Guiver *et al.* 2001) was used to confirm that isolates were *N. meningitidis*. The PCR used the ctrA-U and ctrA-F primers (Table 2.2). Firstly denaturation at 95°C for 2 minutes was followed by 10 cycles of denaturation at 95°C for 20 seconds, annealing at 55°C for 20 seconds and extension at 72°C for 10 seconds. This was followed by a further 10 cycles where the extension step was increased to 12 seconds. This was subsequently followed by a further 10 cycles with the extension step increased to 15 seconds.

2.5.2 Confirmation of PCR products by agarose gel electrophoresis

'Hi-Pure' Low EEO Agarose (Biogene Ltd, Kimbolton, Cambs., UK) was added to 1x ELFO buffer (SHLMPRL) to make a 1.5 % (w/v) gel and heated until molten using a standard microwave. 4µl of 1mg/ml ethidium bromide (SHLMPRL) was added to the molten gel and this was poured into a gel cast and allowed to set. Once the gel had set, 5µl - 10µl of each PCR product was used for agarose gel electrophoresis and visualised using a UV transilluminator (VWR International Ltd., Poole, Dorset, UK) or Bio-Rad gel doc 2000 gel analysis system and Quantity One 1-D analysis software (Bio-Rad Laboratories Ltd, Hemel Hempsted, UK). The ReddyMix PCR Master Mix w/ 1.5mM $MgCl_2$ (Thermo Scientific, Epsom, Surrey, UK) used in all PCR reactions contains a dye and a precipitant to facilitate gel loading. Agarose gels were disposed of following the standard operating procedures in place at the SHLMPRL (designated Ethidium bromide agarose gel waste bins were used and these were uplifted by an outside contractor for safe disposal). Examples of agarose gel electrophoresis pictures for the genotypic serogrouping PCRs, *porA* and MLST gene amplification are shown in the Appendix, section 9.3 (Figures 9.1 – 9.8).

2.5.3 Multi-locus sequence typing (MLST)

2.5.3.1 Amplification reaction primers and conditions

MLST was performed using semi-automated methods as described previously (Sullivan, Jefferies *et al.* 2006). Amplification of the MLST gene fragments was performed using the oligonucleotide primer pairs abcZ-M-AF and abcZ-M-AR, adk-M-AF and adk-M-AR, aroE-M-AF and aroE-M-AR, fumC-M-AF and fumC-M-AR, gdh-M-AF and gdh-M-AR, pdhC-M-AF and pdhC-M-AR, pgm-M-AF and pgm-M-AR as listed in Table 2.3. In addition, primer pairs ending in -SF and -SR were used for amplification if a particular gene fragment was unsuccessfully amplified using the -AF and -AR primer pairs. Amplification of the gene fragments was carried out to a final volume of 25µl using the following: 1µl of each primer (forward

and reverse) + 20µl of 1.1x Reddymix™ PCR Master Mix (1.5mM $MgCl_2$) (Thermo Fisher Scientific, Epsom, Surrey, UK) + 3µl of template DNA.

Table 2.3 – Oligonucleotides used for DNA amplification and sequencing of MLST alleles

Primer Name	*Primer Sequence 5' - 3'*
abcZ-M-AF	TGT TCC GCT TCG ACT GCC AAC
abcZ-M-AR	TCC CCG TCG TAA AAA ACA ATC
adk-M-AF	CCA AGC CGT GTA GAA TCG TAA ACC
adk-M-AR	TGC CCA ATG CGC CCA ATA C
aroE-M-AF	TTT GAA ACA GGC GGT TGC GG
aroE-M-AR	CAG CGG TAA TCC AGT GCG AC
fumC-M-AF	TCC CCG CCG TAA AAG CCC TG
fumC-M-AR	GCC CGT CAG CAA GCC CAA C
gdh-M-AF	CTG CCC CCG GGG TTT TCA TCT
gdh-M-AR	TGT TGC GCG TTA TTT CAA AGA AGG
pdhC-M-AF	CCG GCC GTA CGA CGC TGA AC
pdhC-M-AR	GAT GTC GGA ATG GGG CAA ACA
pgm-M-AF	CTT CAA AGC CTA CGA CAT CCG
pgm-M-AR	CGG ATT GCT TTC GAT GAC GGC
abcZ-M-SF	AAT CGT TTA TGT ACC GCA GR
abcZ-M-SR	GAG AAC GAG CCG GGA TAG GA
adk-M-SF	AGG CWG GCA CGC CCT TGG
adk-M-SR	CAA TAC TTC GGC TTT CAC GG
aroE-M-SF	GCG GTC AAY ACG CTG RTK
aroE-M-SR	ATG ATG TTG CCG TAC ACA TA
fumC-M-SF	TCC GGC TTG CCG TTT GTC AG
fumC-M-SR	TTG TAG GCG GTT TTG GCG AC
gdh-M-SF	CCT TGG CAA AGA AAG CCT GC
gdh-M-SR	RCG CAC GGA TTC ATR YGG
pdhC-M-SF	TCT ACT ACA TCA CCC TGA TG
pdhC-M-SR	ATC GGC TTT GAT GCC GTA TTT
pgm-M-SF	CGG CGA TGC CGA CCG CTT GG
pgm-M-SR	GGT GAT GAT TTC GGT YGC RCC

All sequences obtained from http://pubmlst.org/neisseria/

In addition, primer pairs ending in -SF and -SR were used for amplification if a particular gene fragment was unsuccessfully amplified using the -AF and -AR primer pairs. Amplification of the gene fragments was carried out to a final volume of 25µl using the following: 1µl of each primer (forward and reverse) + 20µl of 1.1x Reddymix™ PCR Master Mix (1.5mM $MgCl_2$) (Thermo Fisher Scientific, Epsom, Surrey, UK) + 3µl of template DNA.

Firstly denaturation at 95°C for two minutes was followed by ten cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 40 seconds and extension at 72°C for 1 minute 30 seconds. Subsequently, twelve cycles with an altered annealing step at 55°C and extension step of 1 minute 35 seconds and a further fourteen cycles with an altered annealing step at 50°C and extension

step of 1 minute 40 seconds. Finally this was completed by an extension step at 72°C for five minutes. PCR products were confirmed by agarose gel electrophoresis as described earlier.

2.5.3.2 Sequencing reaction primers and conditions

Sequencing of the MLST gene fragments was performed as described in (Sullivan, Jefferies *et al.* 2006). Briefly; each fragment was sequenced on both strands using 1µl of each of the corresponding -SF and -SR primers at 5µM/ml (Table 2.3), 4µl of DYEnamic ET Terminator™ (GE Healthcare, Little Chalfont, UK) and 6µl of clean DNA. The sequencing reaction was performed using thirty cycles at the following thermal parameters; denaturation at 95°C for 20 seconds, annealing at 50°C for 15 seconds and extension at 60°C for 60 seconds.

Following the sequencing reaction; sequencing products were prepared using MultiScreen®$_{384}$-SEQ filter plates (Millipore Ltd., Watford, Herts., UK) and a TheOnyx liquid handling system (Aviso, GmbH, Germany) according to the manufacturers' instructions. DNA sequencing was performed using the MegaBACE 1000 DNA Analysis System (GE Healthcare, Little Chalfont, UK) according to the manufacturers' instructions.

2.5.3.3 MegaBACE 1000 automated DNA sequencer

Operation of the MegaBACE 1000 automated DNA sequencer was conducted following standard operating procedures in place at the SHLMPRL. In the Electrophoresis parameters tab within the Instrument Control Manager the standard sequencing run parameters were entered at the following settings; Sample Injection Voltage = 3KV; Sample Injection Time = 38 seconds; Run Voltage = 9KV; Run Time = 120 minutes. On occasion these settings were altered to improve the sequencing read length where there was evidence of late injection or over injection of a sample.

2.5.4 PorA genotypic characterisation

The amplification conditions, confirmation of PCR products, sequencing reactions, preparation of sequencing products and DNA sequencing of PorA variable regions were performed as for the MLST gene fragments with the exception of using the primers listed in Table 2.4. Amplification was performed primarily using porA-M-AF(210) and porA-M-AR(211). In instances where amplification was unsuccessful, primers porA-M-AF(AU) and porA-M-AR(AL) were used together or in combination with the aforementioned pair. DNA sequencing of VR1, VR2 and VR3 on both strands was performed using the primers listed in Table 2.4.

Table 2.4 – Oligonucleotides used for PCR and sequencing of PorA variable regions

Primer Name	Primer sequence 5' – 3'	VR[1]; direction sequenced	Reference
porA-M-AF(210)	ATG CGA AAA AAA CTT ACC GCC CTC	VR1; 5'-3'	(Feavers and Maiden 1998)
porA-M-AR(211)	AAT GAA GGC AAG CCG TCA AAA ACA	ND[2]	(Feavers and Maiden 1998)
porA-M-AF(AU)	GCA CGA GGT CTG CGC	ND	(Molling, Unemo *et al.* 2000)
porA-M-AR(AL)	ATT AAT TTG AGT GTA GTT GCC	ND	(Molling, Unemo *et al.* 2000)
porA-M-SF(103L)	AAC GGA TAC GTC TTG CTC	VR1; 3'-5'	(Suker, Feavers *et al.* 1994)
porA-M-SR(8U)	TCC GTA CGC TAC GAT TCT CC	VR2/3; 5'-3'	(Maiden, Suker *et al.* 1991)
porA-M-SF(21U)	GCG AAA TCA AAG CCG GCG T	VR2/3; 5'-3'	(Maiden, Suker *et al.* 1991)
porA-M-SR(122L)	GGC GAG ATT CAA GCC GCC	VR2/3; 3'-5'	(Suker, Feavers *et al.* 1994)

[1] VR, variable region; [2] ND, not determined

2.5.5 DNA sequencing of the capsule null locus

DNA sequencing of the capsule null locus was performed following the method of Claus *et al* 2002 with modifications. The preparation of amplified DNA and sequencing reaction mixes and the sequencing reaction for nucleotide

sequencing of the *cnl* was performed as for MLST and PorA sequencing using the equivalent primer pair as used in the amplification reaction to obtain the nucleotide sequences for both strands.

2.5.6 DNA sequence analysis

For MLST, sequence data analysis was performed as described previously (Diggle and Clarke 2002). The sequence data were read automatically from the sequencer using the integrated image analysis and data collection software. DNA sequence data were analyzed using the MegaBACE Sequence Analysis Software, Version 2.4, Build 20010115, Molecular Dynamics © 1996 – 2001, using the Cimarron 2.19 Slim Phredify or Cimarron 1.53 Slim Phredify Base caller). MLST allele sequences were downloaded in FASTA format from the MLST database website and generated sequences were compared using the DiscoverIR software, version 1, Build 1.19, LI-COR Inc, Michigan, USA. New allelic variants and new sequence types were submitted to the *N. meningitidis* MLST database curator for assignment (http://pubmlst.org/neisseria/).

For *porA* genosubtyping, the sequence data were read automatically from the sequencer using the integrated image analysis and data collection software. The nucleotide sequences were translated into amino acid sequences using the Translate program (http://expasy.cbr.nrc.cu/tools/dna.html) and compared to known variants on the PorA database (www.neisseria.org/nm/typing) or directly using the PorA web-site database for VR1 and VR2. For VR3 subtyping the nucleotide sequences were translated into amino acid sequences using the Translate program and compared with those previously sequenced by the SHLMPRL or other collaborators (Molling, Unemo *et al.* 2000; Molling, Backman *et al.* 2001; Molling, Jacobsson *et al.* 2002; Clarke, Diggle *et al.* 2003b).

Sequence analysis of the capsule null locus was performed using the sequence trace files (SCF) from the integrated MegaBACE software and imported directly into the CLC DNA Workbench 3.6.2 (CLC bio A/S, Aarhus, Denmark) for reverse complementation (where required) and alignment with published sequences of *cnl* alleles; see Table 2.5. *cnl* alleles were aligned using ClustalX version 2.0

(Larkin, Blackshields *et al.* 2007). New DNA sequences were submitted to GenBank.

Table 2.5 – Capsule null locus allele sequences used for *cnl* DNA sequence comparison

Capsule null locus allele	*Source strain description*	*Accession number*
cnl-1	*N. meningitidis* ST-53 *tex/galE* intergenic sequence replacing the regions A + C of cps locus	AJ308327
cnl-2	*N. meningitidis tex/galE* intergenic sequence, strain α30	AM086166
cnl-3	*N. meningitidis tex/galE* intergenic sequence, strain α627	AM086167
cnl-4	*N. lactamica tex/galE* intergenic sequence, strain ß154	AM086168
cnl-5	*N. lactamica tex/galE* intergenic sequence, strain ß270	AM086169
cnl-6	*N. gonorrhoeae tex/galE* intergenic sequence, strain FA1090	AM086170
cnl-7	*N. lactamica tex/galE* intergenic sequence, strain 10618 V1	AM086171

2.5.7 Analysis of MLST alleles and sequence types

MLST allelic profiles were analysed using eBURSTv3 (Feil, Li *et al.* 2004; Spratt, Hanage *et al.* 2004). The algorithms employed in the eBUSRTv3 software allow STs to be grouped based on relatedness at individual gene loci. BURST, unlike cluster diagrams, trees or dendograms, uses a simple but appropriate model of bacterial evolution in which an ancestral (or founding) genotype increases in frequency in the population and, while doing so, begins to diversify to produce a cluster of closely-related genotypes that are all descended from the founding genotype. This cluster of related genotypes is often referred to as a "clonal complex". eBURSTv3 has been developed and is hosted at Imperial College London. The prevalence of sequence types within carriage and invasive meningococcal disease (IMD) isolate datasets was compared using the comparison tool in the eBURSTv3 software to identify STs unique to each dataset or shared between the two datasets.

Sequence Type Analysis and Recombinational Tests version 2 (STARTv2), written by Keith Jolley (Jolley, Feil *et al.* 2001), was used to perform a number of analyses on the carried *N. meningitidis* strain collection dataset generated during this study and on the invasive meningococcal disease isolate dataset (covering the same temporal period) that was kindly provided by the SHLMPRL. Within the software the MLST scheme for *N. meningitidis* was defined and known allelic profiles and allele sequences were imported from the *N. meningitidis* PubMLST website (www.pubmlst.org/data). The individual data sets containing the allelic profiles and STs for the carried specimens sequenced in this study and for the IMD dataset obtained from the SHLMPRL were entered. Allele and ST frequencies were calculated for each dataset from the corresponding allelic profiles. The number of polymorphic sites was calculated from the concatenated sequences. Lineage assignment was investigated using the BURST method from the allelic profiles and using the UPGMA and Neighbor-joining methods from the concatenated sequences.

2.6 MLST clonal complexes

As of May 2010 the defined clonal complexes listed on the Neisseria MLST website were as follows: ST-11 complex/ET-37 complex, ST-8 complex/Cluster A4, ST-41/44 complex/Lineage 3, ST-32 complex/ET-5 complex, ST-1 complex/subgroup I/II, ST-4 complex/subgroup IV, ST-5 complex/subgroup III, ST-106 complex, ST-116 complex, ST-18 complex, ST-198 complex, ST-23 complex/Cluster A3, ST-231 complex, ST-254 complex, ST-292 complex, ST-334 complex, ST-35 complex, ST-364 complex, ST269 complex, ST-376 complex, ST-53 complex, ST-549 complex, ST-92 complex, ST-22 complex, ST-162 complex, ST-213 complex, ST-461 complex, ST-226 complex, ST-60 complex, ST-174 complex, ST-103 complex, ST-167 complex, ST-750 complex, ST-1157 complex, ST-865 complex, ST-4821 complex, ST-37 complex, ST-175 complex, ST-1117 complex, ST-1136 complex, ST-212 complex, ST-178 complex and ST-282 complex. In this publication, the author used a modified version of these clonal complexes. For some MLST clonal complexes that have dual names to reflect the groupings defined by MLEE, those MLEE designations were, for the most part,

dropped from the text. Additionally, for convenience, the word "complex" was dropped from the name and the prefix "ST-" was replaced with "cc" denoting "clonal complex". For example, "ST-11 complex/ET-37 complex" became "cc11" and "ST-213 complex" was modified to "cc213".

2.7 Measurement of genetic diversity, *D*

Simpson's index of diversity (*D*) was used to assess the level of diversity of sequence types or PorA subtypes within the carriage and IMD strain collections as a whole, or in part to compare temporal distribution, diversity within serogroup or geographic region. It gives the probability that any two randomly selected individuals drawn from an infinitely large community belong to two different species, or in the case of this study, isolates drawn from a population belong to different MLST types (Hunter and Gaston 1988). The value of the index ranges from zero to one, meaning the nearer the value to one the greater the diversity and the nearer to zero the less the diversity. The 95% confidence intervals (CIs) for these indices were calculated following the method of Grundmann and colleagues (Grundmann, Hori *et al.* 2001). Non-overlapping 95% CIs indicate a significant difference in *D*.

2.8 Phylogenetic inference of PorA subtypes (VR1, VR3 & VR3) based on concatenated amino acid sequences

A Pearson (FASTA) file containing the concatenated amino acid sequences of the PorA subtypes (VR1,VR2,VR3) of the carriage strain collection was uploaded into ClustalW2 (Larkin, Blackshields *et al.* 2007) available at www.ebi.ac.uk/clustalw/ and a multiple alignment of the concatenated amino acid sequences was first generated using the default settings. The multiple alignment was subsequently used to construct a phylogenetic tree based on the neighbor-joining (NJ) method (Saitou and Nei 1987).

A phylogram is a branching diagram (tree) that is assumed to be an estimate of a phylogeny. The branch lengths are proportional to the amount of inferred evolutionary change. A cladogram is a branching diagram (tree) assumed to be an estimate of a phylogeny where the branches are of equal length. Therefore, cladograms show common ancestry, but do not indicate the amount of evolutionary "time" separating taxa.

2.9 Estimation of the association of meningococcal serogroups, clonal complexes, STs and PorA subtypes with invasive disease or with a carriage phenotype

An empirical odds ratio (OR) was directly calculated to compare the probability of invasive disease (or carriage), on the basis of individual meningococcal strain characteristics; i.e. serogroup, clonal complex, sequence type or PorA subtype. Where *a* is the number of invasive 'A' serogroups; *b* is the number of carried 'A' serogroups, *c* is the number of invasive non-'A' serogroups and *d* is the number of carried non-'A' serogroups, the odds ratio is calculated using the equation OR = (ad)/(bc). This was repeated for clonal complexes, STs and PorA VR1,2 subtypes. An OR > 1 indicates that a particular meningococcal serogroup, clonal complex, ST or subtype is associated with invasive disease, whilst an OR < 1 indicates that a particular meningococcal serogroup, clonal complex, ST or subtype is associated with carriage. Where an OR indicated carriage the reciprocal OR was calculated in each case. To assess the significance of the ORs, 95% CIs were calculated; if the confidence interval does not include the value 1 then the OR is statistically significant. Furthermore, a narrow confidence interval implies high precision, while a wide confidence interval implies poor precision.

2.10 Invasive meningococcal disease isolate dataset

The meningococcal isolate database at the SHLMPRL was examined to identify invasive meningococcal disease isolates. An invasive meningococcal disease isolate is one which has been grown from a normally sterile site. For the purposes of this study only blood or CSF isolates were considered. IMD isolates were matched temporally to the carriage strain collection; IMD isolates were also received by the reference laboratory during the same period, 1st January 1974 – 31st December 2004. Furthermore, IMD isolates were also matched geographically on the basis that the isolates had been obtained from the same population, i.e. Scotland. The reference laboratory often receives isolates from other locations; therefore isolates were excluded if the submitting laboratory was not located in Scotland. Isolates that had been fully characterised by MLST were selected from the meningococcal isolate database for analysis.

This identified 2,772 invasive isolates, comprising 1,264 blood isolates and 1,508 CSF isolates. This strain collection was designated as the invasive meningococcal disease strain collection; and from this point onwards may be referred to as simply the IMD strain collection or IMD isolates. It should be noted that analysis of the IMD strain collection dataset with STARTv2 (Jolley, Feil *et al.* 2001) identified five duplicate records and ten typographical errors relating to miss-matching ST:allelic profiles. The five duplicate records were removed and the miss-matching ST:allelic profile combinations were re-assigned on the basis that the allelic profile was correct. This gave a final number of isolates in the IMD strain collection of 2,767 from which data were used for all comparisons with the carriage strain collection described herein.

2.11 Meningococcal strain designation

The recommended strain designation for meningococci includes the serogroup, PorA VRs 1 & 2, FetA VR, MLST sequence type and clonal complex (Brehony, Jolley *et al.* 2007). An example being: C: P1.5, 2-1: F5-4: ST-11 (cc11). At the time this project commenced FetA VR sequencing was not employed by the

reference laboratory in Scotland as a means to characterise strains of meningococci, and as such FetA VR sequencing was not included as part of this study. For the purposes of this study in Chapter 6, strain types were designated in the format: sero/geno-group:CC:ST:VR1,VR2,VR3. For example, C:8:8:5,2,36-2.

3 Epidemiological summary and capsular characterisation of the carried meningococci in Scotland, 1974 – 2004

3.1 Introduction

Many of the specimens pre-date the use of electronic data recording by the reference laboratory; any accompanying documentation (i.e. letters or specimen information documents) were archived and a meningococcal isolate register was maintained by the reference laboratory summarising basic information on each specimen. Following the re-location of the reference laboratory to its current location at Stobhill Hospital, epidemiological information recorded in the meningococcal isolate register and any accompanying documentations was compiled onto an electronic database which was examined to identify isolates, based upon the previously mentioned criteria, for inclusion into this study. The serogroup of each isolate included in the study was recorded when the specimens were first processed by the reference laboratory. Unless stated otherwise, only isolates whose serogroup was initially recorded as non-groupable were re-checked by latex- and co-agglutination. A range of serogroup-specific and non-serogroup-specific PCR targets were used to further characterise carried meningococcal specimens.

3.2 Examination of the Reference Laboratory strain archive and the meningococcal isolate database

The project proposal stated that the SHLMPRL isolate archive contained 2091 carried meningococci, of which, 1460 were reportedly carried throat isolates. On commencement of the project the isolate database at the SHLMPRL was examined to identify carried meningococcal throat isolates from the isolate archive. Limited information was available on many of the specimens in the isolate database; particularly for specimens from earlier years. As outlined in Chapter 2 a carriage isolate was defined as coming from an asymptomatic

individual with no epidemiological link to an invasive meningococcal disease case. Based upon these criteria some 523 isolates were included for further study. A considerable number of specimens were initially excluded as limited information was available based upon the "diagnosis" or "clinical details" recorded for the specimen or individual sampled.

Following further investigation and discussion it was decided that we could seek assistance from Health Protection Scotland (HPS) and/or the regional NHS health boards regarding background epidemiological information on the specimens which lacked full details. Following initial contact with HPS it was advised that my request would require a combined approach between the senior management of SHLMPRL and HPS. However no further background information on the specimens was obtained via this route. In addition we also contacted the regional NHS health boards to obtain updated information regarding the specimens listed on the SHLMPRL isolate database.

The isolate database at SHLMPRL was re-examined for isolates for inclusion in this study. The database contained 2,214 upper respiratory tract isolates during the period 1974 – 2004; comprising of 2,006 "throat isolates", 160 "sputum isolates", 23 "nasal isolates", six "nasopharyngeal isolates", six "mouth wash isolates", two "tracheal secretion isolates" and one "bronchial aspirate isolate". Specimens were excluded from the study for various reasons; 588 specimens were not found or the isolate was never archived at the time of receipt; 131 isolates were identified as being from a contact of a notified case of invasive meningococcal disease (IMD), 76 specimens did not yield viable meningococci after 48 hours and were discarded, 30 specimens were from an individual confirmed as having IMD and four specimens were PCR negative for all MLST loci and *porA*. In addition, one isolate was identified as *Neisseria flava* and one was identified as *N. lactamica*; these were also excluded.

The isolate archive at SHLMPRL consisted of isolates stored as freeze-dried (lyophilised) cultures (1972-1996) or isolates stored at -80°C on Protect™ bacterial preservation beads and/or in 5% v/v glycerol (1996 onwards). When isolates were originally archived as freeze-dried (lyophilised) specimens, in some cases, multiple specimens were often stored; for example more than one individual glass vial of freeze-dried (lyophilised) specimen had been prepared.

Where multiple vials existed for individual isolates consecutive vials were prepared for resuscitation and recovery until a viable meningococcal isolate was obtained, or until all vials had been exhausted. In contrast, specimens archived from 1996 onwards were usually stored in pairs using one vial of Protect™ beads and one vial containing 5% glycerol. Furthermore, these specimens were also repeatedly sub-cultured using two beads at a time and each plate was incubated for up to 48 hours until colonies of meningococci had grown. When a sub-culture did not yield any viable meningococci the process was repeated until viable meningococci were obtained or until the vial of Protect™ beads (or 5% glycerol stock) was exhausted. Often it was required to take a single colony pick from the original culture plate for sub-culture either to improve the yield of the culture or to obtain a pure culture; this was more frequent with older specimens.

Successful recovery of isolates from freeze-dried vials was lower than expected during this study. Each vial contained the lyophilised specimen and a strip of paper with the Reference Laboratory specimen number. Specimens more likely to yield viable meningococci were more milky-white in colour, denser and of a powdery consistency. In contrast, the condition of specimens less likely to yield viable meningococci could be described as ranging from yellowish to brown in colour; often they were more transparent and took on a frothy, bubble-like appearance (perhaps due to over-heating during the preparation of the samples). As the project progressed the decision was taken to exclude a further 470 specimens from the study. Due to the visibly poor quality of the specimen in the glass vials it was thought unlikely that any viable meningococci could be recovered from these sub-standard specimens and to attempt to do so would have been a waste of both time and resources. Attempts were made on 695 isolates from 1972 – 1995 using in excess of 1060 individual freeze-dried vials. Of these, 271 isolates failed to grow on sub-culture and no more vials remain in the isolate archive for these isolates.

Many specimens required sub-culturing for purity or for improved growth. Specimens were discarded when significant contamination by another organism had occurred. Initially, when a specimen was resuscitated and no viable meningococci were recovered after incubation for 48 hours the specimen was discarded. However, due to the unexpectedly high number of non-viable specimens being encountered during the resuscitation and recovery process it

was subsequently decided that, for specimens processed thereafter, a portion of any re-suspended specimen be retained should the specimen not yield viable meningococci. Those portions, if required, subsequently underwent the DNA extraction procedure listed in section 2.3.3 or 2.3.4 of Chapter 2 prior to amplification and sequencing. Limited success was achieved however; some 162 specimens were non-viable, while the remaining 629 isolates were successfully sub-cultured from Protect™ beads, 5% v/v glycerol, or freeze-dried (lyophilised) specimens. In total 791 meningococcal specimens plus four ATCC and four NCTC control isolates (see Chapter 2) were included in the study.

The SHLMPRL isolate database was examined for any other relevant information relating to clinical details or any symptoms of the individual sampled. For approximately half of all specimens (n=402; 50.8%) 'Unknown' was recorded in the database and no further information was available. For 294 (37.2%) specimens 'Asymptomatic' or '(Staff) Screen(ing)' was recorded in the database. Several samples (n=58; 7.3%) were recorded as originating from individuals with flu-like or non-specific symptoms (i.e. one or more of 'headache', 'fever', 'neck stiffness', 'rash', 'photophobia', 'pyrexia', 'febrile', 'sore throat', 'pharyngitis', 'tonsillitis', 'drowsy', 'nausea'). A further 23 (2.9%) samples had originated from individuals screened whilst attending a sexually-transmitted infections or genito-urinary medicine facility. Eight (1.0%) more specimens were obtained from individuals recorded as having 'viral illness' or 'encephalitis', or 'anal HSV', 'herpes', 'HIV-positive', 'HPV' or 'measles'. Six (0.8%) more specimens had other details recorded; one each stated 'diabetic', 'pneumonia', 'PUO', 'vomiting', 'student' and 'study'. No accompanying information was available to suggest that any of the source individuals were a contact of a notified case of IMD, or were indeed a notified case themselves and these specimens were accepted into the study.

3.3 Year of isolation

The 791 carried meningococcal specimens included in this study were received by the SHLMPRL during 1st January 1972 – 31st December 2004. Figure 3.1

indicates the number of carried meningococcal specimens per each calendar year was highly variable; from zero in 1991 and 1993 to 234 in 1985. One hundred and thirty (16.4%), 450 (56.9%) and 211 (26.7%) meningococcal specimens were received by the SHLMPRL during the periods 1974 – 1983, 1984 – 1993 and 1994 – 2004, respectively. The carriage strain collection was broadly clustered into three periods; a smaller cluster in the late 1970s, a large cluster from 1996 onwards, and a larger cluster during the mid 1980s that is dominated by the large number of isolates from 1985. In comparison the distribution of the IMD strain collection was more uniform throughout the 31-year period comprising of an average of 89 or 90 fully characterised isolates per calendar year; although this ranged from twenty in 1983 to 144 in 1991 (Figure 3.1).

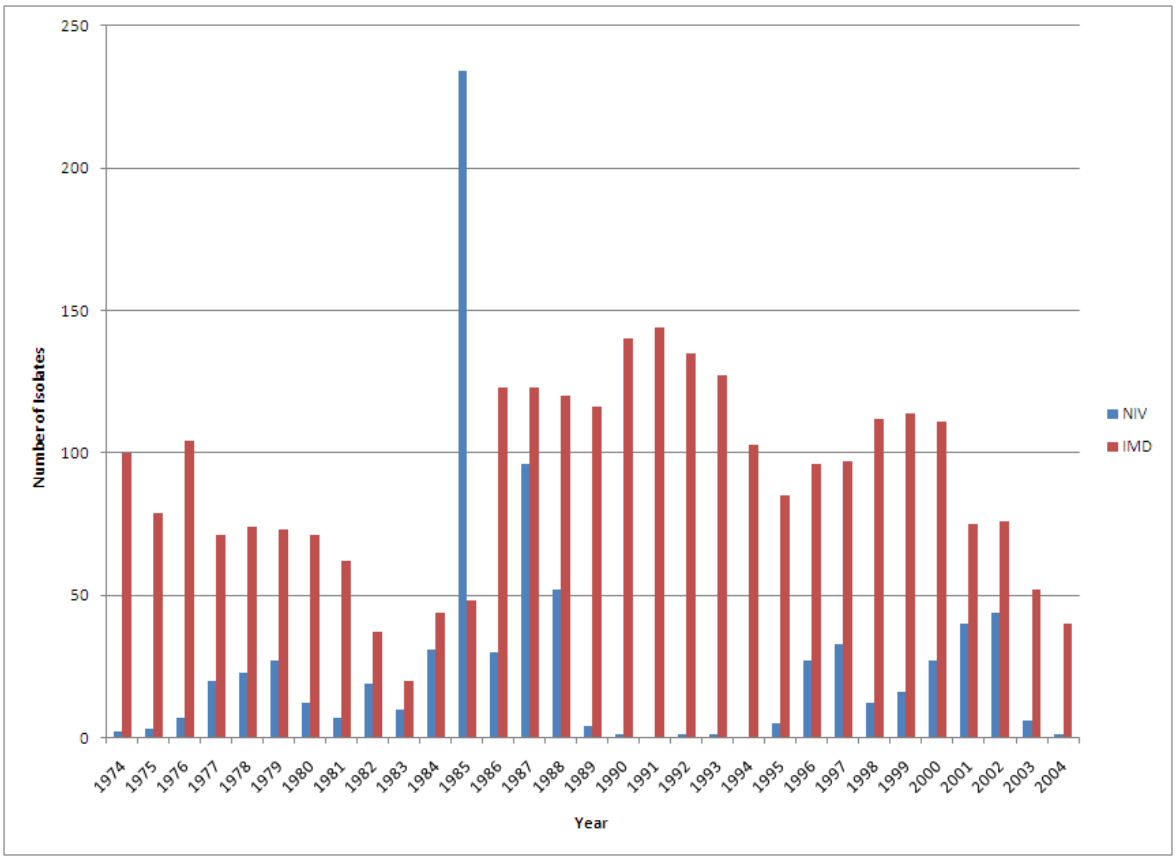

Figure 3.1 - Temporal distribution of meningococci in Scotland, 1974 – 2004

NIV, non-invasive meningococci; IMD, invasive meningococcal disease

3.4 Geographic area (health board)

The NHS health board, under whose jurisdiction each sending laboratory belonged, was used as a predictor of the geographic area of residence of the individual from which each isolate was obtained. In 2006 the NHS health board 'Argyll & Clyde' was sub-divided and the 'Argyll' region was combined with NHS Highland health board to form NHS Highland & Argyll. The 'Clyde' region was combined with Greater Glasgow to form the NHS Greater Glasgow & Clyde health board. Given that the specimens in this study pre-date the re-organisation of those health boards for the purposes of this study the health board names, as defined prior to the re-organisation, were used throughout. This subdivided Scotland into fifteen geographic areas; NHS Argyll & Clyde (AC), NHS Ayrshire & Arran (AA), NHS Borders (BR), NHS Dumfries & Galloway (DG), NHS Fife (FF), NHS Forth Valley (FV), NHS Grampian (GR), NHS Greater Glasgow (GG), NHS Highland (HG), NHS Lanarkshire (LN), NHS Lothian (LO), NHS Orkney (OR), NHS Shetland (SH), NHS Tayside (TY) and NHS Western Isles (WI) (Figure 3.2).

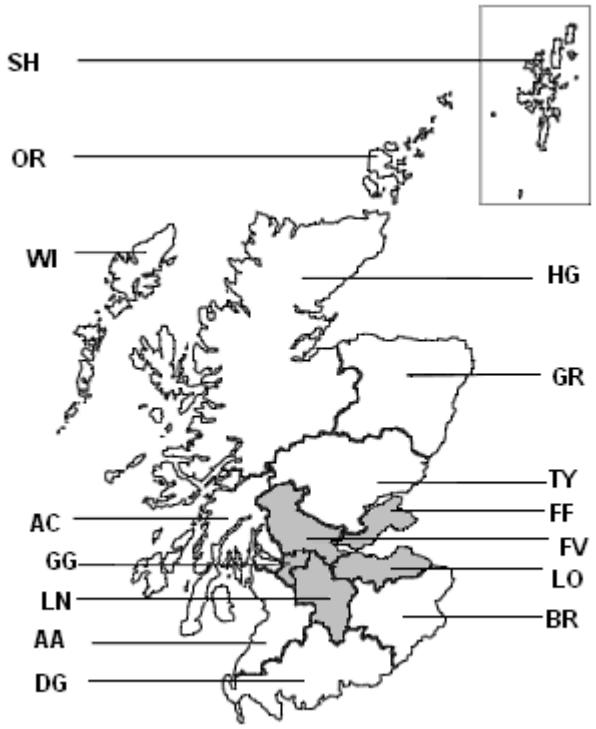

Figure 3.2 - Map of Scotland divided into NHS Health Boards[1]

[1] Correct at the point of commencement of the study (2006); AA = Ayrshire & Arran; AC = Argyll & Clyde; BR = Borders; DG = Dumfries & Galloway; FF = Fife; FV = Forth Valley; GG = Greater Glasgow; GR = Grampian; HG = Highland; LN = Lanarkshire; LO = Lothian; OR = Orkney; SH = Shetland; TY = Tayside; WI = Western Isles.

Figure 3.3 shows the geographic distribution of the carriage strain collection. No specimens were isolated from individuals predicted to reside in the Orkney or Shetland NHS Health board areas. The number of isolates in each geographic area varied between one from the NHS Western Isles health board area to 273 from the NHS Lanarkshire health board area.

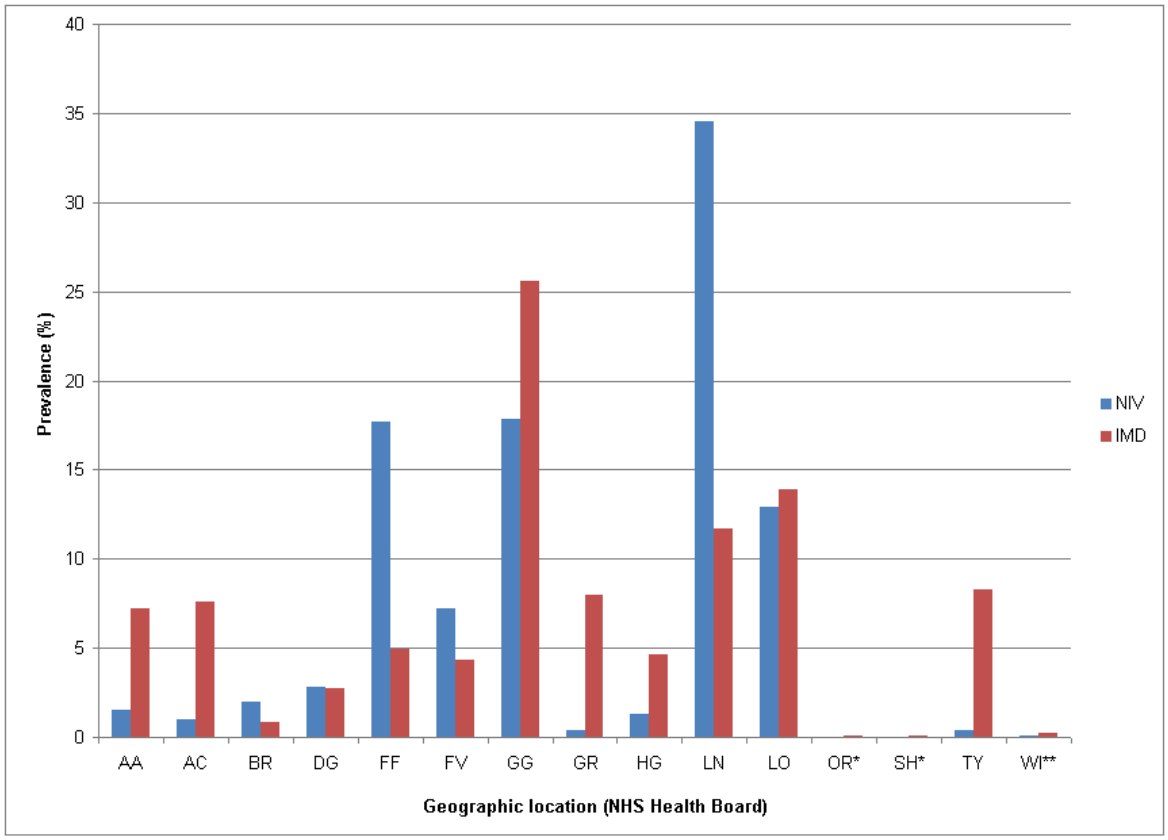

Figure 3.3 - Geographic distribution of meningococci in Scotland, 1974 – 2004
NIV, non-invasive; IMD, invasive meningococcal disease; AA, Ayrshire & Arran; AC, Argyll & Clyde; BR, Borders; DG, Dumfries & Galloway; FF, Fife; FV, Forth Valley; GG, Greater Glasgow; GR, Grampian; HG, Highland; LN, Lanarkshire; LO, Lothian; OR, Orkney, SH, Shetland; TY, Tayside, WI, Western Isles; * One isolate in the IMD strain collection (equivalent to <0.1%) was obtained from individuals resident in each of the Orkney and Shetland NHS Health board regions; ** One isolate in the non-invasive strain collection (equivalent to 0.1%) was obtained from an individual resident in the Western Isles NHS health board region.

Twelve (1.5%) specimens were isolated from individuals predicted to reside in the NHS Ayrshire & Arran Health board area; eight (1.0%) specimens were isolated from individuals predicted to reside in the NHS Argyll & Clyde Health board area; sixteen (2.0%) specimens were isolated from individuals predicted to reside in the NHS Borders Health board area; twenty-two (2.8%) specimens were

isolated from individuals predicted to reside in NHS Dumfries & Galloway Health board area; 140 (17.7%) specimens were isolated from individuals predicted to reside in the NHS Fife Health board area; fifty-seven (7.2%) specimens were isolated from individuals predicted to reside in the NHS Forth Valley Health board area and 141 (17.8%) specimens were isolated from individuals predicted to reside in the NHS Greater Glasgow Health board area. A further three (0.4%) specimens were isolated from individuals predicted to reside in the NHS Grampian Health board area; ten (1.3%) specimens were isolated from individuals predicted to reside in the NHS Highland Health board area; 273 (34.5%) specimens were isolated from individuals predicted to reside in the NHS Lanarkshire Health board area; 102 (12.9%) specimens were isolated from individuals predicted to reside in the NHS Lothian Health board area; three (0.4%) specimens were isolated from individuals predicted to reside in the NHS Tayside Health board area and one (0.1%) isolate was received from an individual predicted to reside in the NHS Western Isles Health board area. In addition, three specimens were coded as being received from BAN:HOS however it was unclear which NHS Health board this submitting laboratory belonged. To summarise, the vast majority (n=613; 77.5%) of carried meningococcal specimens were isolated from individuals predicted to reside in five NHS Health Board areas located across the 'central belt', and hence, the most populated areas of Scotland (FF, FV, GG, LN and LO; shaded grey in Figure 3.2).

Using the data recorded in the SHLMPRL meningococcal isolate database the same fifteen geographic areas based on the NHS health boards were also used to differentiate the IMD strain collection. The number of isolates in each geographic area varied from one each in Orkney and Shetland NHS Health boards to 711 in NHS Greater Glasgow (Figure 3.3). One hundred and ninety-nine (7.2%) specimens were isolated from individuals resident in the Ayrshire & Arran region; 212 (7.6%) specimens were isolated from individuals resident in the Argyll & Clyde region; 21 (0.8%) specimens were isolated from individuals resident in the Borders; 76 (2.7%) specimens were isolated from individuals resident in Dumfries & Galloway; 137 (4.9%) specimens were isolated from individuals resident in the Fife region; 119 (4.3%) specimens were isolated from individuals resident in the Forth Valley region; 711 (25.6%) specimens were isolated from individuals resident in the Greater Glasgow region, 221 (8.0%) specimens were isolated from

individuals resident in the Grampian region; 128 (4.6%) specimens were isolated from individuals resident in the Highland region; 325 (11.7%) specimens were isolated from individuals resident in the Lanarkshire region; 384 (13.9%) specimens were isolated from individuals resident in the Lothian region; one (<0.1%) specimen was isolated from an individual resident in NHS Orkney region; one (<0.1%) specimen was isolated from an individual resident in NHS Shetland region; 230 (8.3%) specimens were isolated from individuals resident in the Tayside region and seven (0.3%) specimens were isolated from individuals resident in the NHS Western Isles region.

In comparison with the carriage strain collection characterised during this study a larger proportion of isolates in the IMD strain collection was obtained from individuals from Ayrshire & Arran, Argyll & Clyde, Greater Glasgow, Grampian, Highland and Tayside NHS health board regions (Figure 3.3). In contrast, a larger proportion of isolates in the carriage strain collection was obtained from individuals resident in the Fife, Forth Valley and Lanarkshire NHS health board regions. A similar proportion of isolates in each strain collection was obtained from individuals resident in the Borders, Dumfries & Galloway and Lothian NHS health board regions.

3.5 Age of individuals sampled

The SHLMPRL meningococcal isolate database was examined for information on the age of the individual sampled. This was available for 459 (58%) specimens of the carriage strain collection. For convenience, specimens were grouped according to age (Figure 3.4). Carried meningococci were isolated from nine (1.1%) persons < 1 year, 14 (1.8%) persons aged 1-4 years, 16 (2.0%) persons aged 5-9 years, 13 (1.6%) persons aged 10-14 years, 181 (22.9%) persons aged 15-19 years, 118 (14.9%) persons aged 20-24 years, 58 (7.3%) persons aged 25-34 years, 33 (4.2%) persons aged 35-44 years, seven (0.9%) persons aged 45-54 years, six (0.8%) persons aged 55-64 years, three (0.4%) persons aged 65-74 years, zero persons aged 75-84 years and one (0.1%) person aged >85yrs. ‘Unknown’ was

recorded for 326 (41.2%) specimens and for six (0.8%) specimens the age-group field was blank.

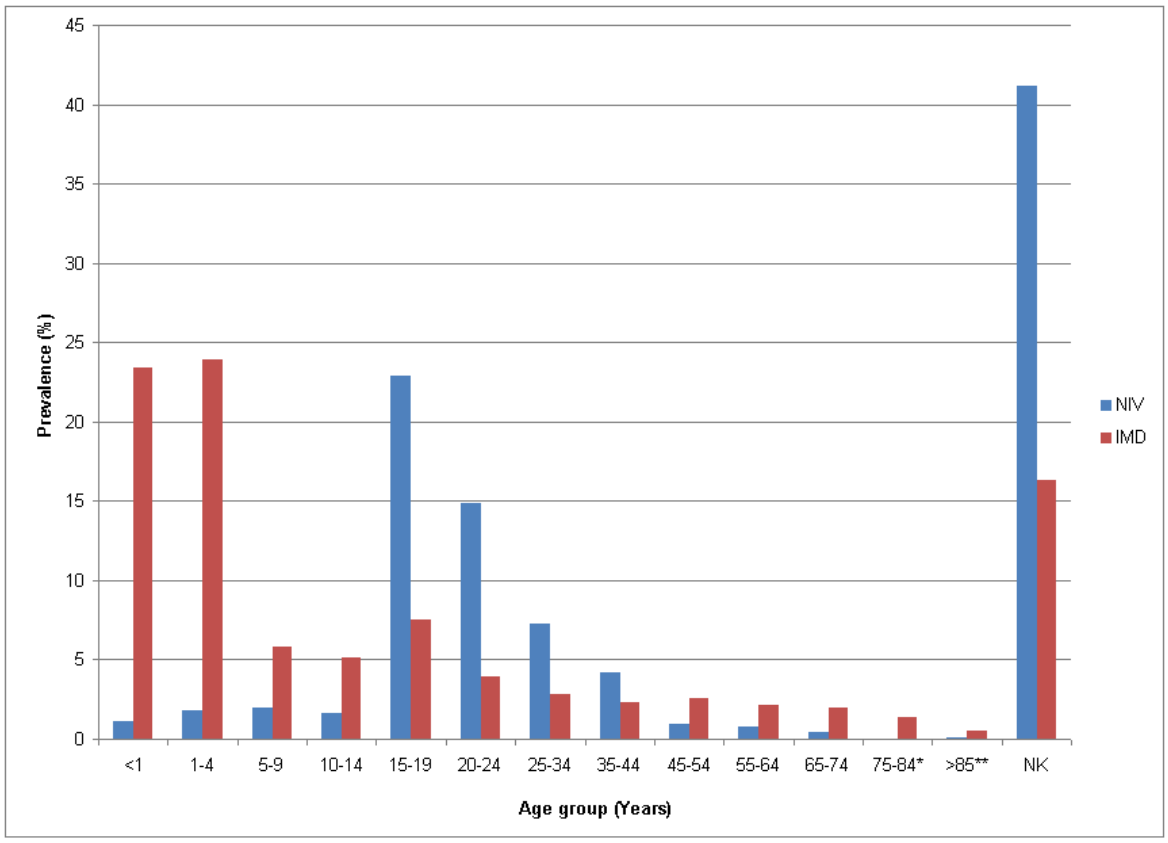

Figure 3.4 - Distribution by age group of the source individual of meningococci in Scotland, 1974 - 2004
NIV, non-invasive; IMD, invasive meningococcal disease; NK, not known; * zero isolates in the non-invasive strain collection were obtained from individuals age 75-84 years; ** one isolate in the non-invasive strain collection (equivalent to 0.1%) was obtained from an individual ≥ 85 years of age

For the IMD strain collection the SHLMPRL isolate database was also examined for information on the age of the individual sampled. This was available for 2,311/2,772 (83.4%) IMD isolates. For convenience, samples were grouped according to age (Figure 3.4). IMD isolates were obtained from 648 (23.4%) persons < 1 year, 662 (23.9%) persons aged 1-4 years, 162 (5.8%) persons aged 5-9 years, 142 (5.1%) persons aged 10-14 years, 207 (7.5%) persons aged 15-19 years, 107 (3.9%) persons aged 20-24 years, 77 (2.8%) persons aged 25-34 years, 65 (2.3%) persons aged 35-44 years, 73 (2.6%) persons aged 45-54 years, 58 (2.1%) persons aged 55-64 years, 55 (2.0%) persons aged 65-74 years, 40 (1.4%) persons aged 75-84 years and fifteen (0.5%) person aged >85yrs. 'Unknown' was recorded for 453 (16.3%) isolates and for a further eight (0.3%) isolates the age-

group field was blank. Nearly half of the IMD strain collection is composed of meningococci isolated from paediatric cases of IMD. Whereas, the majority of the carriage collection is composed of meningococci isolated from individuals in adolescence or early adulthood. It should be noted that for a considerable proportion of the strain collections, more so for the carriage strain collection, information on the age of the individual sampled was unavailable.

3.6 Sex of individuals sampled

For the carriage strain collection examination of the SHLMPRL database showed that information on the sex of the individual sampled was recorded for 303 (38.3%) specimens. 111 (14.0%) and 192 (24.2%) were isolated from females and males respectively. 'Unknown' was recorded for 482 (60.9%) specimens and six (0.8%) were blank. For the IMD strain collection examination of the SHLMPRL database showed that information on the sex of the individual sampled was recorded for 2,511/2,767 (90.7%) isolates. One thousand one hundred and fifty (41.6%) and 1,361 (49.2%) were isolated from females and males respectively. 'Unknown' was recorded for 253 (9.1%) isolates and for eight (0.3%) isolate the 'sex' field in the database was blank. In both strain collections a larger proportion of isolates was also obtained from males compared to females. However, for the majority of isolates (61.7%) in the carriage strain collection this information was not available.

3.7 Serogrouping data

Figure 3.5 shows the serogrouping results for the carried meningococci isolated in Scotland during the period 1974 - 2004. Isolates were characterised into eight distinct serogroups; A (11/791; 1.4%), B (269/791; 34.0%), C (152/791; 19.2%), Y (65/791; 8.2%), W135 (50/791; 6.3%), X (8/791; 1.0%), Z (36/791; 4.6%) and Z' (12/791; 1.5%). The remaining meningococcal isolates were recorded as non-groupable (188/791; 23.8%) in the SHLMPRL meningococcal isolate database.

Also included in Figure 3.5 are each of the eight ATCC/NCTC isolates (one each of serogroups A, B, C, W135, Y, X, Z and 29E).

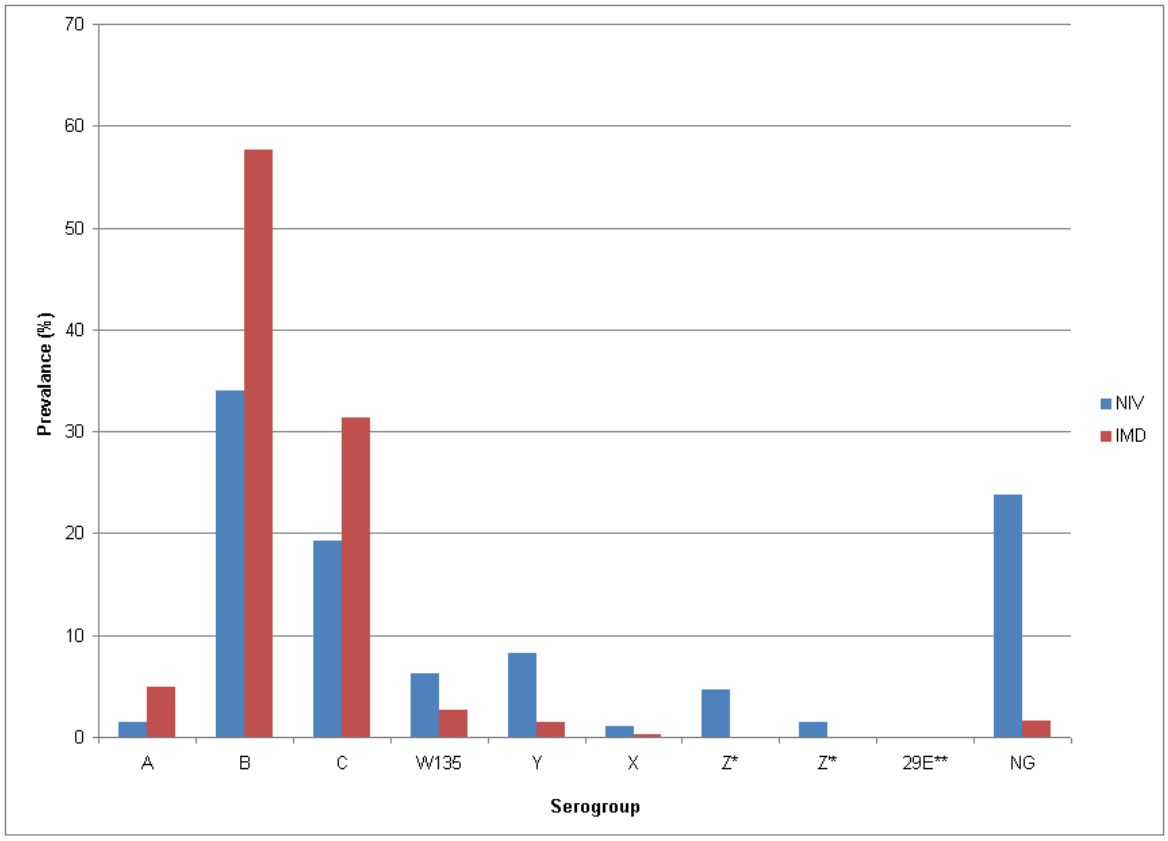

Figure 3.5 - Serogroup prevalence identified in meningococci in Scotland, 1974 – 2004

NIV, non-invasive; IMD, invasive meningococcal disease; NG, non-groupable; * one isolate (equivalent to <0.1%) in the IMD strain collection was of serogroup Z and one isolate (equivalent to <0.1%) was of serogroup Z'; ** one isolate (equivalent to <0.1%) in the IMD strain collection and zero isolates in the non-invasive strain collection were of serogroup 29E

For the IMD strain collection the SHLMPRL meningococcal isolate database was examined for information on the serogroup result recorded at the time each isolate was processed by the reference laboratory. Almost 90% of the IMD strain collection were of serogroup B (n=1597; 57.6%) or serogroup C (n=868; 31.3%) (Figure 3.5). Almost 5% were of serogroup A (n=135), with a further 2.6% (n=73) and 1.4% (n=39) of serogroup W135 and Y, respectively. Nine isolates were serogroup X, and there were one each of serogroups Z, Z' and 29E. For three isolates N/A was recorded in the serogroup field. Nongroupable (NG) was recorded for 45 (1.6%) of the invasive isolates; however genotypic serogrouping PCR had previously characterised 37 of these as B (n=26), C (n=9), A (n=1) or

W135 (n=1) while eight isolates were negative by PCR for serogroup A, B, C, W135 and Y (data not shown).

The most striking difference between the two strain collections is the greater proportion of nongroupable isolates in the carriage strain collection (Figure 3.5). The proportion of meningococci of serogroups W135, Y, X, Z and Z'(29E) is somewhat more pronounced in the carriage strain collection. Contrastingly, the proportion of meningococci of serogroups A, B and C is lower amongst the carriage strain collection. Meningococci of serogroups A, B and C were more likely to be isolated from cases of invasive disease in Scotland during the period 1974 – 2004, as indicated by the odds ratios in Table 3.1. In contrast, meningococci of serogroups W135, Y, Z and 29E were more likely to be found in the carriage strain collection; however, it should be noted that the large odds ratios determined for serogroups Z and 29E were less precise, as indicated by the wide 95%CIs (Table 3.1). Non-groupable meningococci were also more likely to be found in the carriage strain collection.

Table 3.1 – Association of meningococcal serogroups identified in Scotland, 1974 – 2004 with invasive disease or with a carriage phenotype

Invasive Serogroup	**n[1] (IMD[2])**	**n (NIV[3])**	**OR[4]**	**95% CI[5]**
A	135	11	3.64	1.96 to 6.76
B	1597	269	2.65	2.25 to 3.12
C	868	152	1.92	1.58 to 2.33
carriage Serogroup	**n (NIV)**	**n (IMD)**	**OR**	**95% CI**
W135	50	73	2.49	1.72 to 3.60
Y	65	39	6.26	4.18 to 9.39
X	8	9	3.13	1.20 to 8.14
Z	36	1	131.89	18.00 to 960.76
29E	12	2	21.30	4.76 to 95.36
NG	188	48	17.66	12.71 to 24.54

[1] n, number of isolates; [2] IMD, invasive meningococcal disease strain collection; [3] carriage, carriage strain collection; [4] OR, odds ratio; [5] CI, confidence interval

The serogrouping data was divided into three broadly equal time periods to investigate if any temporal changes had occurred in the prevalence of the individual serogroups (Table 3.2). Differences are apparent in the temporal distribution of prevalent serogroups in the carriage strain collection. Serogroup A was most prevalent during 1974 - 1983 (5.4%), decreasing to 0.9% during 1984 –

1993; no serogroup A isolates were found amongst the carriage strain collection beyond 1988. Serogroup B prevalence was similar during the first and third periods, 36.9% vs. 36.0% respectively, and was only slightly lower during 1984 – 1993 (32.2%). The prevalence of serogroup C was distinctly different across the three time periods; during 1974 – 1983 prevalence was 6.9%, however serogroup C accounted for just over 30% of isolates during 1984 – 1993 compared with only 1.9% of isolates during 1994 – 2004 (Table 3.2). The prevalence of serogroup Y increased overall, however it was highest during 1984 – 1993. The prevalence of serogroup X increased overall in contrast with serogroups Z and Z'(29E) which decreased overall. The prevalence of nongroupable isolates was high overall, as noted earlier. During 1974 – 1983 nongroupable isolates accounted for nearly 30% of the carriage strain collection; however these isolates were most prevalent during 1994 – 2004 (46.5%) compared with only 11.6% during 1984 – 1993 (Table 3.2).

Table 3.2 – Temporal analysis of serogroup prevalence amongst meningococci in Scotland, 1974 - 2004

Serogroup	carriage[1]			IMD[2]		
	1974-1983 No. (%)[3]	1984-1993 No. (%)[3]	1994-2004 No. (%)[3]	1974-1983 No. (%)[3]	1984-1993 No. (%)[3]	1994-2004 No. (%)[3]
A	7 (5.4)	4 (0.9)	-	123 (17.9)	9 (0.8)	2 (0.2)
B	48 (36.9)	145 (32.2)	76 (36.0)	414 (60.3)	603 (53.9)	577 (60.0)
C	9 (6.9)	139 (30.9)	4 (1.9)	62 (9.0)	483 (43.2)	332 (34.6)
W135	11 (8.5)	30 (6.7)	9 (4.3)	38 (5.5)	13 (1.2)	22 (2.3)
Y	4 (3.1)	45 (10.0)	16 (7.6)	12 (1.8)	8 (0.7)	19 (2.0)
X	1 (0.8)	1 (0.2)	6 (2.8)	-	2 (0.2)	7 (0.7)
Z	6 (4.6)	29 (6.4)	1 (0.5)	1 (0.2)	-	-
Z'	6 (4.6)	5 (1.1)	1 (0.5)	-	1 (0.1)	-
NG[4]	38 (29.2)	52 (11.6)	98 (46.5)	34 (5.0)	-	11 (1.1)
ND[5]	-	-	-	3 (0.4)	-	-
Total No.	130	450	211	687	1119	961

[1] carriage, carriage strain collection; [2] IMD, invasive meningococcal disease strain collection; [3], expressed as the percentage of isolates of a given serogroup within each time period; [4] ND, not determined; [5] NG, nongroupable

Temporal analysis of the serogrouping data for the IMD strain collection also indicates changes in the prevalence of different serogroups (Table 3.2). The prevalence of serogroup A decreased from 17.9% during 1974 – 1983 to 0.2% during 1994 – 2004; indeed no serogroup A isolates within the IMD strain collection were isolated after 1995. Serogroup B isolates accounted for just over 60% of the IMD strain collection during 1974 – 1983, decreasing to 54% during

1984 -1993 before increasing again to 60% during 1994 - 2004. The prevalence of serogroup C showed the most variation across the three periods; during 1974 – 1983 serogroup C isolates accounted for only 9.0%. The prevalence of serogroup C isolates rose dramatically peaking at 43.2% during 1984 – 1993, before falling during 1994 – 2004 to 34.5%.

Over the 31-year period the prevalence, in the IMD strain collection, of serogroup W135 declined; prevalence was highest during 1974 – 1983 (5.5%), decreasing during 1984 – 1993 (1.2%), before increasing slightly again during 1994 – 2004 to 2.3%. Similarly the prevalence of serogroup Y was lowest during 1984 – 1993 (0.7%) after decreasing compared with 1974 – 1983 (1.8), before recovering again to a similar level during 1994 – 2004 (2.0%). Only a handful of isolates were of any other serogroup. Somewhat surprisingly, a small number of isolates in the IMD strain collection were nongroupable.

The panel of tests available to the reference laboratory would have changed over the years in response to advances in technology (especially molecular biology) and with increased understanding of meningococcal biology. Moreover, carriage studies have shown that meningococci are often serologically non-groupable and that capsular polysaccharide is not transported and/or expressed by strains of meningococci. In order to further characterise non-groupable specimens, some of which were non-viable, different genogrouping PCR tests were used to detect serogroup-specific gene targets.

3.8 Genotypic capsular characterisation of phenotypically nongroupable meningococci

3.8.1 Serogroup-specific genotypic PCR

The encapsulated NCTC and ATCC meningococci (see Table 2.1) were used as positive controls for their respective serogroup-specific genotypic PCR and as negative controls in other serogroup-specific genotypic PCR tests. As a negative control, 18MΩ H_2O was used in each genotypic serogrouping PCR. Figure 3.6

shows the genotypic PCR results for the 188 phenotypically nongroupable carried meningococci.

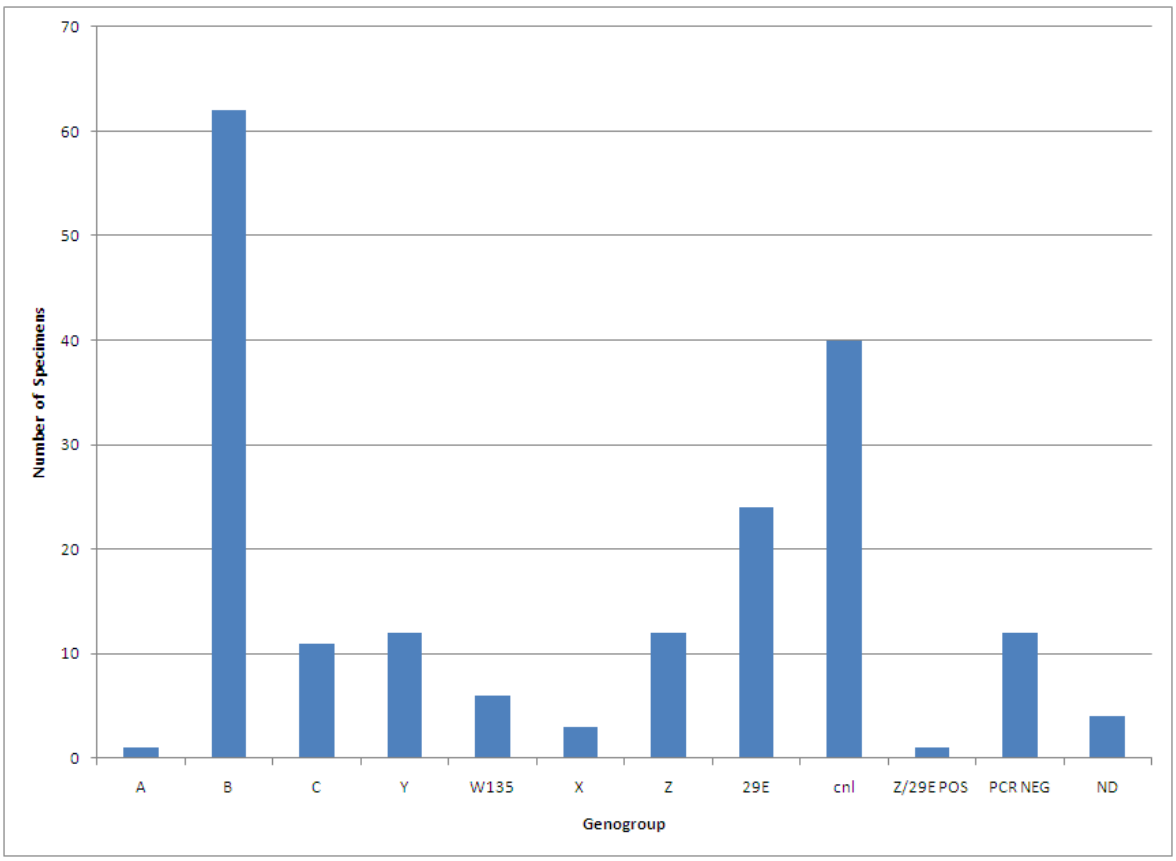

Figure 3.6 - Genogrouping of non-serogroupable carried meningococci in Scotland, 1974 – 2004

cnl, capsule null locus; Z/29E POS, one specimen tested positive for both PCR tests; PCR NEG, samples tested negative for all genogrouping PCR tests; ND, not determined

Serogroup-specific genotypic PCR further characterised phenotypically nongroupable isolates into genogroup A (1/188; 0.5%), B (62/188; 33.0%), C (11/188; 5.9%), Y (12/188; 6.4%), W135 (6/188; 3.2%), X (3/188; 1.6%), Z (12/188; 6.4%) and 29E (24/188; 12.8%). Four NG specimens were not tested fully using the panel of genogrouping PCRs as the specimens were no longer available (recorded as ND in Figure 3.6). Fifty-two (27.7%) meningococcal isolates were negative for all serogroup-specific genogrouping PCR tests and these were tested further for the presence of the *cnl* (see section 3.8.2 below).

Due to their relatively low prevalence it was decided to test carried meningococcal specimens recorded as serogroup X, serogroup Z and serogroup Z' using the *ctrA*-X, *ctrA*-Z and *ctrA*-29E PCRs to confirm the serogrouping results.

Eight of eight (100%) serogroup X isolates and the serogroup X NCTC 10790 tested positive for *ctrA*-X PCR, and negative for the *ctrA*-Z and *ctrA*-29E PCRs. Nine of nine (100%) serogroup Z' isolates and the serogroup 29E NCTC 11202 isolate tested positive for *ctrA*-29E PCR and negative for *ctrA*-X and *ctrA*-Z PCR. The serogroup Z NCTC 10792 isolate tested positive for the *ctrA*-Z PCR and negative for the *ctrA*-X and *ctrA*-29E PCRs. However, of 36 isolates recorded as serogroup Z nine (25%) isolates tested positive for *ctrA*-Z PCR and negative for *ctrA*-X and *ctrA*-29E PCRs. A further 26 (72.2%) serogroup Z isolates tested positive for *ctrA*-29E PCR and negative for the *ctrA*-X and *ctrA*-Z PCRs. Furthermore, a cross-reaction was observed for one (2.8%) isolate which tested positive for both the *ctrA*-Z and *ctrA*-29E PCRs and negative for the *ctrA*-X PCR.

3.8.2 Capsule null locus PCR

Often, carried meningococci lack the genes responsible for the synthesis and transport of capsule polysaccharide and instead posses the capsule null locus (Claus, Maiden *et al.* 2002). Forty (21.3%) nongroupable carried meningococcal specimens tested positive for the capsule null locus by PCR (Figure 3.6) while a further twelve (6.4%) nongroupable carried meningococcal specimens tested negative for the *cnl*. Of these twelve specimens, seven were viable and the organism had been stored on Protect™ beads following initial sub-culture. These seven viable isolates were re-tested phenotypically by agglutination for the presence of capsule and they did not react with the available agglutination reagents. All twelve carried meningococcal specimens were tested genotypically by using the *ctrA* PCR (see section 3.8.3 below) that is routinely used at SHLMPRL to detect meningococcal DNA in clinical specimens from patients with suspected meningococcal disease (Corless, Guiver *et al.* 2001).

3.8.3 ctrA PCR of phenotypically & genotypically NG specimens

The ATCC 13077 serogroup A, ATCC 13090 serogroup B, ATCC 13102 serogroup C, ATCC 35561 serogroup Y, NCTC 11203 serogroup W135, NCTC 10790 serogroup X,

NCTC 10792 serogroup Z and NCTC 11202 serogroup 29E meningococcal isolates were used as positive controls and a *cnl* isolate from the UK carriage study was used as a negative control. As expected, all the encapsulated ATCC and NCTC isolates tested positive for the non-serogroup-specific *ctrA* PCR. *cnl*-positive meningococci lack the capsule transport operon (and the capsule synthesis operon) and as expected this isolate tested negative for the *ctrA* PCR. One of five non-culture carried meningococcal specimens and five of seven viable carriage isolates tested negative for the *ctrA* PCR. Five out of six non-culture carried meningococcal specimens and two out of seven viable carriage isolates tested positive for the *ctrA* PCR (data not shown).

3.9 DNA sequencing of the capsule null locus (*cnl*)

Two different *cnl* alleles, *cnl-2* and a unique allele, were detected amongst *cnl* meningococci in this study. The new *cnl* allele sequence was submitted to GenBank; accession number EU872189, and named as *cnl* allele 8 (*cnl-8*). Figure 3.7 shows the alignment of the *cnl-8* allele with the available *cnl* sequences from GenBank.

```
CLUSTAL 2.0.8 multiple sequence alignment


cnl-1           AAATTGCCTCCGTGATGCCGTCTGAACAGCCGACGGCGCAATGTGGGTAATCGTTTGGAA 60
cnl-5           AAATTGCCTCCGTGATGCCGTCTGAACAGCCGACGGCGCAATGCGGGTAATCGTTTGGAA 60
cnl-2           AAATTGCCTCCGTGATGCCGTCTGAACAGCCGACGGCGCAATGTGGGTAATCGTTTGGAA 60
cnl-4           AAATTGCCTCCGT-ATGCCGTCTGAACAGCCGACGGCGCAATGTGGGTAATCGTTTGGAA 59
cnl-8           AAATTGCCTCCGTGATGCCGTCTGAACAGCCGACGGCGCAATGTGGGTAATCGTTTGGAA 60
cnl-6           AAATTGCCTCCGTGATGCCGTCTGAACAGCCGACGGCGCAATGTGGGTAATCGTTTGGAA 60
cnl-3           AAATTGCCTTTTT-ATGCCGCTCGGACAGCCGGCGGCGCAATGTGGGTAATCGTTTGGAA 59
cnl-7           AAATTGCCTTTTT-ATGCCGCTCGGACAGCCGACGGCGCAATGTGGGTAATCGTTTGGAA 59
                *********   * ******   * ******* ********** ****************

cnl-1           AAGATGAAGATAATACTGATATACTTTGCACGATACAATCTGAAAGGAATGTTT---- 114
cnl-5           AAGATGAAGATAATACTGATATACTTTGCCCGATACAATCTGAAAGGAATGTTT---- 114
cnl-2           AAGATGAAGATAATACTGATATACTTTGCCCGATACAATCTGAAAGGAATGTTT---- 114
cnl-4           AAGATGAAGATAATACTGATATACTTTGCCAGATACAATCTGAAAGGAATGTTT---- 113
cnl-8           AAGATGAAGATAATACTGATATACTTTGCCAGATACAATCTGAAAGGAATGTTT---- 114
cnl-6           AAGATGAAGATAATACTGATATACTTTGCCCGATACAATCTGAAAGGAATGTTTGTTT 118
cnl-3           AAGATGAAGATAATACTGATATACTTTGCCCGATACAATCTGAAAGGAATGTTT---- 113
cnl-7           AAGATGAAGATAATACTGATATACTTTGCCCGATACAATCTGAAAGGAATGTTT---- 113
                ****************************  ***********************
```

Figure 3.7 - DNA sequence alignment of *cnl* alleles

An * underneath the alignment denotes consensus sequence; a space underneath the alignment denotes a polymorphic site; - within the aligned sequences denotes a nucleotide deletion

Capsule null locus allele 8 is different from *cnl-1* at positions 90 and 91; in allele 1 position 90 and position 91 are occupied by an adenine and a cytosine residue respectively, whereas in *cnl-8* there is an inversion of the same two nucleotides at these positions. Furthermore *cnl-8* is only one nucleotide different from *cnl-4*; *cnl-4* has a guanidine deletion at position 14. Table 3.3 shows the *cnl* PCR-positive isolates and the *cnl* allele sequenced.

Table 3.3 – Capsule null locus-containing meningococci identified in Scotland, 1974 – 2004

Year	cc[1]	ST[2]	PorA Subtype [3]VR 1, 2, 3	*cnl* allele	No. of isolates
1975	ND[4]	ND	ND	cnl-2	1
1978	*N. lactamica*	*N. lactamica*	*N. lactamica*	cnl-7	1
1984	cc198	ST-198	18, 25-25, 38-1	cnl-2	1
	cc53	ST-53	7, 30, 38	cnl-8	1
1986	cc198	ST-198	18, 25, 38-1	cnl-2	1
1987	cc198	ST-198	18, 25-1, 38-1	cnl-2	1
	cc198	ST-198	18, 25-7, 38-1	cnl-2	1
1988	cc198	ST-7782	7-2, 13-5, 35-1	cnl-2	1
1996	cc53	ST-53	7, 30-3, 38	cnl-8	1
	cc53	ST-53	7, 30, 38	cnl-8	1
1997	cc53	ST-53	7, 30, 38	cnl-8	1
	cc53	ST-53	7, 30-2, 38	cnl-8	1
	cc53	ST-53	7, 30-3, 38	cnl-8	1
1998	cc53	ST-53	7, 30, 38	cnl-8	1
1999	cc53	ST-53	7, 30, 37-1	cnl-8	1
	cc53	ST-5950	7, 30, 38	cnl-8	1
2000	cc53	ST-53	7, 30-1, 38	cnl-8	1
	cc53	ST-53	7, 30-2, 38	cnl-8	1
	cc53	ST-53	7, 30-3, 38	cnl-8	1
	cc1136	ST-8635	18-4, 25, 38-1	cnl-2	1
2001	cc53	ST-53	7, 30, 38	cnl-8	4
	cc53	ST-53	7, 30-3, 38	cnl-8	3
	cc1117	ST-1117	18-1, 30-3, 38	cnl-8	1
2002	cc53	ST-53	7, 30, 38	cnl-8	3
	cc53	ST-53	7-2, 30, 38	cnl-8	1
	cc53	ST-53	7, 30-1, 38	cnl-8	1
	cc53	ST-53	7, 30-2, 38	cnl-8	1
	cc53	ST-53	7, 30-3, 38	cnl-8	1
	cc198	ST-198	18, 25-1, 38-1	cnl-2	1
	cc198	ST-823	7-2, 13-15, 35-1	cnl-2	1
2003	cc53	ST-53	7, 30, 38	cnl-8	2

[1] cc, MLST clonal complex; [2] ST, Sequence Type; [3] VR, variable region; [4] ND, not determined

One *N. lactamica* isolate from 1978 was also included for *cnl* sequencing as *N. lactamica* is known to possess the *cnl*. With the exception of one specimen all remaining carried meningococcal specimens in this study were characterised by MLST and *porA* VR sequencing. Seven different STs were identified; ST-53 (30

isolates), ST-198 (5 isolates), and a single isolate of the STs 823, 1117, 5950, 7782 and a new ST. These seven STs belonged to four distinct clonal complexes; cc53 (ST-53 and the Single-Locus Variant; SLV ST-5950), cc198 (ST-198 the SLV ST-823 and Double-Locus Variant; DLV ST-7782), cc1117 (ST-1117) and cc1136 (ST-8635; with a new allele at the *pgm* locus is a SLV of ST-1136). Two new STs were identified in this collection of *cnl*-containing meningococci; ST-7782 which had a new *aroE* allele, *aroE-525*, and ST-8635 which had a new *pgm* allele, *pgm-580*.

The *cnl-2* allele was identified in all cc198 isolates and in the new ST (cc1136). The *cnl-7* allele was identified in a *N. lactamica* isolate from 1978 and has previously been described only in *N. lactamica* (Claus, Maiden *et al.* 2002). MLST was not performed on the *N. lactamica* isolate. The new *cnl* allele was identified in all twenty-eight cc53 isolates and in the single ST-1117 isolate and in each of these twenty-nine strains the new allele was identical.

Four reports from three different continents were identified in a review of the literature for evidence of other lineages of capsule null locus-containing meningococci (Table 3.4). In meningococci isolated from carriage or from cases of invasive disease, researchers in Europe, North America and Africa identified a total of twenty-eight STs, accounting for seven distinct lineages of meningococci that have so far been found to contain the *cnl* instead of the genes responsible for polysaccharide capsule synthesis and transport (Claus, Maiden *et al.* 2002; Vogel, Claus *et al.* 2004b; Hoang, Thomas *et al.* 2005; Findlow, Vogel *et al.* 2007). Four of these lineages were identified amongst the carriage isolate collection in Scotland; cc53, cc198, cc1117 and cc1136 (Table 3.4).

Table 3.4 – Comparison of capsule null locus-positive meningococci in Scotland with the published literature

cc[1] (*cnl* allele)	ST	n C[2]	n D[3]	MLST loci							Reference
				abcZ	*adk*	*aroE*	*fumC*	*gdh*	*pdhC*	*pgm*	
53 (*cnl-1*)	53	51	-	16	2	6	25	17	25	22	[a]
	122	2	-	16	2	6	25	8	25	22	[a]
	931	1	-	16	2	6	13	17	25	22	[a]
	932	1	-	16	2	6	25	17	9	22	[a]
	1115	1	-	16	2	6	13	17	25	20	[a]
	1118	1	-	16	2	6	2	17	25	22	[a]
53 (*cnl-8*)	53	24	-	16	2	6	25	17	25	22	[b]
	5950	1	-	16	2	346	25	17	25	22	[b]
1117 (*cnl-1*)	1117	14	-	16	2	159	92	77	25	112	[a]
	1114	2	-	16	2	159	39	77	25	22	[a]
	1116	1	-	16	2	12	92	77	25	112	[a]
	1119	1	-	16	2	159	92	77	25	2	[a]
1117 (*cnl-8*)	1117	1	-	16	2	159	92	77	25	112	[b]
198 (*cnl-2*)	198	26	-	5	4	17	15	14	7	12	[a]
	198	5	-	5	4	17	15	14	7	12	[b]
	39	4	-	5	4	17	15	14	7	16	[a]
	822	3	-	5	4	17	15	14	125	12	[a]
	823	7	-	5	4	17	15	30	7	12	[a]
	823	1	-	5	4	17	15	30	7	12	[b]
	7782	1	-	5	4	525	15	30	7	12	[b]
	824	1	-	5	4	17	15	30	114	12	[a]
	827	1	-	5	79	17	15	14	133	12	[a]
	968	1	-	103	4	17	15	14	7	12	[a]
	1107	1	-	114	4	17	15	14	7	140	[a]
198 (ND)	198	-	1	5	4	17	15	14	7	12	[c]
1136 (*cnl-2*)	1136	3	-	5	4	38	15	22	40	13	[a]
	8635	1	-	5	4	38	15	22	40	580	[b]
	825	1	-	5	4	38	15	8	40	13	[a]
845 (*cnl-2*)	845	7	-	6	41	108	15	9	6	9	[a]
	845	-	1	6	41	108	15	9	6	9	[d]
	847	3	-	6	41	108	118	9	6	9	[a]
	848	1	-	6	41	108	136	9	6	9	[a]
	1138	1	-	6	41	108	15	3	6	9	[a]
44 (*cnl-3*)	43	1	-	12	6	9	9	9	6	9	[a]
UA (*cnl-3*)	192	14	3	35	24	15	48	6	48	41	[e]

[1] cc, MLST clonal complex; [2] number of carriage isolates; [3] number of disease isolates; ND, not determined; UA, unassigned to a clonal complex; [a] (Claus, Maiden *et al.* 2002); [b] This study, highlighted; [c] (Hoang, Thomas *et al.* 2005); [d] (Vogel, Claus *et al.* 2004b); [e] (Findlow, Vogel *et al.* 2007)

3.10 Discussion

The carriage strain collection was assembled from the meningococcal isolate archive maintained by the SHLMPRL. During the study it became apparent that the SHLMPRL isolate database could not be fully cross-matched to the isolate archive as a listing on the database did not guarantee that a specimen or

specimens could be located in the isolate archive. Many meningococcal isolates recorded in the database were absent from the strain collection; presumably they may either have been expended or were never archived at the time the samples were originally processed. Somewhat disappointingly, a considerable number of the missing specimens were found to be NG isolates. Perhaps the isolates were not deemed worthy of further study at the time they were isolated or, due to some constraint, financial or otherwise, the isolates were not retained in the reference laboratory isolate archive. This is highlighted by the difference in prevalence of NG isolates in the temporal distribution of the strain collection.

As a result, some bias may exist through the carriage strain collection. Further bias may have resulted from the exclusion of specimens deemed to be of poorer quality. These poorer quality specimens appeared to be more common during the early years of the 1980s and the early years of the 1990s (perhaps due to quality issues during their preparation). Epidemiological data indicate that isolates of the carriage strain collection were predominantly from males compared to females, of teenage or young adult age, resident in the central belt of Scotland; however the information regarding the sex (60.9%) and age group (42%) of the source individuals was often not known.

Meningococci can currently be differentiated into twelve distinct serogroups based upon differences in polysaccharide capsule structure. A large proportion of carried meningococci in Scotland were found not to react with the available serogrouping antisera and the strains were designated as nongroupable. Carried meningococci are often found not to react with available antisera (Cartwright, Stuart *et al.* 1987; Maiden and Stuart 2002; Caugant, Fogg *et al.* 2006; Climent, Yero *et al.* 2010a). Moreover, a subset of these nongroupable strains in Scotland were found to possess the *cnl*, as previously reported elsewhere in carried meningococci (Claus, Maiden *et al.* 2002; Vogel, Claus *et al.* 2004a; Findlow, Vogel *et al.* 2007) and, in rare cases, from invasive meningococci (Vogel, Claus *et al.* 2004b; Hoang, Thomas *et al.* 2005; Findlow, Vogel *et al.* 2007).

Previously, studies have indicated that meningococci of serogroup C are not isolated in high numbers in carriage studies (Cartwright, Stuart *et al.* 1987; Jolley, Kalmusova *et al.* 2000; Yazdankhah, Kriz *et al.* 2004; Claus, Maiden *et al.* 2005); for example serogroup C carriage was found to be 2.7% during the UK

carriage study (Maiden and Stuart 2002), 3.3% in the Stonehouse survey (Cartwright, Stuart *et al.* 1987) and 4.8% in a study of carried meningococci isolated in the Czech Republic, Norway and Greece (Yazdankhah, Kriz *et al.* 2004). However, in a separate study of carried meningococci from the Czech Republic serogroup C carriage was initially observed as 7.7% (associated with cc11 meningococci) (Jolley, Kalmusova *et al.* 2000) although this was later found to be much higher (11.1% in 15 to 19 year olds and 15.8% in 20 to 24 year olds for cc11 meningococci) (Jolley, Kalmusova *et al.* 2002). Our observation that 19.2% of carried meningococci in this study were serogroup C, associated mostly with cc8, whilst initially appearing to be somewhat unexpected, was similar to the amended report by Jolley and colleagues (Jolley *et al*., 2002). Furthermore, a report by Thomson & Jackson in 1987 described a period during 1984 – 1986 where IMD cases increased to unusually high numbers; particularly characterised by a clustering of 40 cases over the winter of 1984/1985 (Thomson and Jackson 1987). The report only gives details of the 7 cases where disease resulted in fatality, three of which were due to serogroup C.

More significantly, Thomson and Jackson (1987) noted that routine swabbing of contacts revealed many asymptomatic carriers of *N. meningitidis*. It can only be assumed that these disease isolates and those from asymptomatic carriers were forwarded to the reference laboratory for analysis. Consequently, some of these carrier isolates may well be represented in the carriage isolate collection in this study; based on the observation that many carried isolates were obtained from 1985 (Figure 3.1). In addition, 34.5% of the carriage isolate collection was obtained from individuals from Lanarkshire. The account by Mathew & Chaudhuri of the same period of increased disease adds further weight to these observations (Mathew and Chaudhuri 1986). The authors noted that of the twenty isolates in the outbreak, for which serogroups were available, fifteen were of serogroup C and five were of serogroup B. Strains of serogroup B predominated during October to December 1984, while cases from January 1985 onwards were almost exclusively of serogroup C (Mathew and Chaudhuri 1986). When considered together, the reports by Mathew & Chaudhuri (1986), Thomson & Jackson (1987) and the data presented here regarding carriage and disease due to serogroup C meningococci, particularly associated with cc8, may indicate

the resurgence of this clonal complex following a period of transmission throughout central Scotland in particular.

Five of the currently recognised serogroups (A, B, C, W135 and Y) are responsible for the majority of invasive disease cases globally (Rosenstein, Perkins *et al.* 2001; Harrison, Trotter *et al.* 2009). Contrastingly, several of the remaining serogroups are rare and, as such, are seldom reported in the literature; consequently the genetics of those rare serogroups has not been described in great detail. The original serogrouping results recorded when isolates were originally processed was used to define meningococci phenotypically. From the SHLMPRL records it was not possible to ascertain the origins or availability of the agglutination reagents used when the isolates were first processed by the reference laboratory, nor is it evident how laboratory methods may have changed during the period 1974 - 2004. This may have a bearing on the characterisation of the isolates into known serogroups. The currently-available agglutination reagents may not be the same as those available at the time the isolates were originally processed and could potentially yield different results had all the isolates been re-tested during this study. A result of NG recorded against any isolate of the strain collection is indicative of that particular isolate not reacting with the particular reagents in use at the time the test was performed. The use of PCR tests to determine the presence of different serogroup-specific genes was used to further characterise the NG isolates by group genotypically.

The rarity of serogroup X, Z and Z'(29E) amongst invasive disease isolates prompted further characterisation of isolates of these serogroups amongst the carriage strain collection. The published *ctrA* PCR (Bennett, Mulhall *et al.* 2004) was used to test isolates recorded as serogroup X, serogroup Z or serogroup Z'. The *ctrA*-X PCR results were concordant with the recorded phenotypic serogrouping result for isolates of serogroup X and the serogroup X NCTC 10790 isolate. Serogroup Z' is equivalent to serogroup 29E in current nomenclature; as expected the isolates recorded as serogroup Z' tested positive for the *ctrA*-29E PCR, as did the serogroup 29E NCTC 11202 isolate. The results of the *ctrA*-Z PCR, however, were not in concordance with agglutination results determining 36 isolates as serogroup Z. This may be indicative of a deficiency in the specificity of the *ctrA*-Z PCR; however only one cross-reaction was observed and the three

NCTC isolates tested positive only for their respective serogroup-specific *ctrA* PCR. More likely is the possibility that these results indicate a deficiency in the specificity of the agglutination reagents used at the time the isolates were originally processed; specifically in the miss-characterisation of 26 isolates as serogroup Z. These isolates were not re-tested using the agglutination reagents available during this study. MLST serves to further clarify the characterisation of these isolates. Interestingly, five out of nine serogroup Z'-*ctrA*-Z PCR-positive isolates were of ST-103, while 3 were unassigned to a defined clonal complex (see Chapter 6). The isolate that cross-reacted and tested positive for both *ctrA*-Z and *ctrA*-29E PCR was also ST-103. However, none of the serogroup Z-*ctrA*-29E PCR-positive isolates was of ST-103. These isolates were of different lineages; cc60 (n=7), cc178 (n=5), cc212 (n=1), cc254 (n=12). One isolate was unassigned to a clonal complex. Furthermore, serogroup Z'-*ctrA*-29E isolates were also found to belong to cc60 (n=6) and cc254 (n=5); with one isolate belonging to cc35.

Further characterisation of twelve phenotypically NG meningococci could not be achieved using the serogroup-specific PCR tests utilised during this study. The serogroup non-specific *ctrA* PCR used by the SHLMPRL did confirm that six of twelve phenotypically NG meningococci did possess one gene involved in capsule transport, thus supporting the possibility that these meningococci may produce an alternative capsular polysaccharide. The synthesis of capsular polysaccharide by these *ctrA*-positive meningococci may be achieved using genes encoding for one of the less prevalent serogroups or they may produce an unidentified capsular polysaccharide from a novel gene, or genes. However, as identified by Dolan-Livengood and colleagues, it is possible that strains of meningococci can posses the *ctrABCD* operon whilst lacking the *siaABCD* operon (Dolan-Livengood, Miller *et al.* 2003).

Capsule genetics of meningococci of the most common disease-associated serogroups have been studied in more detail (Frosch, Weisgerber *et al.* 1989; Frosch, Muller *et al.* 1992; Hammerschmidt, Hilse *et al.* 1996; Hammerschmidt, Muller *et al.* 1996; Swartley, Ahn *et al.* 1996; Claus, Vogel *et al.* 1997; Swartley, Marfin *et al.* 1997; Swartley, Liu *et al.* 1998; Tzeng, Swartley *et al.* 2001; Von Loewenich, Wintermeyer *et al.* 2001; Claus, Maiden *et al.* 2002; Dolan-Livengood, Miller *et al.* 2003; Sadler, Fox *et al.* 2003; Tzeng, Noble *et al.* 2003;

Tzeng, Datta *et al.* 2005; Weber, Claus *et al.* 2006). However, rarer serogroups are less well studied. In the early 1980s, three antigenically distinct serogroups, H, I and K, were described. This was followed soon after by the characterisation of their respective capsular polysaccharides (Ashton, Ryan *et al.* 1983; Jennings, Lugowski *et al.* 1983; van der Kaaden, van Doorn-van Wakeren *et al.* 1984; van der Kaaden, Gerwig *et al.* 1985). Since then, however, formal reports in the literature of meningococci of these serogroups have been limited. Neither have there been any reports describing new serogroups. One study investigating *ctrA* did however indicate partial homology between *ctrA* in serogroups X and L (Tzeng, Noble *et al.* 2003), while a high degree of homology was observed in the *ctrA* gene between isolates of serogroup H and isolates with sialic acid-containing capsules (Sadler, Fox *et al.* 2003).

There are several possibilities why meningococci in this study tested negative for the specific genotypic serogrouping PCRs and the serogroup non-specific *ctrA* PCR. Firstly there may be genetic polymorphisms at the primer-binding sites in these specimens that do not allow the primers to anneal adequately. However this seems less likely as most genetic polymorphism within the *ctrA* sequence is located at the 5' end of the gene (Frosch, Muller *et al.* 1992; Sadler, Fox *et al.* 2003). The primers used in the *ctrA* PCR amplify a small fragment of 110bp from positions 617 to 727 (Corless, Guiver *et al.* 2001) and thus anneal at positions internal to the beginning and end of the coding sequence which are downstream from the most variable region of sequence and in a region determined to have 100% homology between the different serogroups (Sadler, Fox *et al.* 2003). The lack of amplified product in the *ctrA* PCR for some samples suggests the *ctrA* gene is absent in these specimens. Conflictingly, however, the *cnl* could not be amplified by PCR in these specimens either. Therefore it would seem plausible that these strains may contain genetic material encoding for alternative capsule transport and synthesis operons.

In serogroup B meningococci, capsule expression of the *ctrABCD* and *siaABCD* operons is initiated by divergent promoters in a common 134bp intergenic region (Swartley, Ahn *et al.* 1996). It is not known whether the *ctrA* gene is being transcribed in the NG genotypic PCR-positive meningococci in this study. Sequencing of the *ctrA* promoter region may reveal whether this gene was being transcribed. A similar strategy has been used successfully to investigate the

promoter region of other genes in the meningococcal genome, such as *porA* (van der Ende, Hopman *et al.* 2000; Gorla, Lemos *et al.* 2003). Additionally, phenotypic analysis using a *ctrA*-specific monoclonal antibody followed by ELISA and OMP analysis using SDS-PAGE could be undertaken in parallel to establish if CtrA is being expressed by the NG genotypic PCR-positive meningococci in this study.

The methods employed by Sadler and colleagues (Sadler, Fox *et al.* 2003) could be applied to these undefined specimens. In their study the *siaA* and *ctrA* genes were used to complement serogroup-specific PCR (for B, C, Y and W135). A *ctrA* PCR strategy was used to specifically distinguish non-sialic acid-containing serogroups (A, H, X, Z, 29E) from the sialic-acid containing serogroups (B or C or Y or W135). This identified 12/89 specimens that were *ctrA* positive which could not be characterised further using the specific *ctrA* PCRs. Furthermore, 28/89 specimens were negative for *ctrA* PCR, possibly due to the replacement of the *ctrABCD* and *siaABCD* operons with the capsule null locus (Claus, Maiden *et al.* 2002); although this was not determined by Sadler and colleagues (Sadler, Fox *et al.* 2003).

Complete characterisation of the genes of the cps complex may reveal the underlying mechanisms responsible for loss of encapsulation in NG meningococci from Scotland. Weber and colleagues undertook PCR amplification and sequence analysis of the regions A, B and C of the cps gene cluster to identify the mechanism(s) responsible for inactivation of capsule expression (Weber, Claus *et al.* 2006). Insertion sequences (IS*1016*-like, IS*1106* & IS*1301*), length variation in homopolymeric tracts (in *siaA* or *siaD*) or other irreversible mutations (insertions, deletions & base exchanges) were identified that could influence encapsulation in carried meningococci. Furthermore, irreversible inactivation events were proposed to be the result of short-term, within-host evolution and no association between genotype and the mechanism for loss of encapsulation was observed (Weber, Claus *et al.* 2006).

The possibility that the genogrouping PCR-negative specimens were not meningococci, or not even *Neisseria spp.*, can be ruled out as MLST (Chapter 4) and *porA* VR sequencing (Chapter 5) was successfully performed. The quality of the specimen DNA was also thought not to have affected the genotypic PCR

results since MLST and *porA* VR sequencing was successfully performed. This was further supported by the observation that genotypic serogrouping PCR-negative results were not restricted to non-viable meningococci as different extracted specimens of viable and non-viable meningococci failed to yield any positive result.

We have shown that in Scotland *cnl*-containing carried meningococci belong to four different lineages. As highlighted in the published literature meningococci of other lineages can also contain the *cnl*. Furthermore, the Neisseria MLST website (http://pubmlst.org/neisseria) contains many more examples of NG meningococci of different STs assigned to these *cnl*-containing lineages that could potentially contain the *cnl* rather than the genes for capsule synthesis and transport. Previous studies on *cnl*-positive meningococcal isolates have highlighted that some *cnl* alleles appear to be restricted to particular lineages or *Neisseria* species (*N. gonorrhoeae* or *N. lactamica*); although the number of strains was small. The work of Claus and colleagues (Claus, Maiden *et al.* 2002) revealed the presence of three *cnl* alleles; *cnl-1* (cc53 and cc1117 isolates), *cnl-2* (ST-198, cc1136 and cc845 isolates), and *cnl-3* (cc44 isolates) in carried meningococci. While strains of *N. lactamica* possessed *cnl-2*, *cnl-3*, *cnl-4* or *cnl-5* and strains of *N. gonorrhoeae* possessed either *cnl-2* or *cnl-6*. Frosch and colleagues also reported that the *ctrA* gene was absent from other acapsulate *Neisseria* species including *N. flava*, *N. perflava*, *N. subflava*, *N. mucosa*, *N. sicca* and *N. cinerea* (Frosch, Muller *et al.* 1992); so it seems plausible that other *Neisseria spp.* may also possess the *cnl*.

Sequencing of the *cnl* from carried strains isolated in Scotland has revealed both differences and similarities with the Bavarian *cnl* isolates (Claus, Maiden *et al.* 2002). Scottish *cnl* strains of cc198 and cc1136 posses an identical allele (*cnl-2*) to that of Bavarian strains of the same clonal complexes. However, Scottish *cnl* strains of cc53 and cc1117 instead possess a unique allele (*cnl-8*) compared to that of Bavarian strains of the same clonal complex (*cnl-1*) (Figure 3.6 & Table 3.4). *cnl-8* is also similar to *cnl-4* which has previously only been documented in *Neisseria lactamica* (Claus, Maiden *et al.* 2002). This unique allele may have evolved in these lineages from a previously identified *cnl* allele, i.e. *cnl-1* or *cnl-4*, or this allele was acquired from an independent meningococcal ancestor or from another *Neisseria* species and has subsequently been successfully

maintained within these lineages in Scotland. The question remains as to the significance of these results since the *cnl* is a region of non-coding DNA located between *tex* and *galE* in *Neisseria* spp. (Claus, Maiden *et al.* 2002; Dolan-Livengood, Miller *et al.* 2003).

It is widely accepted that the meningococcus requires a capsule to confer pathogenicity and to protect itself from opsonophagocytosis. Mutations in the capsule genes result in the abolishment of capsule production and impair bacterial survival in normal human serum (Kahler, Martin *et al.* 1998; Tzeng, Noble *et al.* 2003). Furthermore, acapsulate meningococci were believed to be incapable of causing invasive disease. However, our understanding has been challenged following reports of *cnl*-positive meningococci causing invasive disease in apparently immunocompetent as well as immunocompromised individuals. A *cnl*-positive isolate causing disease in an immunocompromised individual was shown to be sensitive in a serum killing assay (Vogel, Claus *et al.* 2004b). More noteworthy was the description of *cnl*-positive isolates causing invasive disease in immunocompetent individuals (Hoang, Thomas *et al.* 2005; Findlow, Vogel *et al.* 2007). However, Hoang and colleagues did not test their isolate in a serum killing assay and Findlow and colleagues were unable to establish, retrospectively, whether the individuals with disease had any immunodeficiency. Findlow and colleagues were, however, able to show that two *cnl*-positive isolates were resistant in a serum killing assay and the levels were comparable to the fully capsulated serogroup B strain, MC58. The authors also state that the isolates may be virulent in immunocompetent individuals due to an alternative mechanism, to encapsulation, that allows them to survive in the bloodstream.

The emergence of disease-causing *cnl*-positive meningococci could therefore impact greatly upon the ability of microbiology laboratories to accurately detect the presence of *N. meningitidis* in clinical specimens taken from individuals with suspected meningococcal disease. In the SHLMPRL, in such instances, clinical specimens are routinely tested by PCR for the presence of meningococcal DNA. The target of choice in our laboratory, and in others, is the *ctrA* gene (Taha, Alonso *et al.* 2005); the first of four genes in the *ctrABCD* operon involved in transport of capsule to the bacterial outer membrane and one of the genes that is absent in *cnl* meningococci (Claus, Maiden *et al.* 2002). The specificity of any

diagnostic test is fundamental to its utility; this would, however, be compromised as these isolates would give false negative results using the *ctrA* PCR.

Moreover, currently licensed vaccines against meningococci target the capsular polysaccharide of serogroups A, C, W135 or Y in a single or multi combination formulation (either non-conjugated or conjugated). Again these vaccines would be ineffective in protecting against acapsulate strains. Fortunately, reports of *cnl* meningococci causing invasive disease are rare and none have been documented in Scotland[2]. Nevertheless, continued surveillance and improved diagnostic techniques are required to monitor these *cnl* lineages. Recently, Dolan and co-workers presented work on the evaluation of an alternative target for real-time PCR detection of *N. meningitidis* regardless of capsular status. Validation of the assay was performed on isolates of *N. meningitidis* and non-*N. meningitidis* organisms. The *sodC* gene was demonstrated to be highly sensitive and specific for identification and detection of the meningococcus in clinical samples (Dolan 2008; Dolan, Hatcher *et al.* 2009) and in a carriage study (Dolan, J., personal communication) and could be readily introduced into the repertoire of molecular tests utilised by the reference laboratory in Scotland.

The carriage strain collection characterised during this study was compared with data on IMD isolates from Scotland obtained during the period 1974 - 2004. While differences are apparent in the relative distribution of isolates within different years and in different geographic areas, the two strain collections were adequately matched as they cover the same 31-year period and all isolates originated in Scotland. Differences in the age distribution are also apparent from the comparison above. This observation is not entirely unexpected; for more than one reason. Firstly, disease incidence is known to vary with age; it is highest in children, particularly those under 2 years of age and can be correlated with waning maternal antibody levels during the first year of life. An inverse relationship between anti-meningococcal antibody titres or the bactericidal activity of serum and the age-related incidence of meningococcal disease has been observed (Pollard and Frasch 2001). The relationship between disease

[2] Inspection of the SHLMPRL meningococcal isolate database revealed that *cnl*-meningococci have been cultured from eye swabs from individuals with infection of the conjunctiva.

incidence and age is reflected in this study by the observation that nearly 50% of the IMD strain collection has been isolated from individuals less than 5 years of age.

Secondly, other studies have shown that carriage rates also vary with age; differently to the age prevalence of IMD isolates. Carriage studies have shown that the meningococcus is rarely carried in young children, particularly in the first one or two years of life (Cartwright, Stuart *et al.* 1987; Caugant, Kristiansen *et al.* 1988; Caugant, Hoiby *et al.* 1994; Bakir, Yagci *et al.* 2001), when carriage of *N. lactamica* is more prevalent (Cartwright, Stuart *et al.* 1987; Bakir, Yagci *et al.* 2001; Bennett, Griffiths *et al.* 2005). Carriage rates of *N. meningitidis* are highest in teenagers and young adults (Cartwright, Stuart *et al.* 1987; Caugant, Kristiansen *et al.* 1988; Caugant, Hoiby *et al.* 1994). While no age data was available for around 40% of the carriage strain collection in this study, most of the remaining isolates were obtained from individuals in their late-teens or early adulthood. In addition, data on the sex of the individuals sampled was not available for nearly two-thirds of the carriage strain collection whereas this information was lacking from only 10% of the IMD strain collection.

This study has shown that differences are apparent in the serogroup composition of the carriage strain collection compared with an IMD strain collection isolated during the same 31-year period in Scotland. Serogroups A, B and C were more prevalent amongst the IMD strain collection, while sero- or genogroups W135, Y, X, Z, Z'(29E) and *cnl*-strains were more prevalent amongst the carriage strain collection. We have shown that meningococci isolated from the nasopharynx of otherwise healthy individuals are frequently nongroupable, as have others (Cartwright, Stuart *et al.* 1987; Jolley, Kalmusova *et al.* 2000; Yazdankhah, Kriz *et al.* 2004; Claus, Maiden *et al.* 2005). Unusually, for IMD isolates, NG was recorded for 45 isolates of the IMD strain collection. Thirty-seven were resolved by PCR as B, C, A or W135 however the genogroup of eight IMD isolates was not fully determined during work previously conducted by the SHLMPRL. The genotypic PCR tests performed on the carriage strain collection in this study were not all in use by the SHLMPRL at the time these NG isolates from the IMD strain collection were tested. Performing the other genogrouping tests (*ctrA* for serogroups X, Z and 29E or capsule null locus) or re-testing with the currently available agglutination reagents may provide further information on the eight NG

isolates, however it is possible these isolates may be of an unknown serogroup which cannot be tested for or they may be of another unidentified serogroup.

Capsule expression is considered essential for the meningococcus to survive extracellularly in the bloodstream or CSF of the human host therefore; it seems unusual that any of the isolates obtained from cases of IMD should not express a capsule. It is possible that these NG IMD isolates may react with the available anti-sera currently used by the reference laboratory if they were to be re-tested; however this was beyond the scope of this study. Alternatively, immunodeficiency or host genetic susceptibility can predispose an individual to meningococcal disease (Figueroa and Densen 1991; Emonts, Hazelzet *et al.* 2003; Vazquez-Bermudez, Barroso *et al.* 2003; Debard, Lamy *et al.* 2005) and the meningococcus may, or may not, require capsule to be expressed to cause infection in an immunodeficient individual.

In Scotland during the 31-year period 1974 – 2004 serogroup B has been the most prevalent of the meningococcal serogroups isolated from cases of IMD and from the carriage strain collection. Moreover, in the years following this 31-year period (i.e. 2005 – to June 2010) the prevalence of serogroup B amongst IMD isolates in Scotland has increased to around 85% as cases of serogroup C disease are becoming more of a rarity following continued use of the MCC vaccine (Shakir, Cameron *et al.* 2008; Ure 2008; McDonald 2010a). The prevalence of serogroup C meningococci in Scotland has been variable over the past 30 years. In the mid 1970s, serogroup C strains accounted for only a few percent of disease isolates, increasing during the early 1980s to overtake serogroup B for a short while as the most prevalent serogroup. Serogroup C declined over the next few years before increasing again to overtake serogroup B as the most prevalent serogroup in the late 1990s (Fallon, Brown *et al.* 1984; Clarke 1999b; 2001; Clarke, Reid *et al.* 2002a; Clarke 2002c; 2003a; 2003c; 2005).

Although few in number amongst the carriage strain collection, serogroup A isolates accounted for a large proportion of the IMD strain collection during 1974 – 1983. This serogroup was not present in either strain collection beyond 1995. Subsequently, serogroup A has not been observed by the reference laboratory from cases of IMD in Scotland (Ure 2008). Similarly changes in serogroup prevalence have been observed in England and Wales (Gray, Trotter *et al.* 2006;

Russell, Urwin *et al.* 2008; Gray, Campbell *et al.* 2009) and in other countries around the globe (Rosenstein, Perkins *et al.* 1999; Rosenstein, Perkins *et al.* 2001). Russell and colleagues observed that, over a 20-year period, the prevalence of serogroup B decreased, serogroup C increased and serogroup A disappeared altogether (Russell, Urwin *et al.* 2008). Today, serogroup A remains an important cause of meningococcal disease in Africa and in parts of Asia and Russia (Caugant and Nicolas 2007; Harrison, Trotter *et al.* 2009; Koroleva, Beloshitskij *et al.* 2009).

4 Multi-locus Sequence Typing (MLST) of carried meningococci in Scotland, 1974 – 2004

4.1 Introduction

The following chapter details the frequency of MLST alleles, clonal complexes (ccs) and their associated sequence types (STs). This is followed by an analysis of the allelic profiles to identify lineages within the carriage strain collection and a discussion of the prevalence of these STs and lineages within data from other countries. Throughout this chapter the carriage strain collection is compared to data on the IMD strain collection mentioned previously. The relationships of STs with serogroup and PorA subtype is discussed later (Chapter 6).

As outlined in the Neisseria Multi Locus Sequence Typing website, sequence types are grouped into clonal complexes by their similarity to a central allelic profile (genotype). The central genotypes are identified by a number of heuristic means, including BURST and split decomposition, along with feedback from public health laboratories and epidemiologists. For the Neisseria database, once a central genotype has been identified, clonal complexes are defined as including any sequence type (ST) that matches the central genotype at four or more loci unless it more closely matches another central genotype. STs are assigned to clonal complexes automatically by a Perl script that runs against the database each evening. The management committee for the Neisseria MLST website and databases is responsible for the designation of clonal complexes; as recently as 21/04/2010 the ST-1117, ST-1136, ST-212, ST-178 and ST-282 complexes were defined.

4.2 Frequency of Multi-locus Sequence Typing alleles

Initial analysis of the carriage strain MLST data set was performed using STARTv2 to identify MLST allele frequencies. This identified 34 unique *abcZ* alleles, 22 unique *adk* alleles, 50 unique *aroE* alleles, 45 unique *fumC* alleles, 46 unique *gdh*

alleles, 49 unique *pdhC* alleles and 36 unique *pgm* alleles (Table 4.1). This included 35 newly identified alleles that were submitted to the *N. meningitidis* MLST database curator for assignment. These were *abcZ-454*, *abcZ-462*, *abcZ-474*, *abcZ-475*, *abcZ-486*, *adk-307*, *adk-322*, *adk-325*, *adk-327, aroE-513*, *aroE-514*, *aroE-519*, *aroE-525*, *aroE-532*, *aroE-533*, *fumC-468*, *fumC-476*, *fumC-477*, *fumC-483*, *fumC-497*, *fumC-498*, *gdh-496*, *gdh-498*, *gdh-502*, *gdh-508*, *gdh-525*, *pdhC-475*, *pdhC-477*, *pdhC-478*, *pdhC-481*, *pdhC-506*, *pgm-497*, *pgm-498*, *pgm-499*, *pgm-501* and *pgm-580*. STARTv2 was also used to determined the number of polymorphic sites from the allele sequences of the carriage strain collection as 85, 43, 188, 56, 86, 90 and 93 for *abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC* and *pgm* respectively (Table 4.1).

Table 4.1 – Diversity in MLST alleles in carried meningococci in Scotland, 1974 – 2004

allele	n[1]	P_{sites}[2]	d_N/d_S[3]	Top 5 (%)[4]	Top 10 (%)[4]
abcZ	34	85	0.0467	57.1	79.2
adk	22	43	0.0190	83.0	95.9
aroE	50	188	0.2699	58.2	82.7
fumC	45	56	0.0217	63.8	82.9
gdh	46	86	0.0478	68.1	85.9
pdhC	49	90	0.0653	50.7	73.0
pgm	36	93	0.1115	57.6	80.9

[1] n, number of allelic variants; [2] P_{sites}, polymorphic sites; [3] d_N/d_S, ratio of non-synonymous to synonymous sites ; [4] percentage of isolates covered by the most common allelic variants

4.3 Frequency of Sequence Types

STARTv2 calculated 297 distinct allelic profiles (i.e. STs) corresponding to the 791 carried meningococci and eight control strains. This did not include 11 incomplete profiles in the dataset. Table 4.2 shows the prevalence of individual STs identified in the carriage strain collection. The twenty most frequently identified STs accounted for 46.2% (369/799) of carried meningococci. In rank order these were; ST-8 (77/799; 9.6%), ST-22 (36/799; 4.5%), ST-53 (31/799; 3.9%), ST-23 (29/799; 3.6%), ST-213 (24/799; 3.0%), ST-457 (22/799; 2.8%), ST-43 (16/799; 2.0%), ST-11 (15/799; 1.9%), ST-254 (14/799; 1.8%), ST-289; 12/799; 1.5%), ST-334 (12/799; 1.5%), ST-60 (11/799; 1.4%), ST-167 (10/799; 1.3%), ST-269 (9/799; 1.1%), ST-44 (9/799; 1.1%), ST-577 (9/799; 1.1%), ST-41 (8/799; 1.0%), ST-178 (8/799; 1.0%), ST-103 (8/799; 1.0%) and ST-114 (8/799; 1.0%).

Table 4.2 – Prevalence of sequence types identified in carried meningococci in Scotland, 1974 – 2004

Frequency (%)	Sequence Type(s)	% of Specimens
77 (9.64)	8	9.64
36 (4.51)	22	4.51
31 (3.88)	53	3.88
29 (3.63)	23	3.63
24 (3.00)	213	3.00
22 (2.75)	457	2.75
16 (2.00)	43	2.00
15 (1.88)	11	1.88
14 (1.75)	254	1.75
12 (1.50)	289, 334	3.00
11 (1.38)	60	1.38
10 (1.25)	167	1.25
9 (1.13)	44, 269, 577	3.39
8 (1.00)	41, 103, 114, 178	4.00
7 (0.88)	198, 259, 1162	2.64
6 (0.75)	32, 33, 750, 1157, 1574, 4221	4.50
5 (0.63)	1, 212, 1251, **6657**	2.52
4 (0.50)	153, 206, 461, 472, 766, 1034, 1455, 2154, 2520, 3296	5.00
3 (0.38)	136, 168, 283, 336, 413, 437, 468, 1097, **7409**	3.42
2 (0.25)	5, 35, 170, 189, 275, 284, 288, 344, 1031, 1117, 1218, 1224, 1466, 1655, 1710, 2415, 2514, 2532, 2552, 2875, 3294, 3676, 4650, **6654**, **6660**, **6911**, **6913**, **7264**, **7407**, **7731**, **7781**	7.75
1 (0.125)	13, 15, 24, 42, 67, 94, 162, 180, 183, 185, 278, 337, 340, 352, 460, 462, 464, 473, 479, 482, 492, 571, 823, 833, 835, 865, 961, 1011, 1057, 1161, 1197, 1220, 1226, 1229, 1246, 1273, 1284, 1292, 1361, 1362, 1416, 1433, 1489, 1650, 1651, 1780, 1794, 1802, 1811, 1839, 1908, 1915, 1947, 1953, 2002, 2104, 2120, 2152, 2155, 2166, 2239, 2380, 2433, 2508, 2526, 2528, 2534, 2539, 2547, 2549, 2592, 2631, 2701, 2803, 3258, 3278, 3285, 3286, 3287, 3293, 3295, 3464, 3621, 3866, 4037, 4053, 4385, 4597, 4677, 4874, 4878, 4881, 5120, 5471, 5544, **5945**, **5946**, **5947**, **5948**, **5949**, **5950**, **5951**, **5952**, **5953**, 5984, **6031**, **6032**, **6033**, **6034**, **6402**, **6403**, 6430, **6655**, **6656**, **6658**, **6659**, **6661**, **6662**, **6663**, 6800, **6903**, **6904**, **6905**, **6906**, **6907**, **6908**, **6909**, **6910**, **6912**, **6914**, **6915**, **7203**, **7265**, **7266**, **7267**, **7268**, **7269**, **7270**, **7271, 7272**, **7398**, **7399**, **7404**, **7405**, **7406**, **7408**, **7410**, **7411**, **7412**, **7413**, **7618**, **7619**, **7620**, **7621**, **7622**, **7624**, **7720**, **7721**, **7722**, **7723**, **7724**, **7725**, **7726**, **7727**, **7728**, **7729**, **7730**, **7732**, **7733**, **7782**, **7797**, **7798**, **7799**, **7800**, **7801**, **7802**, **7803**, **7903**, **7904**, **7905**, 7927, **7930**, **7964**, **7965**, **7966**, **7967**, **7968**, **7969**, **7970**, **7971**, **7973**, **7974**, **7975**, **7987**, **7988**, **7989**, **7990**, **7991**, **7992**, **7997**, **7998**, **7999**, **8000**, **8001**, **8002**, **8003**, **8066**, **8220**, **8221**, **8222**, **8231**, **8232, 8635, 8646, 8647**	26.90

Sequence Types in bold face were identified as new during this study

The prevalence of the remaining STs were each individually less than 1.0% (or fewer than eight in number); three STs were identified seven times accounting for 2.6% of specimens; six STs were identified six times accounting for 4.5% of specimens; four STs were identified five times accounting for 2.5% of specimens; ten STs were identified four times accounting for 5.0% of specimens; nine STs were identified three times accounting for 3.4% of specimens and thirty-one STs

were identified twice accounting for 7.8% of specimens. The remaining 215 STs were each identified once, accounting for over a quarter (26.9%) of specimens (Table 4.2).

During this study 125 new allelic profiles were sequenced and submitted to the *N. meningitidis* MLST database curators for assignment of the new ST. The STs were STs-5945-5953, STs-6031-6034, STs-6402-6403, STs-6654-6663, STs-6903-6915, ST-7203, STs-7264-7272, STs-7398-7399, STs-7404-7413, STs-7618-7622, ST-7624, STs-7720-7733, STs-7781-7782, STs-7797-7803, STs-7903-7905, ST-7930, STs-7964-7975, STs-7987-7992, STs-7997-8003, ST-8066, STs-8220-8222 and STs-8231-8232, ST-8635 and ST-8646-8647 (in bold face in Tables 4.2 - 4.4).

4.4 Frequency of MLST clonal complexes

Seven hundred and one (87.7%) carried meningococci were assigned to thirty-four ccs (Table 4.3) while the remaining 12.3% could not be assigned to a known cc (Table 4.4). Twenty-five ccs contained the central genotype after which the cc was named, whilst eight did not. In twenty-two ccs the central genotype was the most prevalent ST. Nine ccs were comprised of a single ST, while twenty-five ccs comprised greater than one ST. The five most common ccs accounted for 45.9% (367/799) of carriage isolates, while the ten most common ccs accounted for 65.1% (520/799) of carriage isolates. The ten most common ccs, in rank order, were cc41-44 complex/Lineage 3 (n=113; 14.1%), cc8/Cluster A4 (n=82; 10.3%), cc22 (n=65; 8.1%), cc35 complex (n=65; 8.1%), cc23/Cluster A3 (n=43; 5.4%), cc254 complex (n=35; 4.4%), cc269 complex (n=33; 4.1%), cc53 complex (n=32; 4.0%), cc213 complex (n=27; 3.4%) and cc334 complex (n=26; 3.3%).

Table 4.3 – Clonal complexes and STs identified in carried meningococci in Scotland, 1974 – 2004

cc	n^I	n^{ST}	Sequence Type (n^I)
cc41/44	113	51	41 (8), 42 (1), 43 (16), 44 (9), 136 (3), 170 (2), 180 (1), 206 (4), 337 (1), 340 (1), 437 (3), 464 (1), 482 (1), 571 (1), 577 (9), 833 (1), 835 (1), 1097 (3), 1361 (1), 1362 (1), 1433 (1), 1489 (1), 1574 (6), 1908 (1), 1915 (1), 1947 (1), 2120 (1), 2433 (1), 2459 (1), 2514 (2), 2532 (2), 2631 (1), 3278 (1), 3296 (4), 3866 (1), 5120 (1), 5471 (1), **6034** (1), **6655** (1), **6657** (5), **6909** (1), **7266** (1), **7270** (1), **7621** (1), **7624** (1), **7720** (1), **7728** (1), **7730** (1), **7799** (1), **7971** (1), **8220** (1)
cc8	82	2	8 (77), 153 (4), incomplete (1)
cc22	65	9	22 (36), 114 (8), 289 (12), 1224 (2), 1226 (1), 2878 (1), **5948** (1), **7409** (3), **7904** (1)
cc35	65	33	35 (2), 278 (1), 457 (22), 472 (4), 1197 (1), 1292 (1), 1651 (1), 1710 (2), 2380 (1), 3295 (1), 3676 (2), **5945** (1), **6033** (1), **6402** (1), **6658** (1), **6660** (2), **6904** (1), **6905** (1), **6907** (1), **6911** (2), **6913** (2), **7264** (2), **7271** (1), **7408** (1), **7410** (1), **7797** (1), **7798** (1), **7903** (1), **7969** (1), **7988** (1), **7997** (1), **8001** (1), **8232** (1), incomplete (1)
cc23	43	12	23 (29), 183 (1), 1655 (2), 1794 (1), 1811 (1), 5544 (1), 6800 (1), **7406** (1), **7731** (2), **7781** (2), **7973** (1), **8002** (1)
cc254	35	16	254 (14), 468 (3), 1839 (1), 1953 (1), 2520 (4), 2701 (1), 3294 (2), 4881 (1), **6914** (1), **6915** (1), **7203** (1), **7964** (1), **7968** (1), **7991** (1), **8221** (1), new (1)
cc269	33	22	13 (1), 269 (9), 275 (2), 283 (3), 352 (1), 479 (1), 492 (1), 1161 (1), 1273 (1), 1284 (1), 1416 (1), 2166 (1), 2239 (1), 2592 (1), 2803 (1), 3286 (1), 5984 (1), **6908** (1), **7269** (1), **7992** (1), **8000** (1), **8231** (1)
cc53	32	2	53 (31), **5950** (1)
cc213	27	3	213 (24), 1218 (2), **5951** (1)
cc334	26	9	189 (2), 334 (12), 1031 (2), 1034 (4), 2508 (1), 2526 (1), 2552 (2), 3285 (1), 3464 (1)
cc167	21	6	167 (10), 168 (3), 766 (4), 2415 (2), **6031** (1), **7803** (1)
cc11	20	6	11 (15), 67 (1), 473 (1), 1246 (1), 4037 (1), 4677 (1)
cc32	19	3	32 (6), 33 (6), 259 (7)
cc60	19	8	60 (11), 1650 (1), 1780 (1), 2002 (1), 2104 (1), 2155 (1), **6903** (1), **7724** (1), incomplete (1)
cc178	14	7	178 (8), **5949** (1), **6662** (1), **6912** (1), **7974** (1), **7987** (1), new allele (1)
cc364	11	8	15 (1), 413 (3), 462 (1), 2534 (1), 4650 (2), **7725** (1), **7733** (1), **7965** (1)
cc198	9	3	198 (7), 823 (1), **7782** (1)
cc103	8	1	103 (8)
cc461	8	5	461 (4), **6403** (1), 6430 (1), **6910** (1), **7972** (1)
cc174	7	6	185 (1), 1057 (1), 1466 (2), 2547 (1), 3293 (1), **7930** (1)
cc212	7	3	212 (5), 1229 (1), **7618** (1)
cc750	7	2	24 (1), 750 (6)
cc1157	7	2	1157 (6), **7619** (1)
cc1	5	1	1 (5)
cc92	4	4	94 (1), **7721** (1), **7722** (1), **7966** (1)
cc5	2	1	5 (2)
cc376	2	2	**7265** (1), **7405** (1)
cc865	2	2	460 (1), 865 (1)
cc1117	2	1	1117 (2)
cc162	1	1	162 (1)
cc175	1	1	**7800** (1)
cc231	1	1	1011 (1)
cc282	1	1	1802 (1)
cc1136	1	1	**8635** (1)

cc, clonal complex; n^I, number of isolates; n^{ST}, number of sequence types; Sequence Types in bold face were identified as new during this study

Twenty-two new STs were assigned to the cc35 while a further thirteen new STs were assigned to the cc41-44/Lineage 3 (Table 4.3). Seven new STs were assigned to the cc254, while 5 new STs were assigned to each of the cc23/A3, cc178 and cc269. Three new STs were assigned to each of the cc22, cc92, cc364 and cc461 while two new STs were assigned to each of the cc60, cc167 and cc376. A single new ST was also assigned to each of the clonal complexes cc53, cc174, cc175, cc198, cc212, cc213 and cc1157. Forty-two new STs were not assigned to a known MLST clonal complex (Table 4.4).

Table 4.4 – Sequence Types identified in carried meningococci in Scotland, 1974 – 2004 not assigned to a defined clonal complex

ST[1]	n[2]	ST	n	ST	n	ST	n	ST	n	ST	n	ST	n
284	2	2154	4	4597	1	**6659**	1	**7407**	2	**7732**	1	**7999**	1
288	2	2528	1	4874	1	**6661**	1	**7411**	1	**7801**	1	**8003**	1
336	3	2539	1	4878	1	**6663**	1	**7412**	1	**7802**	1	**8066**	1
344	2	2875	2	**5946**	1	**6906**	1	**7413**	1	**7905**	1	**8222**	1
961	1	3258	1	**5947**	1	**7267**	1	**7620**	1	**7967**	1	**8646**	1
1162	7	3287	1	**5952**	1	**7268**	1	**7622**	1	**7970**	1	**8647**	1
1220	1	3621	1	**5953**	1	**7272**	1	**7723**	1	**7975**	**1**		
1251	5	4053	1	**6032**	1	**7398**	1	**7726**	1	**7989**	1		
1455	4	4221	6	**6654**	2	**7399**	1	**7727**	1	**7990**	1		
2152	1	4385	1	**6656**	1	**7404**	1	**7729**	1	**7998**	1		

[1] ST, Sequence Type; [2] n, number of isolates; STs in bold face were identified as new during this study; three additional isolates had a new *gdh* allele; two additional isolates had an incomplete allelic profile

Inspection of the SHLMPRL isolate database revealed that isolates of the IMD strain collection could be assigned to thirty-one of the currently defined *N. meningitidis* clonal complexes (ccs). One hundred and sixty-five (6.0%) isolates were not assigned to a known clonal complex (cc). The five most common ccs accounted for 71.5% (n=1,981) of invasive isolates, while the ten most common ccs accounted for 85.2% (n=2364; 85.3%). The ten most common ccs were ST-8/Cluster A4 (n=547; 19.7%), ST-41/-44/Lineage 3 (n=493; 17.8%), ST-11/ET-37 (n=491, 17.7%), ST-32/ET-5 (n=262; 9.5%), ST-269 (n=188; 6.8%), ST-1/subgroup I/II (n=125; 4.5%), ST-334 (n=95; 3.4%), ST-60 (n=74; 2.7%), ST-213 (n=45; 1.6%) and ST-35 (n=44; 1.6%).

Figure 4.1 shows a comparison of the prevalence of ccs identified in the IMD and carriage strain collections. Amongst the two strain collections there were similarities and differences in the prevalence of particular ccs. Moreover the

comparison highlights that some ccs are more prevalent in one strain collection than the other. Specifically, cc18, cc37 and cc549 were present in the IMD strain collection, but absent from the carriage strain collection. Contrastingly, cc53, cc175, cc178 and cc198 were present in the carriage strain collection, but absent from the IMD strain collection.

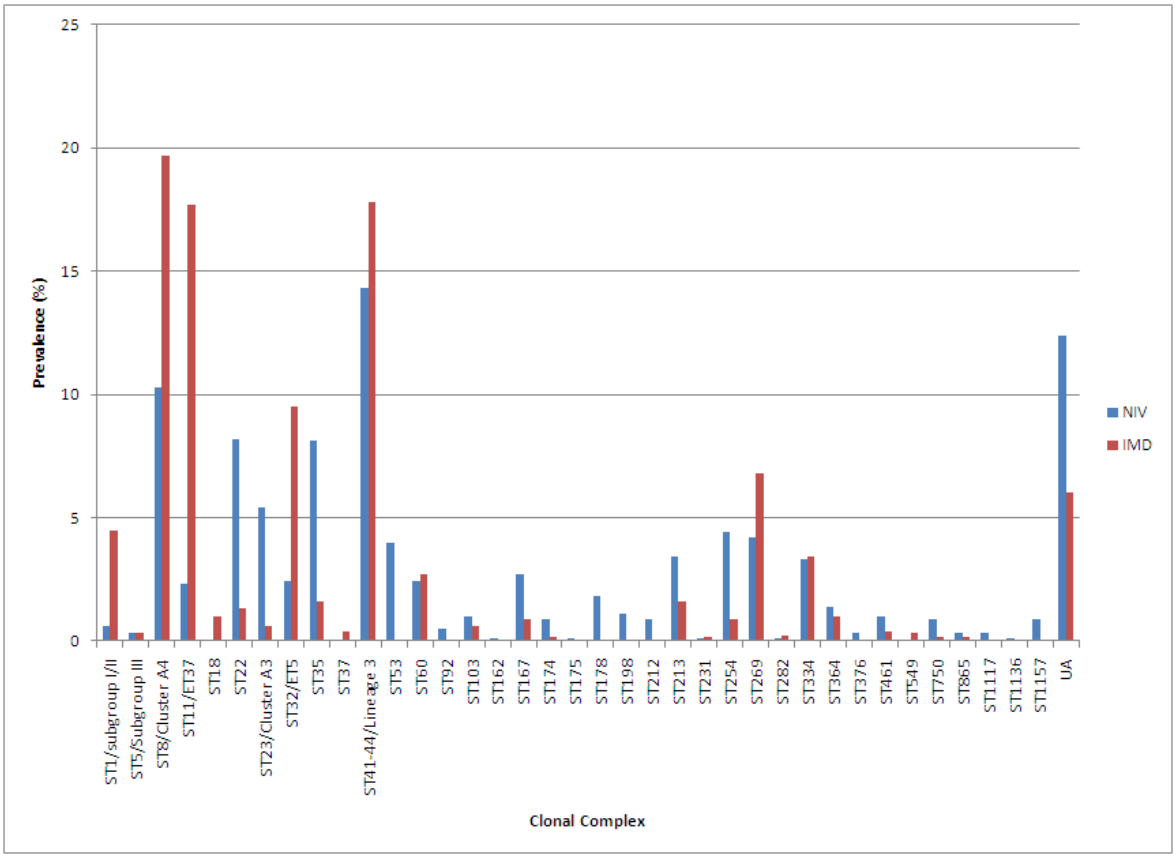

Figure 4.1 - Prevalence of clonal complexes identified in invasive meningococcal disease and carried meningococcal strain collections in Scotland, 1974 – 2004
NIV, non-invasive (carried meningococci); IMD, invasive meningococcal disease; UA, unassigned

Furthermore, cc1, cc8, cc11, cc32, cc41/44 and cc269 were all more prevalent amongst the IMD strain collection than the carriage strain collection. Whereas, cc22, cc23, cc35, cc167, cc174, cc212, cc254, cc461 and isolates not assigned to a clonal complex were all more prevalent amongst the carriage strain collection than the IMD strain collection. A few clonal complexes, cc5, cc60, cc103, cc334 and cc364, were found in similar proportions amongst the two strain collections.

An odds ratio was calculated for each clonal complex to estimate the probability of invasive disease or a carriage phenotype (i.e. carriage) due to individual ccs

(Table 4.5). Invasive disease due to cc1, cc8, cc11, cc32, cc41-44 and cc269 was estimated to be a more likely outcome; whereas cc22, cc23, cc35, cc92, cc167, cc174, cc212, cc213, cc254, cc461, cc750, cc1157 and meningococci unassigned to a clonal complex were more likely to be associated with a carriage phenotype. A varied range of confidence intervals for ccs more likely to be associated with a carriage phenotype indicates that for some ccs this association is less precise. For example, the widest confidence intervals were observed for ccs isolated less frequently over the course of the study, i.e. cc212 (Table 4.5).

Table 4.5 – Association of meningococcal clonal complexes identified in Scotland, 1974 – 2004 with invasive disease or with a carriage phenotype

Invasive cc[1]	**n**[2] **(IMD**[3]**)**	**n (carriage**[4]**)**	**OR**[5] **(95% CI**[6]**)**
cc1	124	5	7.45 (3.04 to 18.28)
cc8	547	82	2.15 (1.68 to 2.76)
cc11	490	18	9.30 (5.77 to 14.98)
cc32	262	17	4.81 (2.93 to 7.91)
cc41-44	491	113	1.31 (1.05 to 1.63)
cc269	188	33	1.69 (1.16 to 2.47)
carriage cc	**n (carriage)**	**n (IMD)**	**OR (95% CI)**
cc22	65	36	6.72 (4.43 to 10.18)
cc23	43	17	9.20 (5.22 to 16.23)
cc35	64	43	5.52 (3.71 to 8.19)
cc92	4	2	6.96 (1.27 to 38.05)
cc167	21	23	3.22 (1.77 to 5.85)
cc174	7	4	6.11 (1.78 to 20.91)
cc212	7	1	24.45 (3.00 to 199.00)
cc213	27	45	2.12 (1.30 to 3.43)
cc254	35	26	4.83 (2.89 to 8.07)
cc461	8	11	2.53 (1.02 to 6.34)
cc750	7	4	6.11 (1.78 to 20.91)
cc1157	7	2	12.22 (2.53 to 58.94)
UA	93	165	2.08 (1.59 to 2.71)

[1] cc, clonal complex; [2] n, number of isolates; [3] IMD, invasive meningococcal disease isolates; [4] carriage, carriage strain collection; [5] OR odds ratio; [6] CI confidence interval

Approximately 70% of the STs identified in the carriage strain collection were unique to the carriage strain collection, whereas just fewer than 30% of the STs were present in both the IMD and carriage strain collections. The shared STs corresponded to less than one third of the total number of STs identified in the carriage strain collection and around one fifth of the STs identified in the IMD strain collection. This corresponded to approximately 60% and 81% of isolates from the carriage and IMD strain collections, respectively (Table 4.6).

Simpsons' index of diversity, *D*, was used to assess the genetic diversity of the two strain collections on the basis of sequence type. This was calculated on the strain collections as a whole and when split into three roughly equal time periods, 1974-1983, 1984-1993 and 1994-2004 (Table 4.6). The indices were calculated as 0.981 (95% confidence interval, CI; 0.955, 1.006) for the full carriage strain collection and 0.989 (95%CI 0.878, 1.100), 0.960 (95%CI 0.929, 0.991) and 0.964 (95%CI 0.899, 1.028), respectively, for the three periods (Table 4.6). This does not indicate any significant difference in the diversity of the strain collection over the 31 year period, given the overlapping 95% CIs.

Table 4.6 – Summary of the genetic diversity in meningococci in Scotland, 1974 – 2004

	carriage[1]				IMD[2]			
	'74-'83	**'84-'93**	**'94-'04**	**ALL**	**'74-'83**	**'84-'93**	**'94-'04**	**ALL**
Isolates	127[a]	445[b]	208[c]	780[d]	687	1119	961	2767
Alleles								
abcZ	20	31	20	34 (11)[g]	20	23	26	34 (11)[g]
adk	17	18	14	22 (7)	11	13	19	24 (9)
aroE	25	36	28	50 (20)	19	33	30	46 (16)
fumC	24	34	28	45 (13)	20	35	31	43 (11)
gdh	21	30	26	47 (19)	16	28	33	44 (16)
pdhC	30	36	26	49 (17)	26	34	30	44 (12)
pgm	23	29	20	36 (14)	17	24	21	28 (6)
STs								
Total	89	153	97	294	96	198	195	406
Unique[e]				209 (71.1%)				321 (79.1%)
Shared[e]				85 (28.9%)				85 (20.9%)
% of isolates[f]				60.3				80.6
***D*[h]**	0.989	0.960	0.964	0.981	0.900	0.938	0.892	0.938
95% CIs[i]	(0.878, 1.100)	(0.929, 0.991)	(0.899, 1.028)	(0.955, 1.007)	(0.892, 0.907)	(0.929, 0.946)	(0.881, 0.903)	(0.934, 0.942)

[1] carriage, carriage strain collection; [2] IMD, invasive meningococcal disease strain collection; [a] Three incomplete isolates/profiles not included; [b] Five incomplete isolates/profiles not included; [c] Three incomplete isolates/profiles not included; [d] Eight NCTC/ATCC isolates and eleven incomplete isolates/profiles not included; [e] Represented as the number & percentage of STs in the strain collection; [f] Represented as the percentage of the strain collection characterised by the shared STs; [g] number of alleles unique to each strain collection in parentheses; [h] *D*, Simpson's index of diversity (Hunter & Gaston 1988); [i] 95% confidence intervals calculated as described in Grundmann *et a*l 2001.

Genetic diversity of the IMD strain collection was 0.938 (95%CI 0.934, 0.942) in comparison with the carriage strain collection and the non-overlapping 95% CIs suggests that the level of genetic diversity of the two strain collections is significantly different (Table 4.6). Furthermore, the level of genetic diversity within the IMD strain collection is different across the different time periods. *D*

was calculated to be 0.900 (95% CI 0.892, 0.907) for the period 1974-1983; compared to 0.938 (95% CI 0.929, 0.946) for the period 1984-1993; and 0.892 (95% CI 0.881, 0.903) for the period 1994-2004 (Table 4.6). The 95% CIs for the periods 1974-1983 and 1994-2004 both overlap with each other, however the 95% CIs for the period 1984-1993 does not overlap with the 95% CI of either of the other two periods. Hence, the level of diversity, *D*, of the IMD strain collection for the periods 1974-1983 and 1994-2004 can be said to be significantly less compared with that for the period 1984-1993.

To assess the probability of invasive disease or carriage by individual STs, odds ratios were again calculated (Table 4.7).

Table 4.7 – Association of meningococcal Sequence Types identified in Scotland, 1974 – 2004 with invasive disease or with a carriage phenotype

Invasive ST[1]	**n**[2] **(IMD**[3]**)**	**n (carriage**[4]**)**	**OR**[5] **(95% CI**[6]**)**
ST1	120	5	7.20 (2.93, 17.67)
ST8	356	77	1.38 (1.07, 1.80)
ST11	468	15	10.64 (6.32, 17.90)
ST32	106	6	5.26 (2.30, 12.03)
ST33	79	6	3.88 (1.69, 8.94)
ST41	242	8	9.48 (4.66, 19.25)
ST153	133	4	10.04 (3.70, 27.22)
ST180	29	1	8.45 (1.15, 62.15)
ST206	50	4	3.66 (1.32, 10.16)
ST269	77	9	2.51 (1.25, 5.03)

carriage ST	**n (carriage)**	**n (IMD)**	**OR (95% CI)**
ST22	36	13	10.00 (5.27, 18.94)
ST23	29	15	6.91 (3.69, 12.95)
ST43	16	21	2.67 (1.39, 5.15)
ST44	9	10	3.14 (1.27, 7.76)
ST136	3	1	10.42 (1.08, 100.36)
ST167	10	8	4.37 (1.72, 11.11)
ST213	24	38	2.22 (1.33, 3.73)
ST254	14	15	3.27 (1.57, 6.81)
ST289	12	9	4.67 (1.96, 11.13)
ST336	3	1	10.42 (1.08, 100.36)
ST457	22	25	3.11 (1.74, 5.54)
ST577	9	1	31.51 (3.99, 249.11)
ST750	6	3	6.97 (1.74, 27.94)
ST1157	6	2	10.46 (2.11, 51.93)

[1] ST, sequence type; [2] n, number of isolates; [3] IMD, invasive meningococcal disease strain collection; [4] carriage, carriage strain collection; [5] OR, odds ratio; [6] CI, confidence interval

Founder STs of the ccs significantly associated with invasiveness (Table 4.5) were also shown to be significantly associated with invasiveness in addition to a second ST belonging to cc8 (ST-153) and two other STs belonging to cc41-44 (ST180 & ST206) (Table 4.7). Founder STs of seven of twelve ccs previously shown to be associated with a carriage phenotype; cc22, cc23, cc167, cc213, cc254, cc750 and cc1157 had significant ORs indicating association with a carriage phenotype (Table 4.7).

Interestingly, ORs of four STs (ST-43, -44, -136 & -577) belonging to cc41-44 were shown to be significant for association with a carriage phenotype (more so for ST43 and ST44 which had much narrower 95% CIs than that calculated for ST136 and ST577) despite this clonal complex being significantly associated with an invasive phenotype (Table 4.5). A second ST belonging to cc22 (ST-289) was significantly associated with a carriage phenotype. ST457 (cc35) was also shown to be significantly associated with a carriage phenotype, as was one ST not assigned to a clonal complex (ST-336); although the wide 95% CI indicates the OR is less precise (Table 4.7).

4.5 Temporal trends in the prevalence of MLST clonal complexes

Data on the prevalence of the MLST clonal complexes was examined for evidence of any temporal changes (Table 4.8). Temporal variation in the prevalence of ccs was observed in the carriage strain collection. Ten ccs were observed during one of the three time periods only; cc5, cc92, cc162, cc175, cc213, cc231, cc282, cc750, cc1117 and cc1136. Nine ccs were observed during two of the three time periods; cc1, cc8, cc53, cc103, cc167, cc198, cc376, cc865 and cc1157. The remaining fifteen ccs were observed during all three time periods; cc11, cc22, cc23, cc32, cc35, cc41-44, cc60, cc174, cc178, cc212, cc254, cc269, cc334, cc364 and cc461.

Table 4.8 – Temporal analysis of clonal complexes identified in meningococci in Scotland, 1974 - 2004: carriage strain collection versus invasive meningococcal disease isolates

cc[1]	carriage[3]			IMD[4]		
	'74 – '83 No. (%)	'84 – '93 No. (%)	'94 – '04 No. (%)	'74 – '83 No. (%)	'84 – '93 No. (%)	'94 – '04 No. (%)
cc1	4 (3.1)	1 (0.2)	-	118 (17.1)	6 (0.5)	-
cc5	2 (1.5)	-	-	5 (0.7)	2 (0.2)	2 (0.2)
cc8	2 (1.5)	80 (17.8)	-	168 (24.5)	329 (29.4)	50 (5.2)
cc11	6 (4.6)	10 (2.2)	2 (0.95)	44 (6.4)	159 (14.2)	287 (29.9)
cc18	-	-	-	-	2 (0.2)	25 (2.6)
cc22	12 (9.2)	42 (9.3)	11 (5.2)	5 (0.7)	11 (1.0)	20 (2.1)
cc23	2 (1.5)	34 (7.6)	7 (3.3)	8 (1.6)	6 (0.5)	3 (0.3)
cc32	3 (3.1)	13 (2.9)	2 (1.0)	76 (11.1)	121 (10.8)	65 (6.8)
cc35	17 (13.1)	39 (8.7)	8 (3.8)	23 (3.3)	14 (1.3)	6 (0.6)
cc37	-	-	-	7 (1.0)	3 (0.3)	-
cc41-44	15 (11.5)	64 (14.2)	34 (16.1)	83 (12.1)	211 (18.9)	197 (20.5)
cc53	-	4 (0.9)	28 (13.3)	-	-	-
cc60	2 (1.5)	11 (2.4)	5 (2.4)	14 (2.0)	33 (3.0)	27 (2.8)
cc92	4 (3.1)	-	-	1 (0.2)	-	1 (0.1)
cc103	-	5 (1.1)	3 (1.4)	-	14 (1.3)	2 (0.2)
cc162	-	-	1 (0.5)	-	1 (0.1)	-
cc167	-	11 (2.4)	10 (4.7)	4 (0.6)	5 (0.5)	14 (1.5)
cc174	1 (0.8)	4 (0.9)	2 (1.0)	1 (0.2)	1 (0.1)	2 (0.2)
cc175	-	1 (0.2)	-	-	-	-
cc178	1 (0.8)	10 (2.2)	3 (1.4)	-	-	-
cc198	-	7 (1.6)	2 (1.0)	-	-	-
cc212	1 (0.8)	2 (0.4)	4 (1.9)	-	1 (0.1)	-
cc213	-	-	27 (12.8)	-	-	45 (4.7)
cc231	-	1 (0.2)	-	3 (0.5)	1 (0.1)	-
cc254	15 (11.5)	19 (4.2)	1 (0.5)	11 (1.6)	11 (1.0)	4 (0.4)
cc269	5 (3.9)	10 (2.2)	18 (8.5)	11 (1.6)	56 (5.0)	121 (12.6)
cc282	-	1 (0.2)	-	-	3 (0.3)	3 (0.3)
cc334	5 (3.9)	19 (4.2)	2 (1.0)	50 (7.3)	44 (3.9)	1 (0.1)
cc364	6 (4.6)	4 (0.9)	1 (0.5)	17 (1.5)	10 (0.9)	2 (0.2)
cc376	1 (0.8)	1 (0.2)	-	1 (0.2)	1 (0.1)	-
cc461	1 (0.8)	4 (0.9)	3 (1.4)	-	-	11 (1.1)
cc549	-	-	-	3 (0.5)	5 (0.5)	-
cc750	-	-	7 (3.3)	-	1 (0.1)	3 (0.3)
cc865	-	1 (0.2)	1 (0.5)	1 (0.2)	3 (0.3)	1 (0.1)
cc1117	-	-	2 (1.0)	-	-	-
cc1136	-	-	1 (0.5)	-	-	-
cc1157	1 (0.8)	-	6 (2.8)	-	-	2 (0.2)
UA[2]	22 (16.9)	52 (11.6)	19 (9.0)	33 (4.8)	65 (5.8)	67 (7.0)

[1] cc, clonal complex; [2] UA, unassigned; [3] carriage, carriage strain collection; [4] IMD, invasive meningococcal disease strain collection

Furthermore, differences were observed in the trend in prevalence of particular ccs; cc1, cc5, cc8, cc92 and cc376 would appear to have disappeared from the carriage strain collection given that they were not observed during at least the most recent time period. Whilst being observed through all three time periods several ccs, cc11, cc32, cc35, cc254, cc334 and cc364, have declined in prevalence across the whole period covered by the carriage strain collection. The prevalence of other ccs would appear to have increased across the whole

period covered by the carriage strain collection; cc22, cc41/44, cc60 and cc269. Moreover, a few ccs would appear to have emerged during the period covered by the carriage strain collection as they were absent from at least the earliest time period; cc53, cc103, cc162, cc167, cc198, cc213, cc750, cc865 and cc1117. However, for some of these lineages the numbers are small, with the exception of cc53 and cc213. The remaining ccs were observed either sporadically or they persisted at fairly low levels (Table 4.8).

In the IMD strain collection, the prevalence of cc1, cc5, cc32, cc35, cc37, cc254, cc334 and cc364 have all decreased over the three time periods. Moreover, cc1 and cc37 were not present in the final period, 1994 – 2004. Furthermore, despite being prevalent during 1994 – 2004, cc5 was not present beyond 1995. Cc8 accounted for nearly a quarter of IMD strains in the first period and subsequently increased by 5% during the second time period; however this lineage appears to have rapidly declined, only accounting for approximately 5% of IMD strains during the final period. Indeed cc8 was not present beyond 2001. Cc334 also declined steadily over the three periods and was not present amongst the IMD strain collection beyond 1996.

Conversely three clonal complexes, cc11, cc41-44 and cc269, increased substantially over the three time periods. Cc11 increased from 6.4% during 1974 – 1983 to 14.2% during 1984 – 1993, and further to nearly 30% in the final time period. Cc41-44 accounted for a modest 12% of the IMD isolates during 1974 – 1983, increasing to almost 19% during 1984 – 1993 and increasing by a further 2% during 1994 – 2004. Cc269 accounted for only 1.6% of IMD isolates during 1974 – 1983 increasing to 5% during 1984 – 1993 and increasing in prevalence by more than double to 12.6% during 1994 – 2004. The table also indicates the emergence of one clonal complex, cc213, which was absent during the first two periods but was found to account for nearly 5% of isolates of the IMD strain collection and nearly 13% of the carriage strain collection during the period 1994 – 2004.

4.6 Analysis of MLST allelic profiles using eBURST

The eBURST software was used to compare the IMD and carriage strain collections. The two datasets gave a total of 3558 isolates, comprising of 615 STs. eBURST identified 85 STs that were common to both strain collections, 214 STs were unique to the carriage strain collection and 321 STs were unique to the IMD strain collection. Lineage analysis of allelic profiles of the 791 carried meningococci and the eight NCTC/ATCC isolates were performed using BURST, implemented in STARTv2 (Jolley, Feil *et al.* 2001) with the group definition set such that each ST in a group matches at least one other ST in the group at five or more loci. BURST analysis categorised the dataset into 37 groups and 49 singletons. Group 1 consisted of three STs assigned to cc213; ST-213 was predicted as the central genotype with two SLVs of ST-1218 and ST-5951 (Figure 4.2).

Figure 4.2 - Representation of BURST Group 1 of carried meningococci in Scotland, 1974 – 2004
Numbers refer to sequence types; ST within black circle is the founder genotype; an ST within red circle is a single-locus variant (SLV) of the founder genotype.

Group 2 consisted of 20 STs belonging to cc269 (ST-13, -269, -275, -283, -352, -479, -492, -1161, -1273, -1284, -1416, -2166, -2592, -2803, -3286, -5984, -7411, -7992, -8000 and -8231) with ST-269 as the predicted central genotype; whilst ST-6906 was not assigned to a known cc (Figure 4.3). In addition two other STs belonging to cc269 (ST-2239 & ST-6908) were assigned to a different group by BURST; Group 36. Furthermore a third ST assigned to cc269, ST-7269, was designated a singleton by BURST. Within this BURST group, a number of other STs (ST-275, ST-352 & ST-7411), besides the predicted central genotype ST-269,

have a number of SLVs of their own and as such are depicted as subgroups (Figure 4.3).

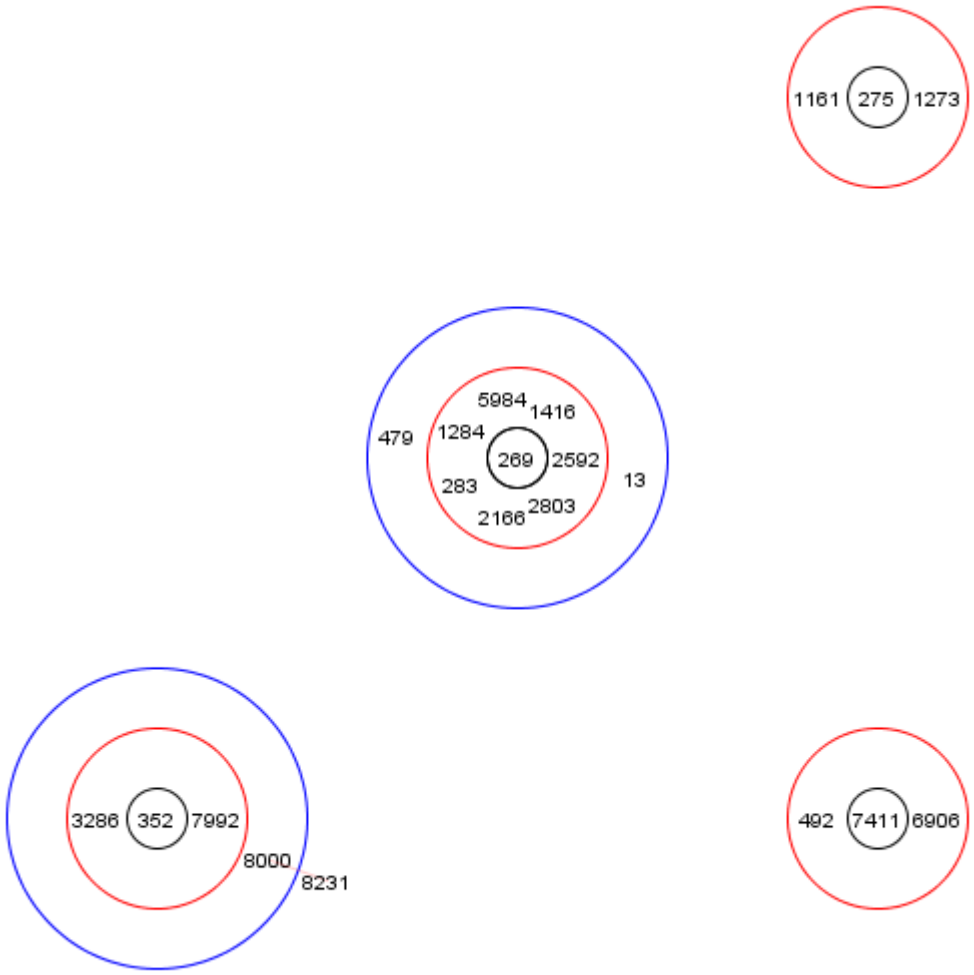

Figure 4.3 - Representation of BURST Group 2 of carried meningococci in Scotland, 1974 – 2004
Numbers refer to sequence types; ST within a black circle is the founder genotype or the subgroup founder genotype; an ST within a red circle is a single-locus variant (SLV) of the founder genotype; an ST within a blue circle is a DLV of the founder genotype; A faint red line connecting a satellite ST with an ST within a group, or subgroup, indicates they are SLVs of each other.

Group 3 consisted of 48 STs almost completely of cc41-44/Lineage 3 with ST-206 as the predicted central genotype. The 48 STs were ST-41, -42, -43, -44, -136, -170, -180, -206, -337, -340, -437, -464, -482, -571, -577, -833, -835, -1097, -1361, -1362, -1433, -1489, -1908, -1915, -2120, -2433, -2459, -2514, -2532, -2631, -3278, -3296, -3866, -5120, -5471, -6034, -6655, -6909, -7266, -7270, -7398, -7624, -7720, -7728, -7730, -7799 and ST-7971 (Figure 4.4). As defined by

the *N. meningitidis* MLST website ST-7398 is not assigned to a known MLST complex, however BURST analysis placed this ST alongside 47 other STs of cc41-44/Lineage 3. Furthermore four STs assigned to cc41-44/Lineage 3 (STs-1574, -1947, -7621 and -8220) were not assigned to this group using BURST and were designated as singletons. Within this BURST group a number of other STs (ST-41, ST-43, ST-180, & ST-2532), besides the predicted central genotype ST-206, have a number of SLVs of their own and as such are depicted as subgroups (Figure 4.4).

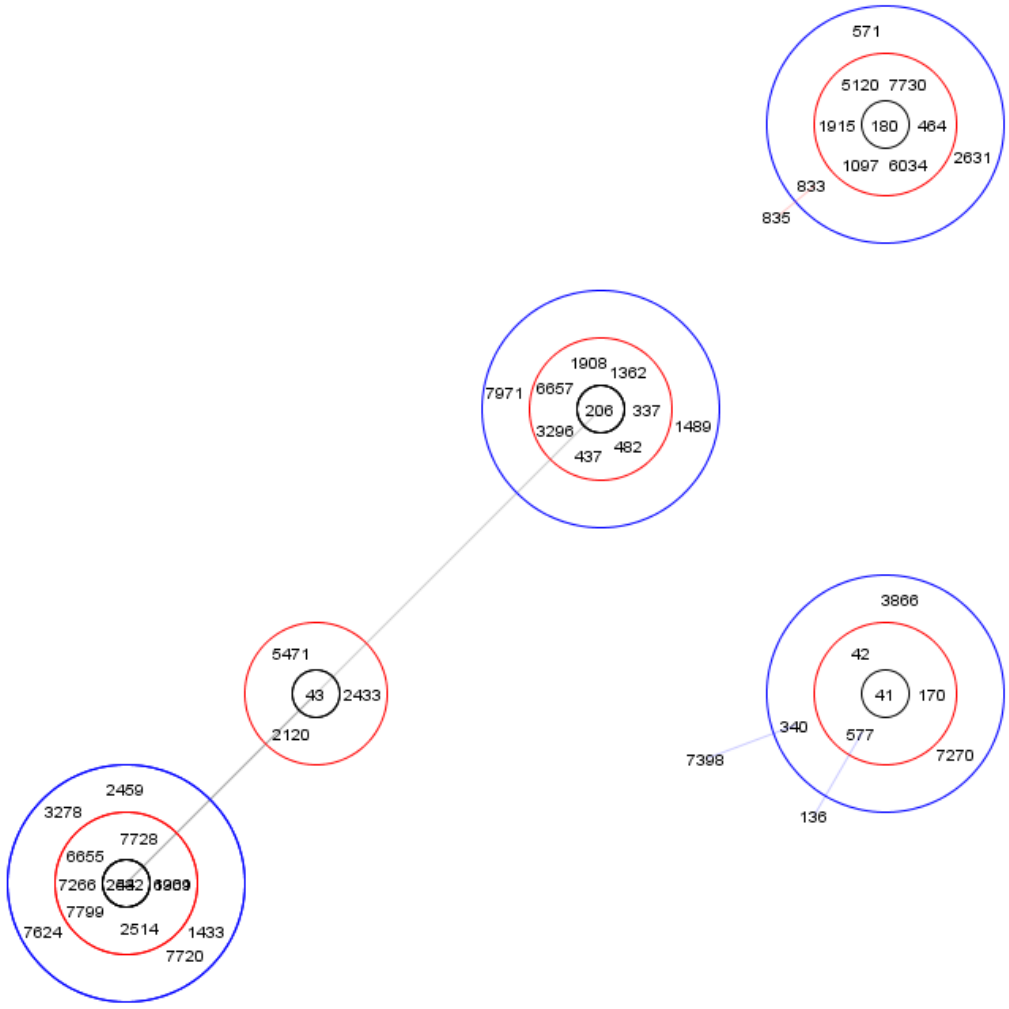

Figure 4.4 - Representation of BURST Group 3 of carried meningococci in Scotland, 1974 – 2004
Numbers refer to sequence types; ST within a black circle is the founder genotype or the subgroup founder genotype; an ST within a red circle is a single-locus variant (SLV) of the founder genotype; an ST within a blue circle is a DLV of the founder genotype; A faint red line connecting a satellite ST with an ST within a group, or subgroup, indicates they are SLVs of each other; A grey line connecting a satellite ST with a ST within a group, or subgroup, indicates these STs are DLVs of each other. A grey line connecting central genotypes within different subgroups indicates these STs are SLVs of each other.

Group 4 consisted of six STs assigned to cc167 with ST-766 as the predicted central genotype. The six STs were ST-766, four SLVs (ST-167, -168, -6031 and -7803) and the DLV ST-2415 (Figure 4.5).

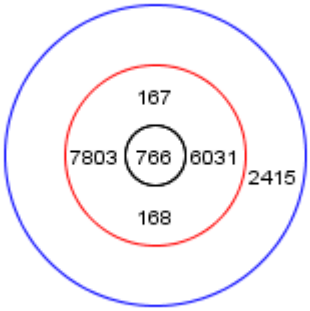

Figure 4.5 - Representation of BURST Group 4 of carried meningococci in Scotland, 1974 – 2004

Numbers refer to sequence types; ST within a black circle is the founder genotype; an ST within a red circle is a single-locus variant (SLV) of the founder genotype; an ST within a blue circle is a DLV of the founder genotype.

Group 5 consisted of two STs, ST-24 and ST-750, which are SLVs of each other, belong to the cc750. Group 6 consisted of two STs, ST-53 and ST-5950, which are SLVs of each other and belong to cc53. Similarly Group 7 also consisted of two STs, ST-1157 and ST-7619, which are DLVs of each other and belong to cc1157. Group 8 consisted of 33 STs that almost completely assigned to cc35 with ST-472 predicted as the central genotype (Figure 4.6). The 33 STs were, ST-35, -278, -457, -472, -1197, -1292, -1651, -1710, -2380, -3295, -3676, -5945, -6033, -6402, -6658, -6660, -6904, -6905, -6907, -6911, -6913, -7271, -7408, -7410, -7797, -7798, -7802, -7903, -7988, -7969, -7997, -8001 and ST-8232. One ST, ST-7802, is currently not assigned to a known cc however it was assigned to this group using BURST. In addition, ST-7264 is part of the cc35 however BURST deemed this ST to be a singleton This BURST group also consisted of a subgroup with ST-457 predicted as the subgroup founder (Figure 4.6). Group 9 consisted of 11 STs that mostly belonged to cc22 with ST-22 predicted as the central genotype (Figure 4.7).

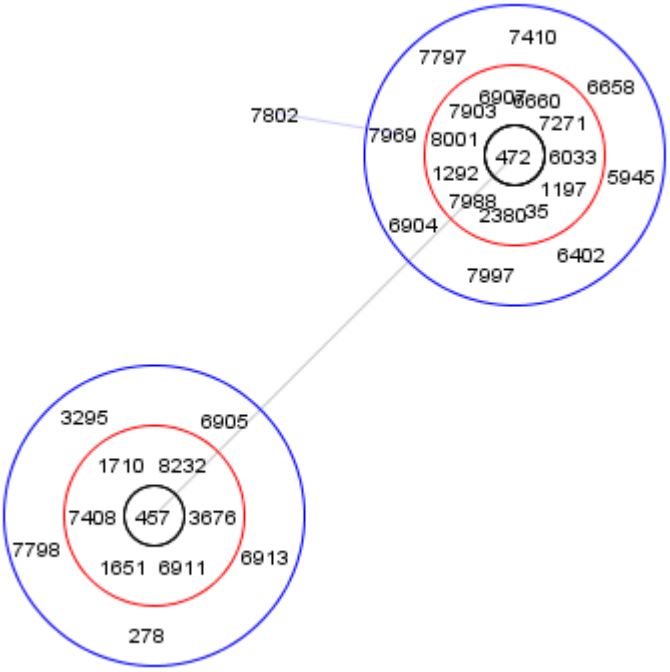

Figure 4.6 - Representation of BURST Group 8 of carried meningococci in Scotland, 1974 – 2004

Numbers refer to sequence types; ST within a black circle is the founder genotype or the subgroup founder genotype; an ST within a red circle is a single-locus variant (SLV) of the founder genotype; an ST within a blue circle is a DLV of the founder genotype; A grey line connecting a satellite ST with a ST within a group, or subgroup, indicates these STs are DLVs of each other. A grey line connecting central genotypes within different subgroups indicates these STs are SLVs of each other.

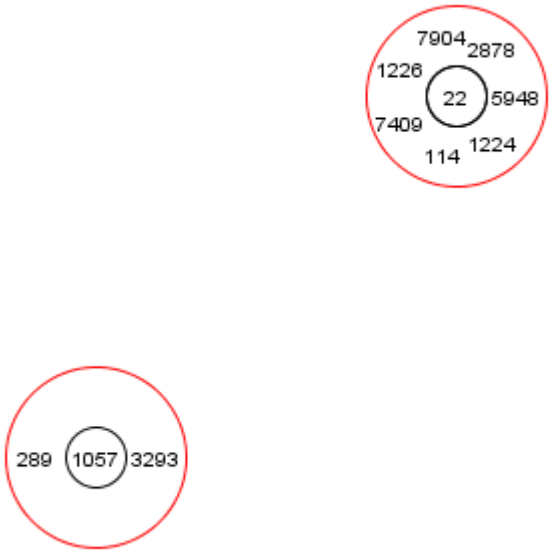

Figure 4.7 - Representation of BURST Group 9 of carried meningococci in Scotland, 1974 – 2004

Numbers refer to sequence types; ST within black circle is the founder genotype or the subgroup founder genotype; an ST within red circle is a single-locus variant (SLV) of the founder genotype.

A subgroup, with ST-1057 as its founder genotype, was also predicted. The STs were, ST-22, -114, -289, -1957, -1224, -1226, -2878, -3293, -5948, -7409 and ST-7904. Interestingly, ST-1057 and ST-3293 belong to cc174 yet they were assigned to this group using BURST. Group 10 consisted of three STs; the central genotype ST-212 and two SLVs of ST-1229 and ST-7618 which belong to the recently assigned cc212 (Figure 4.8).

Figure 4.8 - Representation of BURST Group 10 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.2

Group 11 consisted of twelve STs assigned to cc23/A3 with ST-23 predicted as the central genotype (Figure 4.9).

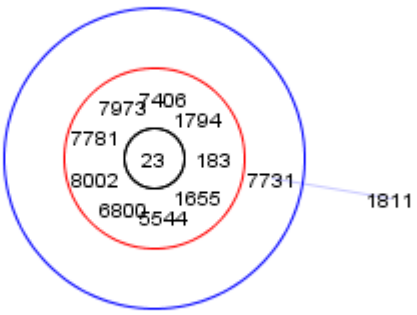

Figure 4.9 - Representation of BURST Group 11 of carried meningococci in Scotland, 1974 – 2004
Numbers refer to sequence types; ST within a black circle is the founder genotype; an ST within a red circle is a single-locus variant (SLV) of the founder genotype; an ST within a blue circle is a DLV of the founder genotype; A grey line connecting a satellite ST with a ST within a group, or subgroup, indicates these STs are DLVs of each other.

The STs were, ST-23, -183, -1655, -1794, -1811, -5544, -6800, -7406, -7731, -7781, -7973 and 8002 (Figure 4.9). Group 12 consisted of four STs that were not assigned to a known MLST complex; these were ST-336, -3621, -4221 and -7999. BURST predicted ST-336 to be the central genotype with the other three STs as SLVs of the central genotype (Figure 4.10).

Figure 4.10 - Representation of BURST Group 12 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.2

Group 13 consisted of three STs assigned to cc32/ET-5; the central genotype ST-32 and two SLVs of ST-33 and ST-259 (Figure 4.11).

Figure 4.11 - Representation of BURST Group 13 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.2

Group 14 consisted of five STs assigned to cc461; the central genotype ST-461, three SLVs (STs-6430, -6910 and -7972) and a DLV of ST-6403 (Figure 4.12).

Figure 4.12 - Representation of BURST Group 14 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.5

Group 15 consisted of 14 STs assigned to cc254 with BURST predicting ST-254 as the central genotype (Figure 4.13). Nine STs were SLVs (STs-1953, -2520, -2701, -3294, -4881, -6914, -6915, -7203 and -7968); two STs were DLVs (ST-468 and ST-7964) and two more were satellites (ST-7991 and ST-8221).

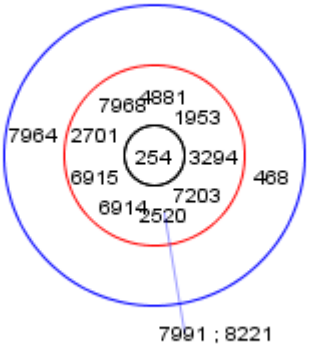

Figure 4.13 - Representation of BURST Group 15 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.9

Group 16 consisted of three STs assigned to cc198; STs-198, -823 and -7782. BURST predicted ST-823 to be the founding genotype; the other two STs being SLVs of ST-823 (Figure 4.14). Group 17 consisted of eight STs assigned to cc60; the central genotype ST-60, five SLVs (STs-1650, -2002, -2104, -2155 and -7724) and two DLVs (STs-1780 and -6903) (Figure 4.15). Group 18 consisted of three STs that were not assigned to a known cc; STs-5953, -5946 and -6663. BURST could not assign a central genotype. The former two STs are SLVs of each other, while the latter ST is a DLV of the former pair.

Figure 4.14 - Representation of BURST Group 16 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.2

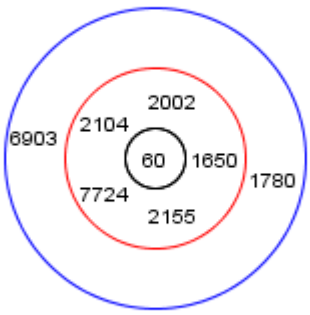

Figure 4.15 - Representation of BURST Group 17 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.5

Group 19 consisted of eight STs assigned to cc334 with BURST predicting ST-334 as the central genotype (Figure 4.16). These STs were ST-189, -334, -1031, -1034, -2508, -2526, -3285 and -3464. Three STs, ST-189, -1034 and -2526, are SLVs of ST-334 while two more, ST-1031 and ST-3285, are DLVs of ST-334. ST-2552 is also part of cc334 but was not assigned to this group by BURST.

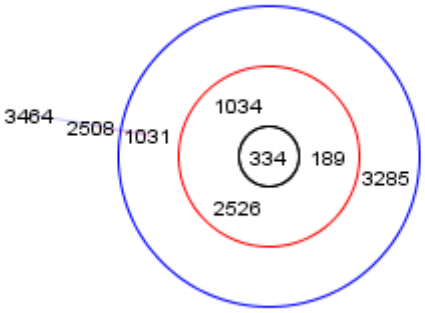

Figure 4.16 - Representation of BURST Group 19 of carried meningococci in Scotland, 1974 – 2004
Numbers refer to sequence types; ST within a black circle is the founder genotype; an ST within a red circle is a single-locus variant (SLV) of the founder genotype; an ST within a blue circle is a DLV of the founder genotype; A faint red line connecting a satellite ST with an ST within a group, or subgroup, indicates these STs are SLVs of each other; A grey line connecting a satellite ST with a ST within a group, or subgroup, indicates these STs are DLVs of each other.

Group 20 consisted of six STs assigned to cc11/ET-37 with ST-11 as the central genotype. Four STs, ST-67, -473, -1246 and -4677 are SLVs of ST-11 while ST-4037 is a DLV (Figure 4.17).

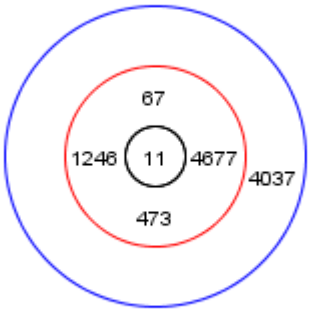

Figure 4.17 - Representation of BURST Group 20 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.5

Group 21 consisted of two STs, ST-7405 and ST-7265, assigned to cc376. Group 22 consisted of two STs, ST-2552 and ST-4597, the former being assigned to cc334 while the latter was unassigned. Group 23 consisted of four STs, ST-94, -7721, -7722 and -7966 assigned to cc92. ST-7966 was the predicted central genotype while the other three STs were DLVs (Figure 4.18).

Figure 4.18 - Representation of BURST Group 23 of carried meningococci in Scotland, 1974 – 2004
Legend as described in Figure 4.2

Group 24 consisted of two STs, ST-8 and ST-153 assigned to cc8. Group 25 consisted of four STs, ST-2154, -4878, -7967 and -7801 which were not assigned to a known cc and BURST did not predict a central genotype. Group 26 consisted of eight STs assigned to cc364; however no central genotype was assigned. These STs were ST-15, -413, -462, -2534, -4650, -7725, -7733 and -7965. Group 27 consisted of six STs assigned to cc178; however no central genotype was predicted by BURST. The six STs were ST-178, -5949, -6662, -6912, -7987 and -7974. Group 28 consisted of three STs which are not assigned to a known cc; these were ST-2528, -3258 and -7267.

Group 29 consisted of two STs; ST-185 assigned to cc174 and ST-1251 that is not assigned to a known cc. Group 30 consisted of three STs that are not assigned to a known cc; ST-3287, -7413 and -8222. Group 31 consisted of two more unassigned STs; ST-288 and ST-7970. Group 32 consisted of a further two unassigned STs; ST-961 and ST-5947. Group 33 also consisted of two STs; ST-2547 assigned to cc174 and the unassigned ST-7930. Group 34 consisted of two more unassigned STs; ST-1162 and ST-7727. Group 35 consisted of two further

unassigned STs; ST-6661 and ST-7412. While Group 36 consisted of a pair of STs, ST-2239 and ST-6908, assigned to cc269 and Group 37 consisted of a pair of STs, ST-460 and ST-865, assigned to cc865.

Many singleton STs (33/49) were not assigned to a known cc; these were ST-284, -344, -1220, -1455, -2152, -2539, -2875, -4053, -4385, -4874, -5952, -6032, -6654, -6656, -6659, -7268, -7272, -7399, -7404, -7407, -7620, -7622, -7723, -7726, -7729, -7732, -7905, -7975, -7989, -7990, -7998, -8003 and -8066. Twenty-three of these singleton genotypes were assigned as new STs during this study. The *N. meningitidis* PubMLST website/database (www.pubmlst.org/neisseria) was inspected for further information regarding the remaining ten singletons not assigned to a known clonal complex. This identified that several of the singleton STs had previously been identified from carriage in the UK and Europe; some were identified as part of the UK carriage study investigating the effects of the meningococcal serogroup C polysaccharide-conjugate (MCC) vaccination campaign conducted during 1999 – 2001. Those STs previously identified from carriage were ST-1220, -2152, -4053 -4385 and ST-4874. A few singleton STs were previously identified from cases of IMD in the UK and Europe; STs- 284, -1220, -1455 and -2539. Additionally, the ATCC 13090 serogroup B isolate sequenced in this study as ST-2875 matched one record on the website as this ST/serogroup combination was previously identified in the strain NCTC 10026.

The *N. meningitidis* MLST website was also inspected for further information regarding the sixteen singleton STs assigned to known clonal complexes. ST-1 is the central genotype of cc1/subgroup I/II that has been a globally prevalent lineage of serogroup A responsible for causing invasive disease at least since the 1960s. Also sampled in carriage, this lineage has at one time or another been prevalent in Europe, Africa, Asia, Australia and New Zealand. This lineage has become less prevalent in more industrialised countries however it currently remains a problem in the African continent. ST-5 is the central genotype of cc5/subgroup III that has been a globally prevalent lineage of serogroup A responsible for causing invasive disease at least since the 1960s. Occasionally isolated from carriers; this lineage has been prevalent in the African continent, Europe, Asia, Saudi Arabia and South America. ST-103 is the central genotype of cc103; particularly found in carriers associated with different serogroups (NG, B, C, Z, A, 29E) and found to cause invasive disease (serogroup B/C). Records on

the MLST website detail isolates originating from Norway, Greece, Czech Republic, Germany, Spain, Ireland, Israel, Cuba and Brazil. ST-162 is the central genotype of cc162 isolated from carriers (mostly B/NG; one A/W135) and invasive disease (mostly B, some NG). Records on the PubMLST website detail isolates originating from several European countries (Germany, Spain, Greece, France, Norway and Czech Republic), Taiwan and the USA during 1998 – 2010.

ST-1011 is assigned to the cc231. Records on the PubMLST website indicate this lineage has been circulating in the Czech Republic since the early 1970s and has been found mainly in carriage; however it is capable of causing disease. These isolates were of serogroup B, C or were designated NG. ST-1117 is the central genotype of cc1117. This lineage has repeatedly been isolated from carriers in Europe during 1999 – 2009, particularly NG isolates during carriage studies in the UK, Germany and the Czech Republic. In addition, records describe isolates from Ireland, France, Norway and the USA. This lineage was also reportedly isolated from a case of invasive disease in the Czech Republic during 2005; the isolate was NG (ST-4788).

Records on the PubMLST website indicate ST-1466 (cc174) has been identified in serogroup Y strains from cases of invasive disease in the UK and France during 2001-2002. ST-1574 (cc41-44) has also been identified during 2001 in the UK from cases of serogroup B invasive disease. ST-1802 is assigned to the cc282 and was previously identified during the UK carriage study (Stockport; 1999) in a serogroup B isolate. Similarly, ST-1839 (cc254) was also identified in Stockport in 1999, in a NG isolates, as part of the UK carriage study. Records on the PubMLST database indicate ST-1947 (cc41-44) was previously identified in Norway during 1996 in carriage isolates of serogroup C or NG and in Cuba during 1998-1999 in carriage isolates of serogroup B or NG. This ST was also identified from strains of serogroup B causing invasive disease in Spain during 2001-2004.

4.7 Analysis of the concatenated MLST DNA sequences

The eBURST algorithm allows lineage assignment on the basis of relatedness at the level of the allelic profile and, as such, any relationships inferred by this method are less reliable than that based upon the direct analysis of the DNA sequences themselves. For this reason, lineage assignment of the carriage strain collection was performed using the UPGMA algorithm in STARTv2 from the concatenated sequences (p-distances) of one isolate of each ST identified in the carriage strain collection in addition to the four ATCC and four NCTC isolates (Figure 4.19). Overlaid onto the UPGMA tree was information on the clonal complex to which each ST was assigned; cc1, solid red circle or sphere; cc5, solid orange circle or sphere; cc8, yellow solid circle or sphere; cc11, light green solid circle or sphere; cc22, dark green solid circle or sphere; cc23, blue solid circle or sphere; cc32, open square or rectangle; cc35, light blue solid circle or sphere; cc41/44, pink solid circle or sphere; cc53, purple solid circle or sphere; cc60, brown solid circle or sphere; cc92, black solid circle or sphere; cc103, light grey solid circle or sphere; cc162, dark grey solid circle or sphere; cc167, peach solid circle or sphere; cc174, pale green solid circle or sphere; cc175; red solid square or rectangle; cc178, red solid square or rectangle; cc198, yellow solid square or rectangle; cc212, bright green solid square or rectangle; cc213, dark green solid square or rectangle; cc231, blue solid square or rectangle; cc254, light blue solid square or rectangle; cc269, pink solid square or rectangle; cc282, purple solid square or rectangle; cc334, brown solid square or rectangle; cc364, black solid square or rectangle; cc376, light grey solid square or rectangle; cc461, dark grey solid square or rectangle; cc750, peach solid square or rectangle; cc865, pale green solid square or rectangle; cc1117 pale pink solid square or rectangle; cc1136, pale blue solid square or rectangle; cc1157, pale purple solid square or rectangle; UA, unassigned, open circle (Figures 4.20 – 4.23).

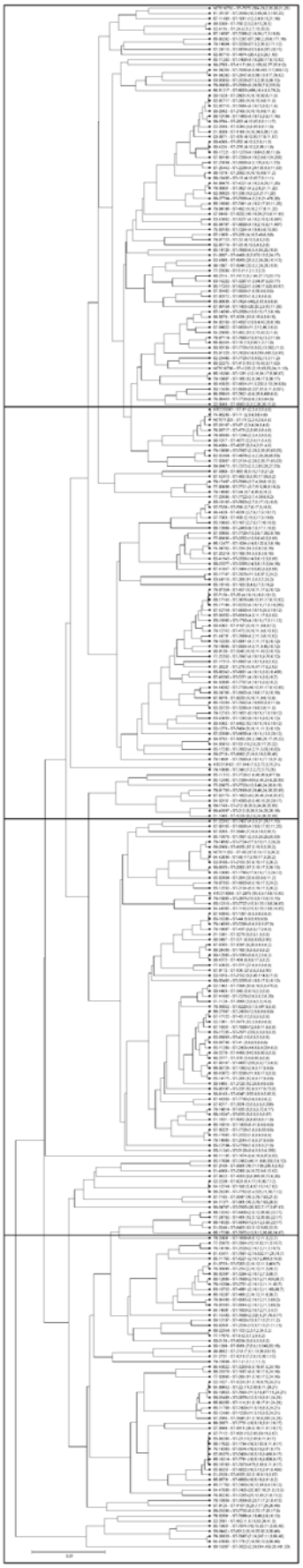

Figure 4.19 - UPGMA tree generated from the concatenated MLST DNA sequences of carried meningococci in Scotland, 1974 – 2004

a, area of UPGMA tree shown in Figure 4.20; b, area of UPGMA tree shown in Figure 4.21; c, area of UPGMA tree shown in Figure 4.22; d, area of UPGMA tree shown in Figure 4.23

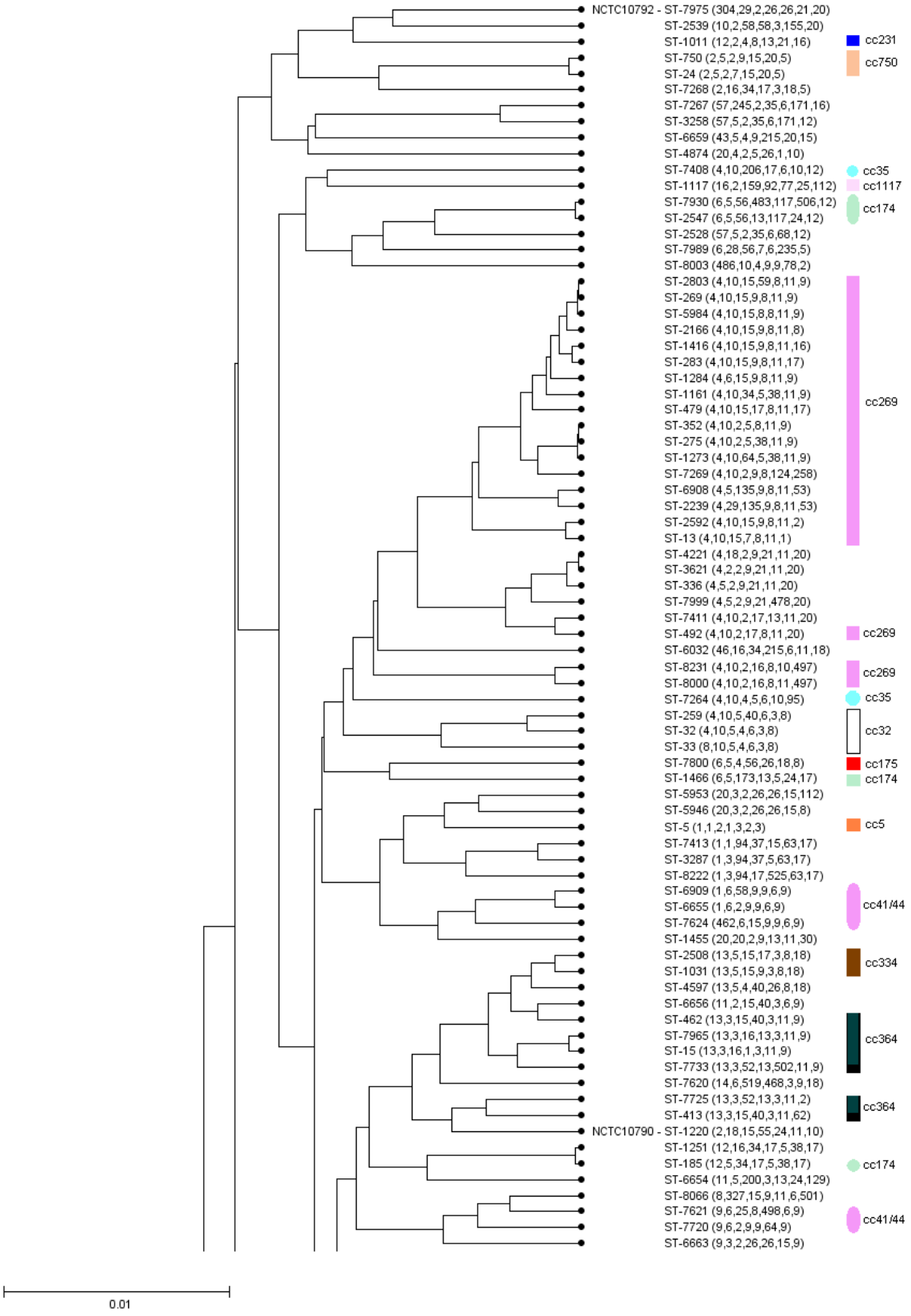

Figure 4.20 - Enlargement of section a of the UPGMA tree in Figure 4.19

For legend see the text on page 146

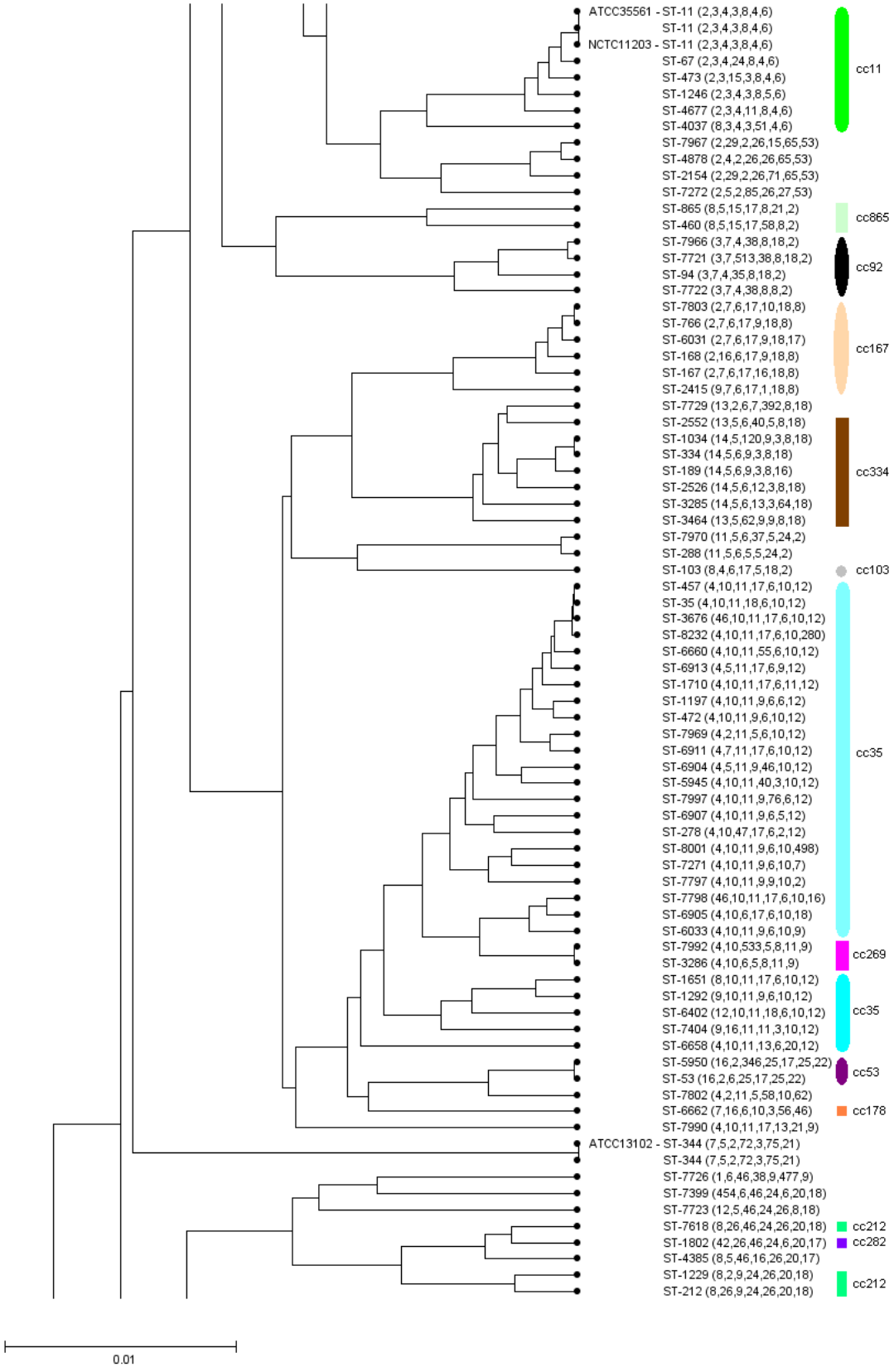

Figure 4.21 - Enlargement of section b of the UPGMA tree in Figure 4.19

For legend see the text on page 146

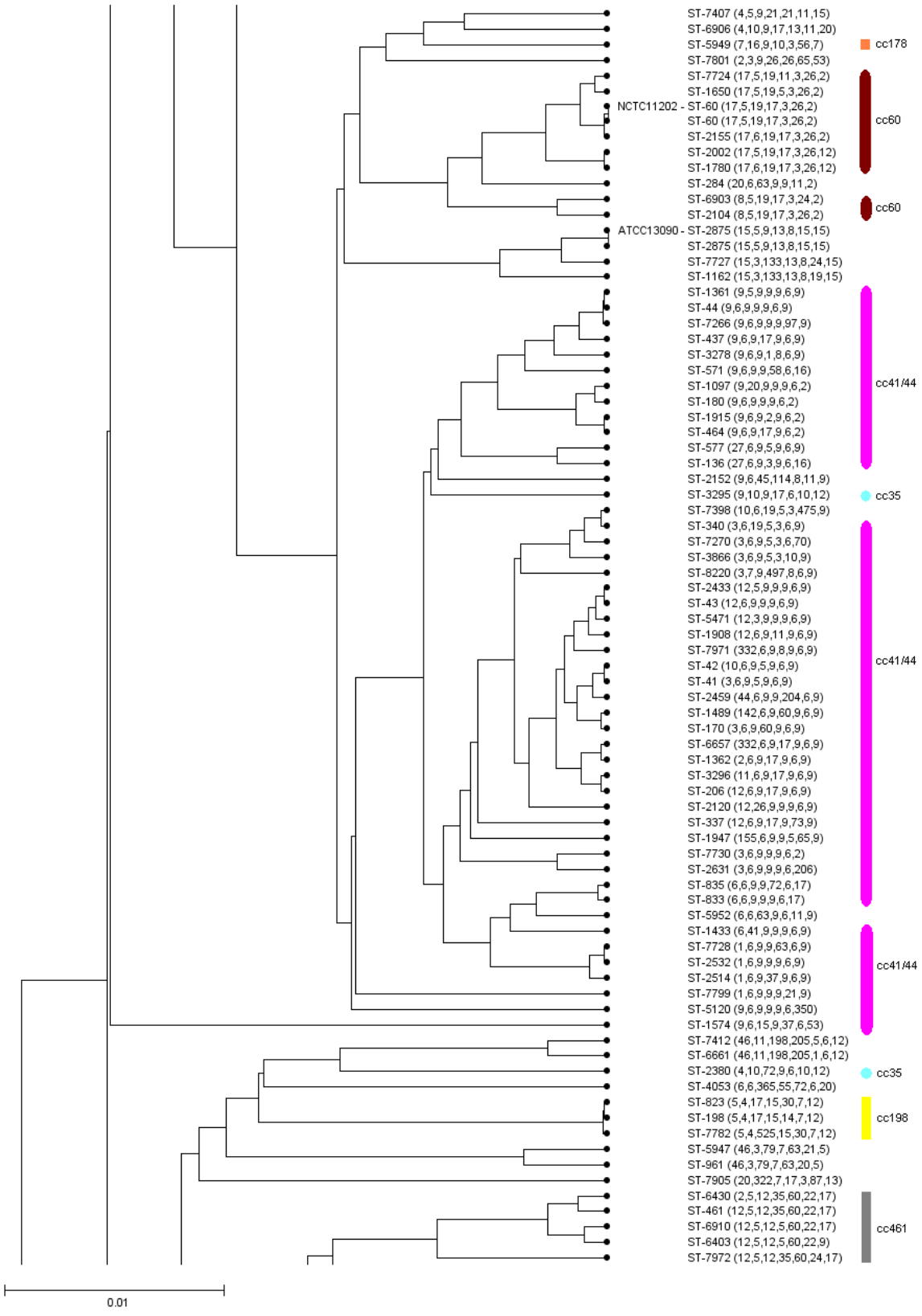

Figure 4.22 - Enlargement of section c of the UPGMA tree in Figure 4.19

For legend see the text on page 146

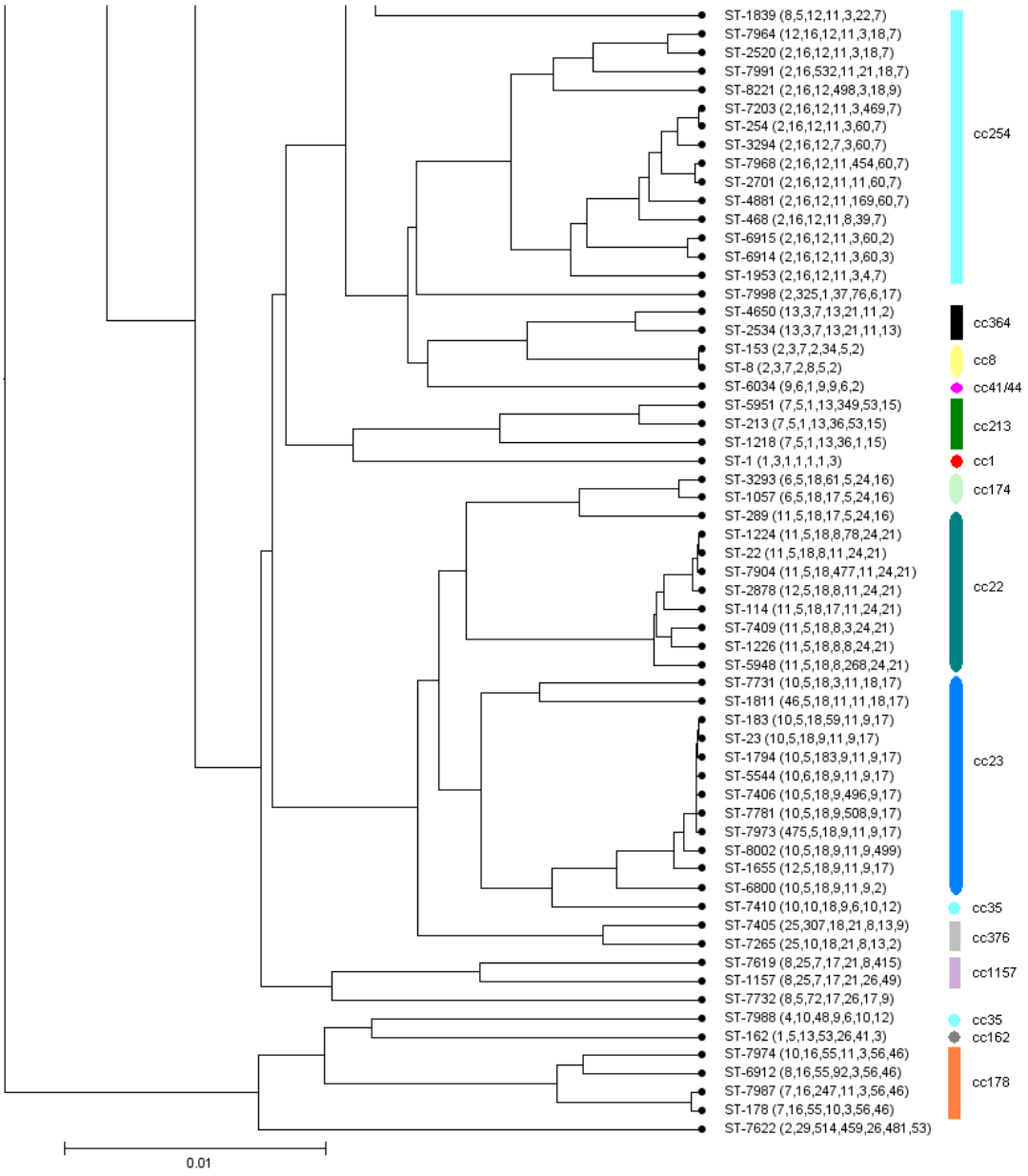

Figure 4.23 - Enlargement of section d of the UPGMA tree in Figure 4.19

For legend see the text on page 146

In the previous section (4.6), BURST was unable to group a few STs, known to be assigned to a particular MLST cc, with other members of the cc. For example, ST-2239 and ST-6908 were not grouped with other members of cc269 (BURST Group 2; Figure 4.3). However, they were placed together in Group 36. A third ST, ST-7269 was designated a singleton by BURST. In section a of the UPGMA tree of the concatenated MLST DNA sequences (Figure 4.20) we can see that the three aforementioned STs do indeed cluster with other STs of cc269. Furthermore, in the previous section (4.6) BURST was unable to group ST-1574,

ST-1947, ST-7621 and ST-8220 with other members of cc41/44 (BURST Group 3; Figure 4.4). Three of these aforementioned STs, ST-1574, ST-1947 and ST-8220, do however cluster together with other STs of cc41/44 in section c of the UPGMA tree of the concatenated MLST DNA sequences (Figure 4.22). ST-7621 does not cluster with the main group of cc41/44 STs but instead clusters in section a (Figure 4.20) next to ST-7720. Similarly, ST-7264 which did not group with other cc35 STs in BURST Group 8 (Figure 4.6) also did not cluster with the main group of cc35 STs in the UPGMA tree (section b; Figure 4.21). Instead, ST-7264 can be found in section a (Figure 4.20). In section 4.6, ST-2552 could not be assigned by BURST with other members of cc334 (Group 19; Figure 4.16). In the UPGMA tree, ST-2552 does cluster with ST-1031 in section a (Figure 4.20) however, neither of these STs cluster with the main group of cc334 STs in section b (Figure 4.21). If we consider STs unassigned to a clonal complex, several STs which had been grouped together by BURST also cluster together in the UPGMA tree of the concatenated MLST DNA sequences. For example, STs in BURST Group 12 (ST-336, ST-3621, ST-4221 and ST-7999) cluster together in section a (Figure 4.20) as do STs in BURST Group 30 (ST-3287, ST-7413 and ST-8222). STs in BURST Group 31 (ST-288 and ST-7970) cluster together in section b (Figure 4.21). Furthermore, STs in BURST Group 32 (ST-961 and ST-5947), Group 34 (ST-1162 and ST-7727) and Group 35 (ST-6661 and ST-7412) all cluster in section c (Figure 4.22).

In contrast, some STs assigned together in groups by BURST do not cluster together fully in the UPGMA tree of the concatenated MLST DNA sequences. In BURST Group 18, ST-6663 does not cluster together in section a (Figure 4.20) with ST-5953 and ST-5946. In BURST Group 25, ST-7801 (section c; Figure 4.22) does not cluster together with ST-2154, ST-4878 and ST-7967 (section b; Figure 4.21). In BURST Group 28, ST-2528 does not cluster together with ST-3258 and ST-7267 in section a (Figure 4.20). In the UPGMA tree of the concatenated MLST DNA sequences, there are several examples of STs belonging to designated ccs which do not cluster together with other STs of the same designated cc. For example, ST-7408 (section a; Figure 4.20), ST-7264 (section a; Figure 4.20), ST-3295 (section c; Figure 4.22), ST-2380 (section c; Figure 4.22), ST-7410 (section d; Figure 4.23) and ST-7988 (section d; Figure 4.23) cluster separately from the main cc35 cluster (section b; Figure 4.21). Furthermore, the main cc35 cluster is interrupted by two STs, ST-3286 and ST-7992, assigned to cc269. ST-6909, ST-

6655 and ST-7624 cluster together, as do ST-7621 and ST-7720, in section a of the UPGMA tree (Figure 4.20) but not with the main cc41/44 cluster in section c (Figure 4.22). Furthermore, the main cc41/44 cluster is also interrupted by STs not assigned to this cc. Similarly, the main cc269 cluster in section a (Figure 4.20) is interrupted by STs not assigned to this cc.

4.8 Discussion

This study is the first of its kind to characterise a collection of carried meningococci in Scotland spanning more than three decades. MLST of carried meningococci from Scotland over a 31-year period has identified unique allele sequences and unique allelic profiles. Predominantly, the new STs were not assigned to any of the currently defined clonal complexes, further indicating the extensive variation within circulating meningococcal strains that remains unidentified. Continued surveillance of circulating meningococcal lineages is of particular importance as this allows the identification of previously unrecognised clonal complexes, such as the recently defined cc1117, cc1136, cc212, cc178 and cc282. Furthermore, it enables the relative prevalence of lineages to be effectively monitored, at the national level, should any temporal changes occur.

The majority of carriage isolates (87.7%) were assigned to a currently defined cc; a comparatively small proportion of isolates were not. Other studies have observed a higher proportion of unassigned sequence types. This may be due, in part, to the increasing number of defined ccs. In 2003 the ST-41 complex was expanded to include all STs that match ST-44 at four or more loci, and as such, was renamed the ST-41/44 complex. Since then a further nineteen ccs have been defined; one in 2003, one in 2004, nine in 2005, two in 2006, one in 2009 and five, so far, during 2010 (www.pubmlst.org/neisseria). It would seem unlikely that all the possible variation in meningococcal strains could be catalogued; however it may be possible that, in the future, fewer strains would be characterised as “unassigned” to a clonal complex as more laboratories adopt MLST as a typing tool. Many of the original MLST ccs were based on the clonal groupings assigned to strains on the basis of MLEE and its electrophoretic types

(ETs) such that these lineages are named using both nomenclature e.g. the ST-11/ET-37 complex.

Historically, studies of carried meningococci, based on MLEE studies conducted during the 1980s, found that the prevalence of particular clones responsible for the majority of systemic disease was greatly reduced in carried meningococci. Furthermore, the most prevalent clonal complex amongst carriage isolates had not previously been identified amongst disease-associated strains (Caugant, Bovre *et al.* 1986; Caugant, Kristiansen *et al.* 1988). It is particularly important, therefore, to monitor carried meningococci for the emergence of new lineages; especially STs not currently assigned to defined clonal complexes. While many of these STs are not related to defined ccs, groupings between these unassigned STs may still occur; it is also possible that any emergent lineages may come from this pool of unassigned STs. MLST has more commonly been applied to characterise invasive disease isolates. As the scientific community continues to characterise a greater proportion of carried meningococci other carriage-associated lineages may be identified.

Clonal complexes were identified at varying frequencies amongst the carriage strain collection. We have shown, as have others (Jolley, Kalmusova *et al.* 2000; Jolley, Kalmusova *et al.* 2002; Yazdankhah, Kriz *et al.* 2004; Climent, Yero *et al.* 2010a; Climent, Urwin *et al.* 2010b), that "hyperinvasive" clonal complexes can be identified amongst meningococci isolated from healthy carriers. Jolley and colleagues, in their study of carried meningococci isolated in the Czech Republic in 1993, identified three hyperinvasive clonal complexes; cc41-44/Lineage 3, cc11/ET-37 and cc32/ET-5 (Jolley, Kalmusova *et al.* 2000; Jolley, Kalmusova *et al.* 2002). The prevalence of the cc41-44/Lineage 3 was remarkably similar in our study compared to Jolley *et al.* (2002). Cc11/ET-37 (15.2%) was the most prevalent cc amongst the Czech isolates, whereas this cc only accounted for 2.5% of the carriage strain collection presented herein. Cc32/ET5 was identified in 2.4% of the carriage strain collection in this study while only one isolate was identified amongst the Czech carrier strain. Contrastingly, the prevalence of cc8 was much higher in this study (8.0% versus 0.6%) compared to the Czech study (Jolley, Kalmusova *et al.* 2000; Jolley, Kalmusova *et al.* 2002). In the Czech study and in this study the prevalence of serogroup C was higher than expected; likely due to the recent spread of cc11/ET-37 in the Czech Republic in the early

1990s (Jolley, Kalmusova *et al.* 2000; Jolley, Kalmusova *et al.* 2002) and cc8/Cluster A4 in Scotland during the latter part of 1984 and early 1985 (Mathew and Chaudhuri 1986; Thomson and Jackson 1987).

In a comparison of carried and disease isolates from the Czech Republic, Greece and Norway many carried isolates from all countries were not assigned to any of the clonal complexes defined at that time (Yazdankhah, Kriz *et al.* 2004). Geographic variation between countries in the prevalence of some ccs was also observed. In Greece cc162 was the most prevalent cc amongst carried meningococci, however this cc was absent from the carrier collections from Norway and the Czech Republic. Cc23 was absent from the Czech Republic carrier collection but was the second most prevalent cc amongst the carrier collections from Norway and Greece. Cc23 was previously found to be associated with carriage (Yazdankhah, Kriz *et al.* 2004). In the carriage strain collection in this study cc23 was the fifth most prevalent cc, and ST-23 was the fourth most prevalent ST. Cc92, cc106 and cc116 were only found in the Czech Republic carrier collection (Yazdankhah, Kriz *et al.* 2004). These three ccs were also common in the previously reported carrier collection from 1993 (Jolley, Kalmusova *et al.* 2000; Jolley, Kalmusova *et al.* 2002). Cc11, however, was much less prevalent in the carrier collection (Yazdankhah, Kriz *et al.* 2004) despite accounting for the majority of the invasive disease collection during the same period (Yazdankhah, Kriz *et al.* 2004) or the Czech carrier isolate collection (Jolley, Kalmusova *et al.* 2000; Jolley, Kalmusova *et al.* 2002).

We identified, in the carriage strain collection, STs belonging to four lineages, cc53, cc198, cc1117 and cc1136 previously identified in carried meningococci from Bavaria, Germany by Claus and colleagues to lack the genes for capsule synthesis and transport and instead possess the capsule null locus (*cnl*) (Claus, Maiden *et al.* 2002). The four lineages were also identified during the UK meningococcal carriage study in carried meningococci obtained from 16-18 year olds attending schools in Glasgow (Dr Mathew Diggle, personal communication). During the UK carriage study, 1256 meningococci isolated in Glasgow were characterised by MLST into 31 clonal complexes; 30 of which were identified amongst the carriage strain collection in this study. Fewer strains from the UK carriage study compared to the carriage collection described herein were not assigned to currently defined clonal complexes; 7.24% versus 12.3%.

In Cuba, from two recent studies comparing carried and disease isolates collected during 1983 – 2005, cc53 was associated with carriage (Climent, Yero *et al.* 2010a; Climent, Urwin *et al.* 2010b). Interestingly one disease isolate from Cuba was found to belong to the cc53. The Cuban studies also identified cc198 among carrier isolates (Climent, Yero *et al.* 2010a; Climent, Urwin *et al.* 2010b) as did Yazdakhah and colleagues (Yazdankhah, Kriz *et al.* 2004). There have been three reports of *cnl*-containing lineages isolated from cases of IMD; two in immunocompromised individuals and one from an apparently immunocompetent individual (Vogel, Claus *et al.* 2004b; Hoang, Thomas *et al.* 2005; Findlow, Vogel *et al.* 2007). As yet, no reported cases of IMD in Scotland have been caused by *cnl*-containing meningococci (Dr. M. A. Diggle; personal communication). Climent and colleagues did not determine whether NG isolates possessed or lacked genes encoding for capsule polysaccharide synthesis nor did they determine the presence of the capsule null locus so it is unclear whether this isolate possessed the *cnl* or whether the strain had the genetic material to produce capsular polysaccharide but was unable to do so due to some unknown mutation that impaired synthesis or transport the capsular polysaccharide.

Odds ratios were used to estimate the invasiveness of individual clonal complexes and STs identified in Scotland during 1974 – 2004 using the carriage strain collection described herein and data on invasive meningococcal disease isolates provided by the SHLMPRL. Cc1, cc8, cc11, cc32, cc41-44, and cc269 and their respective founder STs were identified as being significantly associated with invasive disease. In addition two more STs belonging to two of the aforementioned ccs were also associated with invasive disease. More recent data from the SHLMPRL indicates that cc269 and cc41-44 are most commonly observed ccs in case of invasive disease in Scotland (Ure 2008). In comparison, Climent and colleagues also found cc8, cc32 and cc41-44 were associated with disease in Cuba; cc32 was the most frequently identified in 61.4% of 373 isolates (163 cases, 210 carriers) over a 20 year period (Climent, Yero *et al.* 2010a). This was followed by cc53 (19.6%), cc41/44 (7.2%) and cc103 (2.7%). Furthermore, cc11/ET-37 was associated with disease in the Czech Republic, Greece and Norway (Yazdankhah, Kriz *et al.* 2004). In England and Wales, cc8, cc11, cc32, cc41-44 and cc269 were also significantly associated with invasive disease (Ure, Gray *et al.* 2004).

The observation that the "hyperinvasive" lineages were identified as being associated with invasive disease in Scotland is not unexpected. Of greater importance is knowledge of the STs and lineages found to be significantly associated with a carriage phenotype in Scotland; this included cc22, cc23, cc167, cc213, cc254, cc750 and cc1175 and their respective founder STs, in addition to other ccs and STs. Interestingly, two STs belonging to cc41-44 were significantly associated with a carriage phenotype despite the observation that cc41-44 was significantly associated with an invasive phenotype. This leads one to question why there should be such a difference in phenotype between members of the same genetic lineage.

Ure and colleagues also observed that cc22, cc23, and cc213 were negatively associated with invasive disease (Ure, Gray *et al.* 2004) whilst Yazdankhah and colleagues also found the cc23 complex was associated with carriage (Yazdankhah, Kriz *et al.* 2004). Climent and colleagues reported that cc53 was associated with carriage (Climent, Yero *et al.* 2010a; Climent, Urwin *et al.* 2010b) and while this lineage was prevalent amongst the carriage strain collection in this study, its absence from the IMD strain collection in Scotland prevents the calculation of an odds ratio; and hence an estimation of its invasive or carriage potential. This was also the case for several other STs or ccs. Cc53 cnl-positive meningococci are commonly carried and often described phenotypically as NG:NT:P1.7. This lineage is clearly associated with carriage and not with IMD.

Analysis of the dataset with eBURST revealed some discrepancies in the assignment of individual STs to particular groupings. Five STs assigned in this study were identified as members of assigned clonal complexes; ST-7264 (cc35), ST-7269 (cc269), ST-7621 (cc41-44), ST-7800 (cc175) and ST-8220 (cc41-44); however eBURST did not assign them to groupings containing other STs belonging to the same cc. eBURST was used with the group definition set where each ST in a group matches at least one other ST in the group at five or more loci. A small number of STs assigned to defined ccs were not grouped by eBURST as these STs had more than two differences in their allelic profile from other STs identified in this study that were assigned to known clonal complexes. Intermediary STs not sampled during this study would link those STs not grouped to the remaining members of the complex with the groupings defined by eBURST.

BURST uses a simple model of bacterial evolution in which an ancestral (or founding) genotype increases in frequency in the population, and while doing so, begins to diversify to produce a cluster of closely-related genotypes that are all descended from the founding genotype. This cluster of related genotypes is referred to as a "clonal complex". The relationships inferred by BURST are based upon relatedness at the level of the allelic profile and not directly at the DNA sequence level. Analysis of the concatenated MLST DNA sequences was undertaken to further examine the relationships between different meningococcal lineages identified during this study. UPGMA is generally not considered a good algorithm for construction of phylogenetic trees as it relies on the rates of evolution among different lineages to be approximately equal. In the study of bacterial population biology this is likely not to be the case, so the method should not be relied upon to cluster strains without artefacts. This was evident in the tree constructed from the concatenated MLST DNA sequences of the carried meningococcal strain collection characterised during this study. There were several examples of STs of different lineages clustering together with seemingly unrelated lineages belonging to different ccs (Figures 4.20 – 4.23). There are clearly deficiencies with both BURST and UPGMA however, analysis of the MLST allelic profiles and the concatenated MLST DNA sequences required the use of both methods.

Despite the observed temporal changes in the prevalence of individual clonal complexes, indices of diversity for the carriage strain collection during different time periods were not significantly different from each other or from the strain collection as a whole. However, the carriage strain collection was genetically more diverse than the IMD strain collection based on Simpson's Index of diversity. Furthermore, measurement of *D* within the IMD strain collection also indicated significant temporal changes in genetic diversity.

Analysis of the temporal distribution of ccs indicated major differences in the prevalence of certain clonal complexes, and trends in the data revealed whether ccs had increased, decreased or remained the same during the 31-year period covered by this study. For example, a dramatic reduction in cc1 has been observed and this lineage appears to have disappeared from the meningococcal population in Scotland. This contrasts with the recent emergence of cc213 strains (Ibarz-Pavon, Brehony *et al.* 2004). Despite an apparent increase in

prevalence between the second and third time periods it should be noted, however, that the prevalence of cc11 lineage has actually declined; mostly as a result of the introduction of the serogroup C polysaccharide-conjugate vaccine into the UK childhood vaccination schedule in November 1999 due to the strong association of serogroup C with the ST-11/ET-37 complex.

As previously discussed, comparisons of IMD and carriage strain collections in other countries have highlighted striking differences. Yazdankhah and colleagues presented a comparison of carried and disease-causing meningococci from three European countries; the Czech Republic, Greece and Norway (Yazdankhah, Kriz *et al.* 2004). However, the isolates studied were not appropriately matched temporally between countries or geographically within in any of the three countries. The carriage isolates were from only a few different distinct regions within the different countries while the disease isolates included represented only a subset of the total collection of disease isolates in each country (Yazdankhah, Kriz *et al.* 2004); as such there may be geographical bias or the isolates may not be entirely representative of the circulating strains within each country.

5 PorA variable region sequence analysis of carried meningococci in Scotland, 1974 - 2004

5.1 Introduction

The following chapter details the frequency of the PorA variable regions VR1, VR2 and VR3 deduced from the generated nucleotide sequences; the frequency of PorA subtypes using VR1 & VR2 data; the frequency of PorA subtypes inclusive of VR3 data; an examination of the temporal distribution of the major PorA subtypes (VR1,VR2) and an examination of the potential coverage of PorA subtypes in carried meningococci by licensed or experimental PorA-based OMV vaccines. This chapter also discusses data on PorA subtypes in the invasive meningococcal (IMD) strain collection. The relationship between PorA subtypes and data on serogroup, clonal complex or sequence type is presented later (Chapter 6). PorA VR1, VR2 and VR3 are located on Loop I, Loop IV and Loop V of the PorA protein, respectively. VRs are grouped into families and variants within families are assigned on the basis of amino acid sequence relationships. Groupings are determined with 80% amino acid identity as a guide, or where identity is below 80%, the presence of a particular motif that is representative of a VR family (Russell, Jolley *et al.* 2004). Variants were assigned in order of discovery following submission of DNA sequence trace files to the PorA database curator (www.pubmlst.org/neisseria). For example, VR families have the prefix P1. , followed by a number corresponding to the family, e.g. P1.5. Each variant is prefixed by the number corresponding to the family, e.g. 5- , followed by a number corresponding to a particular variant, e.g. 5-24.

5.2 Variable region 1 (VR1)

Translation of the *porA* DNA sequences of carried meningococci identified 45 individual variants of VR1 (Table 5.1) that could be grouped into nine of the currently defined VR1 families. Six of these variants had not previously been sequenced before and these were submitted to the PorA database curator for

verification and assignment of a new variant (Table 5.2). The new VR1 variants accounted for 13.3% of all VR1 variants sequenced; this was equivalent to 0.9% of specimens of the carriage strain collection. In eighteen specimens VR1 was not determined because a PCR product could not be amplified using the method outlined in section 2.5.4 (PorA genotypic characterisation). Seventeen (37.8%) VR1 variants were observed only once accounting for 2.4% of the carriage strain collection. This was in contrast with a further 17 variants which were observed over at least 20 years accounting for 719 (90%) of the carriage strain collection.

Table 5.1 – Frequency of PorA variable region peptides identified in carried meningococci in Scotland, 1974-2004

VR1	Frequency (%)	VR1	Frequency (%)
5	101 (12.6)	5-4	2 (0.25)
18-1	91 (11.4)	5-11	2 (0.25)
19	87 (10.9)	17-1	2 (0.25)
5-1	65 (8.1)	**19-33**	2 (0.25)
7-2	61 (7.6)	20	2 (0.25)
22	56 (7.0)	5-18	1 (0.13)
7	53 (6.6)	**5-24**	1 (0.13)
21	46 (5.8)	**5-29**	1 (0.13)
22-1	34 (4.3)	**5-31**	1 (0.13)
5-2	30 (3.8)	**7-32**	1 (0.13)
7-1	22 (2.8)	12	1 (0.13)
12-1	20 (2.5)	12-16	1 (0.13)
18	19 (2.4)	18-4	1 (0.13)
21-2	13 (1.6)	18-9	1 (0.13)
17	11 (1.4)	19-2	1 (0.13)
18-7	11 (1.4)	19-15	1 (0.13)
19-1	10 (1.3)	**19-32**	1 (0.13)
19-3	6 (0.75)	21-10	1 (0.13)
21-1	5 (0.63)	21-13	1 (0.13)
21-2	5 (0.63)	22-2	1 (0.13)
7-4	4 (0.50)	22-11	1 (0.13)
18-3	3 (0.38)	22-21	1 (0.13)
5-3	2 (0.25)	ND	18 (2.2)

VR2	Frequency (%)	VR2	Frequency (%)	VR2	Frequency (%)
2	95 (11.9)	16-3	4 (0.50)	**9-14**	1 (0.13)
3	87 (10.9)	16-4	4 (0.50)	**9-17**	1 (0.13)
15	66 (8.3)	9-5	3 (0.38)	10-12	1 (0.13)
16	62 (7.8)	10-2	3 (0.38)	10-23	1 (0.13)
13-1	53 (6.6)	13-7	3 (0.38)	**10-81**	1 (0.13)
14	48 (6.0)	16-16	3 (0.38)	13-5	1 (0.13)
2-2	37 (4.6)	30-1	3 (0.38)	13-6	1 (0.13)
1	28 (3.5)	30-2	3 (0.38)	14-3	1 (0.13)
10-4	28 (3.5)	34	3 (0.38)	15-10	1 (0.13)
9	24 (3.0)	4-1	2 (0.25)	15-39	1 (0.13)
15-1	21 (2.6)	9-7	2 (0.25)	**15-41**	1 (0.13)
30	21 (2.6)	10-3	2 (0.25)	16-5	1 (0.13)
4	18 (2.3)	10-8	2 (0.25)	16-13	1 (0.13)
16-8	15 (1.9)	10-9	2 (0.25)	16-21	1 (0.13)
28	15 (1.9)	13-15	2 (0.25)	**16-93**	1 (0.13)
10	14 (1.8)	15-11	2 (0.25)	**16-96**	1 (0.13)
13	12 (1.5)	23	2 (0.25)	**16-102**	1 (0.13)
14-6	10 (1.3)	25-1	2 (0.25)	23-2	1 (0.13)
30-3	10 (1.3)	25-6	2 (0.25)	23-3	1 (0.13)
25	8 (1.00)	25-11	2 (0.25)	25-7	1 (0.13)
26	8 (1.00)	2-5	1 (0.13)	25-9	1 (0.13)
2-1	6 (0.75)	**2-58**	1 (0.13)	25-25	1 (0.13)
10-1	6 (0.75)	3-1	1 (0.13)	30-14	1 (0.13)
10-57	6 (0.75)	4-2	1 (0.13)	**44**	1 (0.13)
13-2	5 (0.63)	9-3	1 (0.13)	ND	17 (2.1)

VR3	Frequency (%)
36-2	221 (27.7)
38	142 (17.8)
35-1	139 (17.4)
36	130 (16.3)
37-1	74 (9.3)
37	36 (4.5)
38-1	18 (2.3)
35	15 (1.9)
36-1	5 (0.63)
40	1 (0.13)
new[a]	1 (0.13)
ND	17 (2.1)

VR1, Variable region 1; VR2, Variable region 2; VR3, Variable region 3; ND, not determined; new VR1 and VR2 variants are highlighted in bold type; [a], new VR3 peptide sequence LFGSTSDE

The five and ten most common VR1 variants accounted for 50.6% and 78.1% of the carriage strain collection. The ten most common VR1 variants were P1.5 (n=101; 12.6%), P1.18-1 (n=91; 11.4%), P1.19 (n=87; 10.9%), P1.5-1 (n=65; 8.1%), P1.7-2 (n=61; 7.6%), P1.22 (n=56; 7.0%), P1.7 (n=53; 6.6%), P1.21 (n=46; 5.8%), P1.22-1 (n=34; 4.3%) and P1.5-2 (n=30; 3.8%).

Table 5.2 – PorA Variable Region peptides identified as new variants during this study in carried meningococci in Scotland, 1974 – 2004

Variable Region	Peptide Family	Peptide name	Peptide Sequence
VR1	P1.5	5-24	PPQNIQQPQVTKR
VR1	P1.5	5-29	PLQNIQQPQVTKPQVTKR
VR1	P1.5	5-31	PIPNIQPQVTKR
VR1	P1.7	7-32	AQAANGGAGASAQVKVTKVTKA
VR1	P1.19	19-32	PPSKSQPLVKVTKA
VR1	P1.19	19-33	PPSKSQPPVKVTKA
VR2	P1.2	2-58	HFVQQTLQSQPTLVP
VR2	P1.9	9-14	YVDEQSEYPA
VR2	P1.9	9-17	YVDEPSEYHA
VR2	P1.10	10-81	HFVQNKQNKQNQQNQLPTLVP
VR2	P1.15	15-41	HYTRQDNADVFVP
VR2	P1.16	16-93	TKDKNDNLTLVP (missing YY motif)
VR2	P1.16	16-96	YYTKDTNNNLTLVLTLVP
VR2	P1.16	16-102	YYTKDTNNNLTLNNLTLVP
VR2	P1.44	44	HFVQQLLKVSLLSF
VR3	-	-	LFGSTSDE

Ten variants belonging to the P1.5 family were identified; accounting for 206 specimens (Table 5.1). Three variants were identified more than twice; in rank order these were variant 5 (n=101), variant 5-1 (n=65) and variant 5-2 (n=30). Variants 5-3, 5-4 and 5-11 were each identified twice while variants 5-18, 5-24, 5-29 and 5-31 were identified only once. The latter three variants were newly characterised during this study (Table 5.2). Five variants belonging to the P1.7

family were identified; accounting for 141 specimens (Table 5.1). In rank order these were variant 7-2 (n=61), variant 7 (n=53), variant 7-1 (n=22), variant 7-4 (n=4) and variant 7-32 (n=1). The latter variant was newly characterised during this study (Table 5.2).

Three variants belonging to the P1.12 family were identified; accounting for 22 specimens (Table 5.1). The most common variant was variant 12-1 (n=20). Variants 12 and 12-16 were each only identified only once during the study. Two variants belonging to the P1.17 family were identified; variant 17 and variant 17-1 were identified eleven and two times, respectively (Table 5.1). Six variants belonging to the P1.18 family were identified; accounting for 127 specimens (Table 5.1). Four variants were identified more than once; these were variant 18-1 (n=91), variant 18 (n=19), variant 18-7 (n=11) and variant 18-3 (n=3). Variants 18-4 and 18-9 were each identified once. Seven variants belonging to the P1.19 family were identified; accounting for 108 specimens (Table 5.1). Four variants were identified more than once; these were variant 19 (n=87), variant 19-1 (n=10), variant 19-3 (n=6) and variant 19-33 (n=2). The three remaining variants, 19-2, 19-15 and 19-32 were each identified once. Variants 19-32 and 19-33 were newly characterised during this study (Table 5.2).

Only one variant, 20, belonging to the P1.20 family was identified in two specimens (Table 5.1). Six variants belonging to the P1.21 family were identified; accounting for 71 specimens (Table 5.1). Four variants were identified more than once; in rank order these were variant 21 (n=46), variant 21-2 (n=13) and variants 12-1 and 12-7 (both n=5). Variants 12-10 and 12-13 were each identified once. Five variants belonging to the P1.22 family were identified; accounting for 93 specimens (Table 5.1). The two most common were variants 22 (n=56) and 22-1 (n=34). Three other variants, 22-2, 22-11 and 22-21 were each identified once.

5.3 Variable region 2 (VR2)

Translation of the *porA* DNA sequences of carried meningococci identified 74 individual VR2 variants that grouped into seventeen of the 20 currently defined

families (Table 5.1). Nine of these variants were had not previously been sequenced before and these were submitted to the PorA database curator for verification and assignment of a new variant name (Table 5.2). One of the new variants also defined a new family, P1.44. The new VR2 variants accounted for 12.1% of all VR2 variants identified; this was equivalent to 1.1% of specimens of the carriage strain collection. For seventeen specimens VR2 could not be sequenced. Twenty nine (39.2%) VR2 variants were observed only once accounting for 3.6% of isolates of the carriage strain collection. In contrast, 19 (25.7%) variants were observed over at least a 20 year period, accounting for 613 (76.7%) of isolates of the carriage strain collection. The five and ten most common VR2 variants accounted for 45.4% and 66.1% of the carriage strain collection, respectively. The ten most common VR2 variants were P1.2 (n=95; 11.9%), P1.3 (n=87; 10.9%), P1.15 (n=66; 8.3%), P1.16 (n=62; 7.8%), P1.13-1 (n=53; 6.6%), P1.14 (n=48; 6.0%), P1.2-2 (n=37; 4.6%), P1.1 (n=28, 3.5%) and P1.10-4 (n=28, 3.5%).

One variant, 1, belonging to the P1.1 family was identified in 28 specimens (Table 5.1). Five variants belonging to the P1.2 family were identified; accounting for 140 specimens (Table 5.1). Three variants were identified more than once; variant 2 (n=92), variant 2-2 (n=37) and variant 2-1 (n=6). Variants 2-5 and 2-58 were each identified once; the latter variant was newly characterised during this study (Table 5.2). Two variants belonging to the P1.3 family were identified; accounting for 88 specimens (Table 5.1). All but one was variant 3, while the remaining specimens had variant 3-1. Three variants belonging to the P1.4 family were identified; accounting for 21 specimens (Table 5.1). The most common variant was variant 4 (n=18). Variants 4-1 and 4-2 were identified only twice and once, respectively.

Six variants belonging to the P1.9 family were identified; accounting for 32 specimens (Table 5.1). Two of these variants, 9-14 and 9-17 were newly characterised during this study. The most common variant was variant 9 (n=24). The remaining variants were seldom identified; 9-5 (n=3), 9-7 (n=2) and 9-3 (n=1). Eleven variants belonging to the P1.10 family were identified; accounting for 66 specimens (Table 5.1). In rank order these were variant 10-4 (n=28), variant 10 (n=14), variants 10-1 and 10-57 (both n=6), variant 10-2 (n=3), variants 10-3, 10-8 and 10-9 (each n=2) and variants 10-12, 10-23 and 10-81

(each n=1). The latter variant was newly characterised during this study (Table 5.2).

Seven variants belonging to the P1.13 family were identified; accounting for 77 specimens (Table 5.1). In rank order these were variant 13-1 (n=53), variant 13 (n=12), variant 13-2 (n=5), variant 13-7 (n=3), variant 13-15 (n=2), and variants 13-5 and 13-6 (both n=1). Three variants belonging to the P1.14 family were identified; accounting for 59 specimens (Table 5.1). In rank order these were variant 14 (n=48), variant 14-6 (n=10) and variant 14-3 (n=1). Six variants belonging to the P1.15 family were identified; accounting for 92 specimens (Table 5.1). The most common variants were variant 15 (n=66) and variant 15-1 (n=21). The remaining four variants were seldom identified, variant 15-11 (n=2), and variants 15-10, 15-39 and 15-41 (each n=1). The latter variant was newly characterised during this study (Table 5.2).

Eleven variants belonging to the P1.16 family were identified; accounting for 94 specimens (Table 5.1). Five of these variants were identified more than once; in rank order these were variant 16 (n=62), variant 16-8 (n=15), variants 16-3 and 16-4 (both n=4) and variant 16-16 (n=3). The other six variants, 16-5, 16-13, 16-21, 16-93, 16-96 and 16-102, were each identified once. The latter three variants were newly characterised during this study (Table 5.2). Three variants belonging to the P1.23 family were identified, accounting for only four specimens (Table 5.1). Variant 23 was identified twice, while variants 23-2 and 23-3 were each identified once. Seven variants belonging to the P1.25 family were identified; accounting for 16 specimens (Table 5.1). In rank order these were variant 25 (n=8), variants 25-1, 25-6 and 25-11 (each n=2) and variants 25-7, 25-9 and 25-25 (each n=1). One variant, 26, belonging to the P1.26 family was identified in eight specimens (Table 5.1).

One variant, 28, belonging to the P1.28 family was identified in fifteen specimens (Table 5.1). Five variants belonging to the P1.30 family were identified; accounting for 38 specimens (Table 5.1). In rank order these were variant 30 (n=21), variant 30-3 (n=10), variants 30-1 and 30-2 (both n=3) and variant 30-14 (n=1). One variant, 34, belonging to the P1.34 family was identified in three specimens (Table 5.1). Variant 44 was identified in one

specimen and was newly characterised during this study defining a new peptide family (Table 5.1 & Table 5.2).

5.4 Variable region 3 (VR3)

Translation of the *porA* DNA sequences of carried meningococci identified eleven individual VR3 variants that could be grouped into five families (Table 5.1). One hundred fifty-four (19.3%) specimens possessed a VR3 belonging to the P1.35 Family. Three hundred fifty-six (44.6%) specimens possessed a VR3 belonging to the P1.36 Family. One hundred ten (13.8%) specimens possessed a VR3 belonging to the P1.37 Family. One hundred sixty-one (20.2%) specimens possessed a VR3 belonging to the P1.38 Family. One specimen possessed a VR3 belonging to the P1.40 Family. One peptide sequence did not match any previously identified peptide sequence; peptide LFGSTSDE (Table 5.2). In rank order the VR3 peptides were variant 36-2 (n=221), variant 38 (n=142), variant 35-1 (n=139), variant 36 (n=130), variant 37-1 (n=74), variant 37 (n=36), variant 38-1 (n=18), variant 35 (n=15), variant 36-1 (n=5), variant 40 (n=1) and one new variant. For seventeen specimens VR3 was not sequenced.

5.5 PorA variable region families

Amongst the IMD strain collection 36 VR1 variants could be assigned to nine VR1 Families and 46 VR2 variants could be assigned to fifteen VR2 Families. Figure 5.1 shows the prevalence of the different PorA VR Families amongst the IMD strain collection compared with the carriage strain collection. Certain VR families were more prevalent in the IMD strain collection. Nearly half of the IMD strain collection possesses a VR1 variant belonging to the P1.5 family, compared with less than 30% of the carriage strain collection. The VR2 families P1.2 and P1.10 were also more prevalent in the IMD strain collection. While the VR3 is said to be less diverse than the other two VRs, it is clear from the comparison of

the two strain collections that the P1.38 family is considerably more prevalent amongst the carriage strain collection.

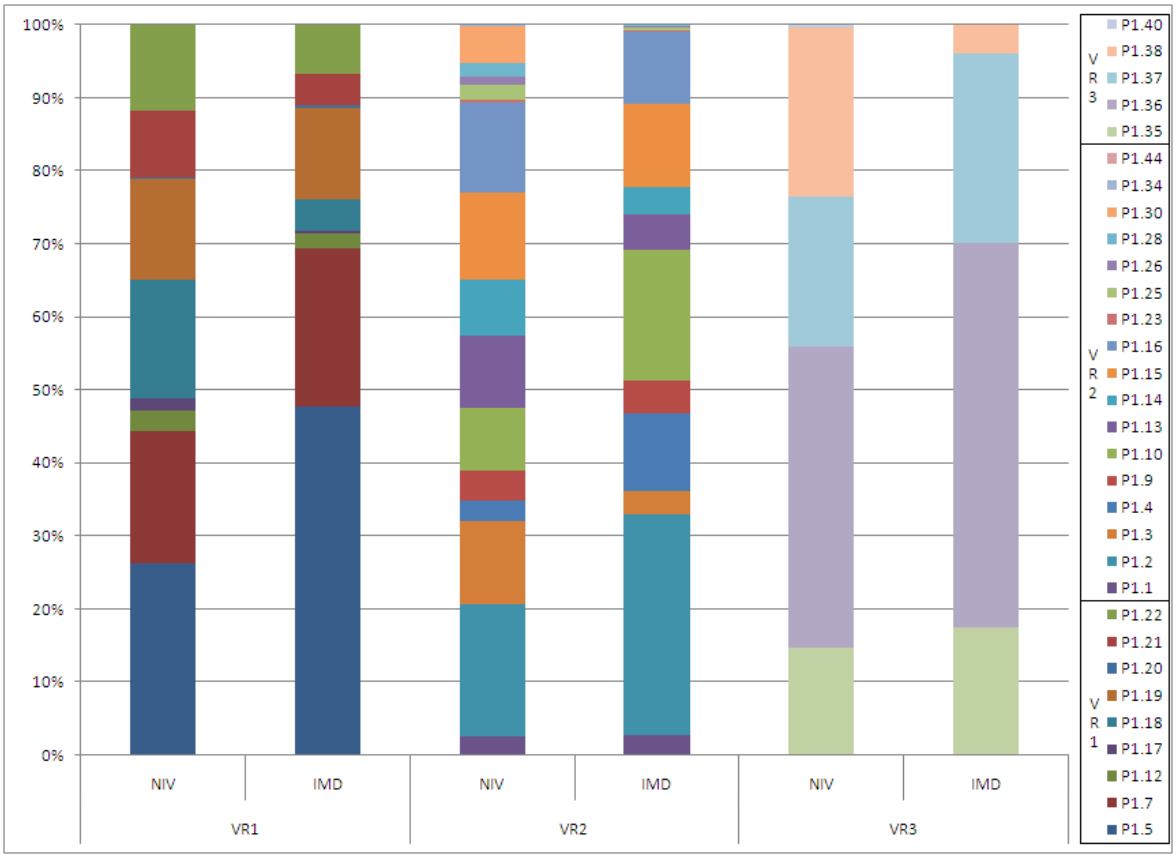

Figure 5.1 - Prevalence of PorA VR Families identified in meningococci in Scotland, 1974 – 2004

NIV, non-invasive; IMD, invasive meningococcal disease; VR1, variable region 1; VR2, variable region 2; VR3, variable region 3

5.6 PorA subtypes

DNA sequencing of *porA* VR1 and VR2 identified 144 unique subtypes (Table 5.3); over half of which were identified only once in carried meningococci (n=77; 53.5%). This accounted for 9.6% of the carriage strain collection. A further 49 subtypes were identified between two and ten times in carried meningococci, accounting for 229 specimens. Nineteen subtypes were identified greater than ten times; in rank order these were subtype 5,2 (n=92), subtype 18-1,3 (n=80), subtype 19,15 (n=56), subtype 21,16 (n=39), subtype 5-1,2-2 (n=33), subtype

22,14 (n=30), subtype 7-2,13-1 (n=29), subtype 5-1,10-4 (n=25), subtype 19,15-1 (n=21), subtype 7-1,1 (n=19), subtype 7,30 (n=17), subtype 7-2,4 (n=17), subtype 22-1 (n=15), subtype 22-1,16-8 (n=15), subtype 5-2,10 (n=13), subtype 21-2,28 (n=13), subtype 7,16 (n=12), subtype 12-1,13-1 (n=12) and subtype 22,9 (n=11). In thirteen specimens, including the ATCC13090 isolate, the PorA subtype could not be determined as no product could be amplified by PCR. Sixteen (10.4%) subtypes were observed over periods of at least 20 years, accounting for 411/799 (51.4%) of the carriage strain collection. A further fourteen (9.7%) subtypes were observed over periods of 15-19 years, accounting for 132/799 (16.5%) of the carriage strain collection.

DNA sequencing of *porA* VR1, VR2 and VR3 identified 163 unique subtypes (Table 5.4); over half of which were identified only once amongst carried meningococci (n=94; 57.7%). This accounted for 11.8% of the carriage strain collection. A further 51 subtypes were identified in more than two but fewer than ten carried meningococci, accounting for 170 specimens. Eighteen subtypes were identified in greater than ten carried meningococci; in rank order these were subtype 5, 2, 36-2 (n=90); subtype 18-1, 3, 38 (n=80); subtype 19, 15, 36 (n=48); subtype 21, 16, 37-1 (n=39); subtype 5-1, 2-2, 36-2 (n=33); subtype 22, 14, 36 (n=30); subtype 7-2, 13-1, 35-1 (n=29); subtype 5-1, 10-4, 36-2 (n=25); subtype 7-1, 1, 35-1 (n=19); subtype 19, 15-1, 36 (n=17); subtypes 7, 30, 38 and 7-2, 4, 37 (both n=16); subtypes 22-1, 14, 38 and 22-1, 16-8, 36-2 (both n=15); subtypes 7, 16, 35; 12-1, 13-1, 35-1 and 21-2, 28, 37 (each n=12) and subtype 22, 9, 35-1 (n=11).

Table 5.3 – Frequency of PorA subtypes (VR1, VR2) identified in carried meningococci in Scotland, 1974 – 2004

PorA subtype (VR1, VR2)	Frequency (%)	PorA subtype (VR1, VR2)	Frequency (%)	PorA subtype (VR1, VR2)	Frequency (%)	PorA subtype (VR1, VR2)	Frequency (%)	PorA subtype (VR1, VR2)	Frequency (%)
5, 2	92 (11.5)	17, 16-4	4 (0.50)	19-33, 13-1	2 (0.25)	7, 16-21	1 (0.13)	18-7, 9-14	1 (0.13)
18-1, 3	80 (10.0)	7, 16-16	3 (0.38)	20, 9	2 (0.25)	7-1, 14-6	1 (0.13)	18-7, 9-17	1 (0.13)
19, 15	56 (7.0)	7, 30-2	3 (0.38)	21, 15	2 (0.25)	7-2, 4-2	1 (0.13)	18-9, 25-9	1 (0.13)
21, 16	39 (4.9)	7-2, 1	3 (0.38)	21-1, 2-2	2 (0.25)	7-2, 13-2	1 (0.13)	19, 13-6	1 (0.13)
5-1, 2-2	33 (4.1)	7-4, 14-6	3 (0.38)	21-1, 16	2 (0.25)	7-2, 13-5	1 (0.13)	19, 15-39	1 (0.13)
22, 14	30 (3.8)	18, 3	3 (0.38)	22, 9-7	2 (0.25)	7-2, 13-7	1 (0.13)	19, 15-41	1 (0.13)
7-2, 13-1	29 (3.6)	18-1, 34	3 (0.38)	22-1, 13-1	2 (0.25)	7-2, 13-15	1 (0.13)	19, 16	1 (0.13)
5-1, 10-4	25 (3.1)	18-3, 1	3 (0.38)	5, 2-2	1 (0.13)	7-2, 16	1 (0.13)	19-1, 3	1 (0.13)
19, 15-1	21 (2.6)	19, 13	3 (0.38)	5, 3	1 (0.13)	7-2, 16	1 (0.13)	19-1, 16	1 (0.13)
7-1, 1	19 (2.4)	5-2, 10-2	2 (0.25)	5, 44	1 (0.13)	7-2, 30	1 (0.13)	19-2, 13-1	1 (0.13)
7, 30	17 (2.1)	5-2, 10-3	2 (0.25)	5-1, 2-58	1 (0.13)	7-4, 13-7	1 (0.13)	19-3, 15-10	1 (0.13)
7-2, 4	17 (2.1)	5-4, 2	2 (0.25)	5-1, 10-8	1 (0.13)	7-32, 13-1	1 (0.13)	19-15, 15	1 (0.13)
22-1, 14	15 (1.9)	7, 1	2 (0.25)	5-1, 10-9	1 (0.13)	12, ND	1 (0.13)	19-32, 13	1 (0.13)
22-1, 16-8	15 (1.9)	7, 9	2 (0.25)	5-2, 3	1 (0.13)	12-1, 13-2	1 (0.13)	21, 16-13	1 (0.13)
5-2, 10	13 (1.6)	7, 30-1	2 (0.25)	5-2, 9-5	1 (0.13)	12-1, 13-7	1 (0.13)	21, 16-96	1 (0.13)
21-2, 28	13 (1.6)	7-1, 4-1	2 (0.25)	5-2, 10-1	1 (0.13)	12-1, 23-3	1 (0.13)	21, 16-102	1 (0.13)
7, 16	12 (1.5)	7-2, 9	2 (0.25)	5-2, 10-9	1 (0.13)	12-16, 13	1 (0.13)	21, 26	1 (0.13)
12-1, 13-1	12 (1.5)	7-2, 13	2 (0.25)	5-2, 10-12	1 (0.13)	17, 4	1 (0.13)	21, 28	1 (0.13)
22, 9	11 (1.4)	7-2, 16	2 (0.25)	5-2, 10-23	1 (0.13)	17, 16-93	1 (0.13)	21-1, 2-5	1 (0.13)
7, 30-3	8 (1.00)	18, 25-1	2 (0.25)	5-2, 10-81	1 (0.13)	17, 23	1 (0.13)	21-10, 16-5	1 (0.13)
18, 25	7 (0.88)	18, 25-6	2 (0.25)	5-3, 2	1 (0.13)	17-1, 23	1 (0.13)	21-13, 30-14	1 (0.13)
5, 2-1	6 (0.75)	18, 25-11	2 (0.25)	5-3, 10-4	1 (0.13)	17-1, 23-2	1 (0.13)	22, 13-1	1 (0.13)
5-2, 10-57	6 (0.75)	18-1, 30	2 (0.25)	5-11, 10-4	1 (0.13)	18, 13-2	1 (0.13)	22, 14-3	1 (0.13)
18-7, 9	6 (0.75)	18-1, 30-3	2 (0.25)	5-11, 10-8	1 (0.13)	18, 25-7	1 (0.13)	22-1, 10-1	1 (0.13)
22, 14-6	6 (0.75)	18-7, 9-5	2 (0.25)	5-18, 10-2	1 (0.13)	18, 25-25	1 (0.13)	22-1, 14	1 (0.13)
12-1, 13	5 (0.63)	19, 13-1	2 (0.25)	5-24, 2-2	1 (0.13)	18-1, 1	1 (0.13)	22-2, 28	1 (0.13)
19-3, 15	5 (0.63)	19, 13-2	2 (0.25)	5-29, 10-4	1 (0.13)	18-1, 3	1 (0.13)	22-11, 14	1 (0.13)
21-7, 16	5 (0.63)	19-1, 15	2 (0.25)	5-31, 10	1 (0.13)	18-1, 3-1	1 (0.13)	22-21, 9	1 (0.13)
22, 26	5 (0.63)	19-1, 13-1	2 (0.25)	7, 13-1	1 (0.13)	18-1, 30-1	1 (0.13)	ND	16 (2.0)
5-1, 10-1	4 (0.50)	19-1, 15-11	2 (0.25)	7, 14	1 (0.13)	18-4, 25	1 (0.13)		
17, 16-3	4 (0.50)	19-1, 26	2 (0.25)	7, 15	1 (0.13)	18-7, 9-3	1 (0.13)		

VR1, Variable Region 1; VR2, Variable region 2; VR3, Variable region 3; ND, not determined

Table 5.4 – Frequency of PorA subtypes (VR1, VR2 & VR3) amongst carried meningococci in Scotland, 1974 – 2004

PorA subtype (VR1, VR2, VR3)	Frequency (%)	PorA subtype (VR1, VR2, VR3)	Frequency (%)	PorA subtype (VR1, VR2, VR3)	Frequency (%)	PorA subtype (VR1, VR2, VR3)	Frequency (%)	PorA subtype (VR1, VR2, VR3)	Frequency (%)
5, 2, 36-2	90 (11.3)	19, 15-1, 36-2	4 (0.50)	21-1, 2-2, 36-2	2 (0.25)	7-2, 4-2, 37	1 (0.13)	19, 13-2, 36	1 (0.13)
18-1, 3, 38	80 (10.0)	5-2, 10, 36-2	3 (0.38)	22, 9-7, 35-1	2 (0.25)	7-2, 13-2, 35-1	1 (0.13)	19, 13-6, 36	1 (0.13)
19, 15, 36	48 (6.0)	7, 16-16, 37-1	3 (0.38)	22-1, 13-1, 35-1	2 (0.25)	7-2, 13-5, 35-1	1 (0.13)	19, 15, 38	1 (0.13)
21, 16, 37-1	39 (4.9)	7, 30-2, 38	3 (0.38)	5, 2, 36	1 (0.13)	7-2, 13-7, 35-1	1 (0.13)	19, 15, new	1 (0.13)
5-1, 2-2, 36-2	33 (4.1)	7-2, 1, 35-1	3 (0.38)	5, 2, 36-1	1 (0.13)	7-2, 13-15, 35-1	1 (0.13)	19, 15-39, 36	1 (0.13)
22, 14, 36	30 (3.8)	7-4, 14-6, 35-1	3 (0.38)	5, 2-2, 36-2	1 (0.13)	7-2, 16, 35	1 (0.13)	19, 15-41, 36	1 (0.13)
7-2, 13-1, 35-1	29 (3.6)	18, 3, 38	3 (0.38)	5, 3, 36-2	1 (0.13)	7-2, 16, 37-1	1 (0.13)	19, 16, 37-1	1 (0.13)
5-1, 10-4, 36-2	25 (3.1)	18-1, 34, 38	3 (0.38)	5, 44, 36-2	1 (0.13)	7-2, 30, 38	1 (0.13)	19-1, 3, 38	1 (0.13)
7-1, 1, 35-1	19 (2.4)	18-3, 1, 35-1	3 (0.38)	5-1, 2-58, 36-2	1 (0.13)	7-4, 13-7, 35-1	1 (0.13)	19-1, 16, 37-1	1 (0.13)
19, 15-1, 36	17 (2.1)	19, 13, 35-1	3 (0.38)	5-1, 10-8, 36-2	1 (0.13)	7-32, 13-1, 37-1	1 (0.13)	19-2, 13-1, 36	1 (0.13)
7, 30, 38	16 (2.0)	5-2, 10-2, 36-1	2 (0.25)	5-1, 10-9, 36-1	1 (0.13)	12, ND, ND	1 (0.13)	19-3, 15-10, 36	1 (0.13)
7-2, 4, 37	16 (2.0)	5-2, 10-3, 37-1	2 (0.25)	5-2, 3, 38	1 (0.13)	12-1, 13-2, 35-1	1 (0.13)	19-15, 15, 36	1 (0.13)
22-1, 14, 38	15 (1.9)	5-4, 2, 36-2	2 (0.25)	5-2, 9-5, 35-1	1 (0.13)	12-1, 13-7, 35-1	1 (0.13)	19-32, 13, 36	1 (0.13)
22-1, 16-8, 36-2	15 (1.9)	7, 1, 35-1	2 (0.25)	5-2, 10-1, 36-2	1 (0.13)	12-1, 23-3, 37	1 (0.13)	21, 16-13, 37-1	1 (0.13)
7, 16, 35	12 (1.5)	7, 9, 35-1	2 (0.25)	5-2, 10-9, 36-2	1 (0.13)	12-16, 13, 35-1	1 (0.13)	21, 16-96, 37-1	1 (0.13)
12-1, 13-1, 35-1	12 (1.5)	7, 30-1, 38	2 (0.25)	5-2, 10-12, 36-2	1 (0.13)	17, 4, 37	1 (0.13)	21, 16-102, 37-1	1 (0.13)
21-2, 28, 37	12 (1.5)	7-1, 4-1, 37-1	2 (0.25)	5-2, 10-23, 36-2	1 (0.13)	17, 16-93, 36	1 (0.13)	21, 26, 35-1	1 (0.13)
22, 9, 35-1	11 (1.4)	7-2, 9, 35-1	2 (0.25)	5-2, 10-81, 36-2	1 (0.13)	17, 23, 37	1 (0.13)	21, 28, 37	1 (0.13)
5-2, 10, 37-1	10 (1.3)	7-2, 13, 35-1	2 (0.25)	5-3, 2, 36-2	1 (0.13)	17-1, 23, 37	1 (0.13)	21-1, 2-5, 36-2	1 (0.13)
7, 30-3, 38	8 (1.00)	18, 25-1, 38-1	2 (0.25)	5-3, 10-4, 36-2	1 (0.13)	17-1, 23-2, 37	1 (0.13)	21-1, 16, 36-2	1 (0.13)
18, 25, 38-1	7 (0.88)	18, 25-6, 38-1	2 (0.25)	5-11, 10-4, 36-2	1 (0.13)	18, 13-2, 35-1	1 (0.13)	21-1, 16, 37-1	1 (0.13)
5, 2-1, 36-2	6 (0.75)	18, 25-11, 38-1	2 (0.25)	5-11, 10-8, 36-2	1 (0.13)	18, 25-7, 38-1	1 (0.13)	21-2, 28, 36-1	1 (0.13)
5-2, 10-57, 36-2	6 (0.75)	18-1, 30, 38	2 (0.25)	5-18, 10-2, 36-2	1 (0.13)	18, 25-25, 38-1	1 (0.13)	21-10, 16-5, 37-1	1 (0.13)
18-7, 9, 35-1	6 (0.75)	18-1, 30-3, 38	2 (0.25)	5-24, 2-2, 36-2	1 (0.13)	18-1, 1, 35-1	1 (0.13)	21-13, 30-14, 36-2	1 (0.13)
22, 14-6, 36-2	6 (0.75)	18-7, 9-5, 35-1	2 (0.25)	5-29, 10-4, 36-2	1 (0.13)	18-1, 3, 40	1 (0.13)	22, 13-1, 36-2	1 (0.13)
12-1, 13, 35-1	5 (0.63)	19, 13-1, 36	2 (0.25)	5-31, 10, 37-1	1 (0.13)	18-1, 3-1, 38	1 (0.13)	22, 14-3, 36-2	1 (0.13)
19, 15, 35-1	5 (0.63)	19-1, 15, 36	2 (0.25)	7, 13-1, 35-1	1 (0.13)	18-1, 30-1, 38	1 (0.13)	22-1, 10-1, 36-2	1 (0.13)
19-3, 15, 36	5 (0.63)	19-1, 13-1, 35-1	2 (0.25)	7, 14, 35	1 (0.13)	18-4, 25, 38-1	1 (0.13)	22-1, 14, 36-1	1 (0.13)
21-7, 16, 37-1	5 (0.63)	19-1, 15-11, 36	2 (0.25)	7, 15, 36	1 (0.13)	18-7, 9-3, 35-1	1 (0.13)	22-2, 28, 37	1 (0.13)
22, 26, 35-1	5 (0.63)	19-1, 26, 36-2	2 (0.25)	7, 16-21, 35	1 (0.13)	18-7, 9-14, 35-1	1 (0.13)	22-11, 14, 38	1 (0.13)
5-1, 10-1, 36-2	4 (0.50)	19-33, 13-1, 36-2	2 (0.25)	7, 30, 37-1	1 (0.13)	18-7, 9-17, 35-1	1 (0.13)	22-21, 9, 35-1	1 (0.13)
17, 16-3, 36	4 (0.50)	20, 9, 35-1	2 (0.25)	7-1, 14-6, 36-2	1 (0.13)	18-9, 25-9, 38-1	1 (0.13)	ND	16 (2.0)
17, 16-4, 36	4 (0.50)	21, 15, 36	2 (0.25)	7-2, 4, 37-1	1 (0.13)	19, 13-2, 35-1	1 (0.13)		

Legend as for Table 5.3; in addition subtypes in red type are those which have been differentiated by the inclusion of VR3 sequencing data

The addition of VR3 sequencing data was able to re-characterise twelve PorA VR1,2 subtypes amongst the carriage strain collection into twenty-seven subtypes (Table 5.3 & Table 5.4). Ninety-two specimens with subtype 5, 2 were identified as having the one of three VR3 peptides; 36-2 (n=90), 36 (n=1) or 36-1 (n=1). Thirteen specimens with subtype 5-2, 10 were identified as having one of two VR3 peptides; 37-1 (n=10) or 36-2 (n=3). Seventeen specimens with subtype 7, 30 were identified as having one of two VR3 peptides; 38 (n=16) or 37-1 (n=1). Seventeen specimens with subtype 7-2, 4 were identified as having one of two VR3 peptides; 37 (n=16) or 37-1 (n=1). Two specimens with subtype 7-2, 16 were identified as having the VR3 peptide 35 or 37-1. Eighty-one specimens, including ATCC 13077, with subtype 18-1, 3 were identified as having one of two VR3 peptides; 38 (n=80) or 40 (n=1). Two specimens with subtype 19, 13-2 were identified as having the VR3 peptide 35-1 or 36. Fifty-six specimens with subtype 19, 15 were identified as having one of four VR3 peptides; 36 (n=48), 35-1 (n=5), 38 (n=1) or a new VR3 peptide; LFGSTSDE (n=1). Twenty-one specimens with subtype 19, 15-1 were identified as having one of two VR3 peptides; 36 (n=17) or 36-2 (n=4). Two specimens with subtype 21-1, 16 were identified as having VR3 peptide 36-2 or 37-1. Thirteen specimens with subtype 21-2, 28 were identified as having on of two VR3 peptides; 37 (n=12) or 36-1 (n=1). Fifteen specimens with subtype 22-1, 14 were identified as having one of two VR3 peptides; 38 (n=14) or 36 (n=1).

The number of VR1 and VR2 variants and PorA subtypes identified amongst the carriage strain collection was greater than in the IMD strain collection (Table 5.5). The five and ten most prevalent VR1 variants accounted for 69.5% and 90.6% of isolates of the IMD strain collection, respectively. The ten most prevalent VR1 variants were 5 (n=557), 5-1 (n=450), 7-2, (n=364), 5-2 (n=301), 19 (n=251), 22 (n=159), 7 (n=146), 21 (n=111), 7-1 (n=87) and 19-1 (n=82) (data not shown). The five and ten most prevalent VR2 variants accounted for 57.2% and 80.7% of isolates of the IMD strain collection, respectively. The ten most prevalent VR2 variants were 2 (n=514), 4 (n=289), 10 (n=281), 2-2 (n=251), 16 (n=248), 15 (n=213), 10-4 (n=124), 9 (n=114), 13-1 (n=107) and 14 (n=91) (data not shown).

The most prevalent PorA subtypes (VR1, VR2) in the IMD strain collection were 5,2 (n=478), 7-2,4 (n=279), 5-2,10 (n=275), 5-1,2-2 (n=232), 19,15 (n=206), 5-

1,10-4 (n=114), 21,16 (n=109), 18-1,3 (n=75), 19-1,15-11 (n=74) and 7,16 (n=69). The five and ten most prevalent PorA subtypes accounted for 53.1% and 69.1% of isolates of the IMD strain collection, respectively. The inclusion of VR3 data re-characterised 130 PorA VR1,VR2 subtypes into 169 PorA VR1,VR2,VR3 subtypes. Simpson's index of diversity, *D*, was calculated for PorA subtype (VR1,VR2) across the full period of the IMD strain collection as 0.928 (95% CI 0.927, 0.928) (Table 5.5). In comparison, the level of *D* of PorA subtypes in the carriage strain collection was greater at 0.958 (95% CI 0.948, 0.968). Similarly, when VR3 data was included the level of D for PorA subtypes was greater in the carriage strain collection than in the IMD strain collection, respectively [0.961 (95% CI 0.949, 0.973) vs. 0.931 (95% CI 0.930, 0.932)]. The non-overlapping 95% CIs indicate that the diversity of PorA subtypes is significantly different between the two strain collections. Therefore, with respect to PorA subtype, the diversity in the carriage strain collection is greater than the diversity in the IMD strain collection.

Table 5.5 – Summary of antigenic diversity in carried and invasive meningococcal disease strain collections in Scotland, 1974 – 2004

	carriage[1]	**IMD[2]**
Isolates	780	2767
PorA		
VR1 peptides	45	35
VR2 peptides	74	46
Subtypes (VR1, 2)	144	130
***D*[3]**	0.958	0.928
95% CIs[4]	(0.948, 0.968)	(0.927, 0.928)
VR3 peptides	10	10
Subtypes (VR1,2,3)	163	169
D	0.961	0.931
95% CIs	(0.949, 0.973)	(0.930, 0.932)

[1] carriage, carriage strain collection; [2] IMD, invasive meningococcal disease strain collection; [3] *D*, Simpson's index of diversity (Hunter and Gaston 1988); [4] 95% confidence intervals calculated as described in (Grundmann, Hori *et al.* 2001).

Figure 5.2 shows the ten most prevalent PorA subtypes (VR1,VR2) from the IMD strain collection, the ten most prevalent PorA subtypes (VR1,VR2) from the carriage strain collection and a comparison of their prevalence in the two strain collections. Subtypes 5,2; 5-1,2-2; 5-1,10-4; 18-1,3; 19,15 and 21,16 ranked in the top ten most prevalent subtypes in both strain collections; with subtype 5,2

the most prevalent in both strain collections. Subtypes 5-2,10; 7,16; 7-2,4 and 19-1,15-11 ranked in the ten most prevalent subtypes of the IMD strain collection; but not in the carriage strain collection. In contrast, subtypes 7-1,1; 7-2,13-1; 19,15-1 and 22,14 ranked in the ten most prevalent subtypes of the carriage strain collection but not in the IMD strain collection. Furthermore, subtypes 5,2; 5-1,2-2; 5-2,10; 7-2,4 and 19-1,15-11 were considerably more prevalent amongst the IMD strain collection. Whereas subtypes 7-1,1; 7-2,13-1; 18-1,3 and 19,15-1 were considerably more prevalent amongst the carriage strain collection. Differences in prevalence of the other prevalent PorA subtypes amongst the two strain collections were less pronounced and tended to be within one percent.

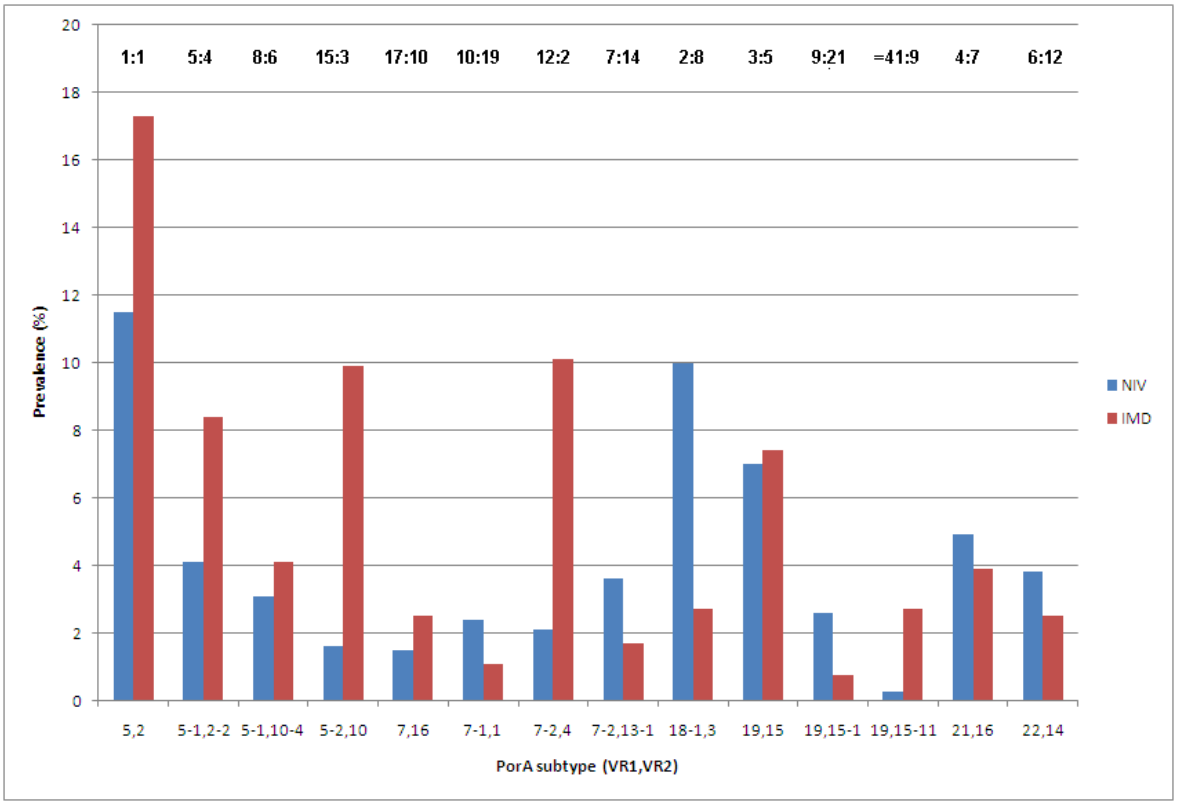

Figure 5.2 - Prevalence of the major PorA subtypes identified in meningococci in Scotland, 1974 – 2004

NIV, non-invasive; IMD, invasive meningococcal disease; numbers along the top of the figure correspond to the rank in prevalence of subtypes in each strain collection, NIV:IMD.

For each PorA VR1,2 subtype an odds ratio was calculated to compare the probability of invasive disease by, or carriage due to, that individual PorA VR1,2 subtype (Table 5.6). Whilst assessing invasiveness, subtypes 5,2; 5,2-1 and 7-2,4

were found to have ORs greater than 1 and the 95% CIs indicated significance. Whilst assessing a carriage phenotype, thirteen subtypes were found to have ORs greater than 1 and the 95% CIs indicated significance. There was, however, variation in the observed in range of the CIs for many subtypes. For example, subtype 7,30-2 had on OR of 10.43 but the wide range of the 95% CI (1.08 to 100.36) indicates the OR to be less precise. In comparison, subtype 7-2,13-1 had an OR of 2.22 with a much narrower 95% CI (1.34 to 3.41) indicating greater precision for this OR.

Table 5.6 – Association of PorA VR1,2 subtypes identified in meningococci in Scotland, 1974 – 2004 with invasive disease or with a carriage phenotype

"Invasive" Subtype	**n[1] (IMD[2])**	**n (carriage[3])**	**OR[4]**	**95% CI[5]**
5,2	478	92	1.60	1.26 to 2.04
5,2-1	66	6	3.23	1.39 to 7.48
7-2,4	279	17	5.16	3.14 to 8.47

"carriage" Subtype	**n (carriage)**	**n (IMD)**	**OR**	**95% CI**
7,30-2	3	1	10.43	1.08 to 100.36
7-1,1	19	30	2.22	1.24 to 3.97
7-2,13-1	29	48	2.13	1.34 to 3.41
17,16-3	4	3	4.64	1.04 to 20.76
18-1,3	81	75	4.05	2.93 to 5.60
18-7,9	6	5	4.18	1.27 to 13.73
19,15-1	21	21	3.53	1.92 to 6.50
19-3,15	5	2	8.71	1.69 to 44.96
21-2,28	13	4	11.43	3.72 to 35.14
21-7,16	5	2	8.71	1.69 to 44.96
22,26	5	4	4.35	1.17 to 16.24
22-1,14	15	15	3.51	1.71 to 7.21
22-1,16-8	15	3	17.63	5.09 to 61.05

[1] n, number of strains; [2] IMD, invasive meningococcal disease strain collection; [3] carriage, carriage strain collection; [4] OR, odds ratio; [5] CI; confidence interval

Figure 5.3 shows the inferred phylogeny generated from the multiple alignments of concatenated amino acid sequences of the individual PorA subtypes identified during this study. The alignment was generated in ClustalW2 using the default settings with the neighbour-joining tree constructed from the alignment. Added to the phylogram is prevalence and temporal data which again highlights that the majority of PorA subtypes were only encountered sporadically while a

smaller number of subtypes are more prevalent and persist within the strain collection over long periods. Moreover, within groups of related subtypes a particular subtype tends to dominate over a number of other less frequently encountered variants.

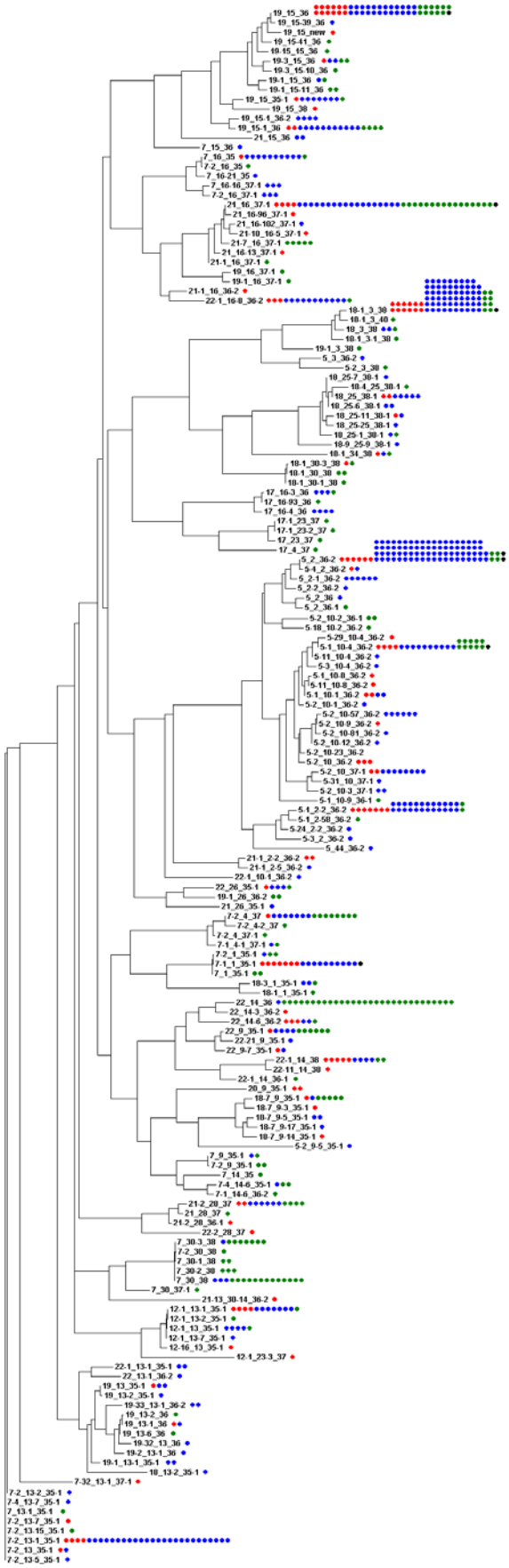

Figure 5.3 - Phylogenetic relationship of PorA subtypes identified in carried meningococci in Scotland, 1974 – 2004

For legend see over page

See previous page

Figure 5.3 - Phylogenetic relationship of PorA subtypes identified in carried meningococci in Scotland, 1974 – 2004

Subtypes are defined by PorA VR1, VR2 and VR3; neighbour-joining tree constructed in ClustalW2 using the pairwise differences from the multiple alignment of concatenated amino acid sequences using default settings. The number of dots is proportional to the number of times individual subtypes were sequenced in the carriage strain collection. Red, blue and green dots indicate the occurrence of a subtype during the period 1974 – 1983, 1984 – 1993 or 1994 – 2004, respectively. Black dots indicate an ATCC or NCTC strain.

5.7 Temporal distribution of PorA subtypes

PorA subtyping data (VR1,VR2) was examined for evidence of any changes in subtype prevalence over the 31 year period covered by the carriage strain collection. As previously, data was partitioned into three roughly equal time periods; 1974-1983, 1984-1993 and 1994-2004. The distribution of the most prevalent subtypes (sequenced in n>10 specimens; as listed in Table 5.3) was determined in each of the three time periods (Table 5.7).

Table 5.7 – Temporal distribution of the major PorA subtypes identified in carried meningococci in Scotland, 1974 – 2004

subtype (VR1,VR2)	Prevalence (%)		
	1974-1983 (n=127)[1]	1984-1993 (n=445)[1]	1994-2004 (n=208)[1]
5, 2	6 (4.7)	79 (17.8)	5 (2.4)
18-1, 3	12 (9.4)	59 (13.3)	9 (4.3)
19, 15	14 (11.0)	28 (6.3)	12 (5.8)
21, 16	4 (3.1)	18 (4.0)	16 (7.7)
5-1, 2-2	7 (5.5)	24 (5.4)	2 (1.0)
22, 14	-	1 (0.2)	29 (13.9)
7-2, 13-1	4 (3.1)	25 (5.6)	-
5-1, 10-4	4 (3.1)	10 (2.2)	10 (4.8)
19, 15-1	2 (1.6)	15 (3.4)	4 (1.9)
7-1, 1	7 (5.5)	11 (2.5)	-
7, 30	-	3 (0.7)	14 (6.7)
7-2, 4	1 (0.8)	7 (1.6)	9 (4.3)
22-1, 14	5 (3.9)	7 (1.6)	3 (1.4)
22-1, 16-8	3 (2.4)	11 (2.5)	1 (0.5)
5-2, 10	5 (3.9)	8 (1.8)	-
21-2, 28	3 (2.4)	6 (1.3)	4 (1.9)
7, 16	1 (0.8)	10 (2.2)	1 (0.5)
12-1, 13-1	4 (3.1)	7 (1.6)	1 (0.5)
22, 9	1 (0.8)	4 (0.9)	6 (2.9)
Total (%)[2]	83 (65.3)	327 (73.5)	126 (60.6)

[1] Number of fully subtyped isolates during each period; [2] major PorA subtypes as the number and percentage of fully subtyped isolates during each period

The majority (14/19) of the subtypes persisted within the carriage strain collection throughout all time periods. Three subtypes were present during the earlier two periods but not in the third period; 7-2,13-1; 7-1,1 and 5-2,10. Two subtypes were absent from the earliest period but present in the latter two periods; 22,14 and 7,30. There was evidence to indicate changes in the distribution of persistent subtypes. The prevalence of five subtypes in the carriage strain collection has decreased; 5-1,2-2; 12-2,13-1; 19,15; 22-1,14 and 22-1,16-8. The prevalence of a further three subtypes (5,2; 7,16 and 18-1,3), following increases from the first to second periods, have decreased to below their prevalence in the earliest period. The prevalence of three subtypes appears to have increased; 7-2,4; 21,16 and 22,9.

5.8 Potential coverage of the carriage strain collection by currently licensed or experimental PorA-based outer-membrane vesicle vaccines

PorA-based OMV vaccines have been used for around two decades in targeted vaccination campaigns to limit the burden of large disease outbreaks in different parts of the world. The concept of designing vaccines that negate the use of capsular polysaccharide is an attractive one, particularly when trying to eradicate disease due to serogroup B meningococci. With this in mind the potential coverage of carried meningococci in Scotland by licensed or experimental PorA-based vaccines reported in the available literature was calculated from the aforementioned subtype frequencies.

Table 5.8 shows the potential coverage of the carriage strain collection by six different PorA-based vaccine formulations reported in the literature; the Cuban VA-MENGOC-BC B: 4: P1.19,15 vaccine developed by the Finlay Institute (Holst, Feiring *et al.* 2003); the Norwegian P1.7, 16 vaccine developed by the Norwegian Institute of Public Health (NIPH) (Bjune, Hoiby *et al.* 1991); the New Zealand MeNZB P1.7-2,4 vaccine developed by the New Zealand Ministry of Health and Chiron (Oster, Lennon *et al.* 2005); the six-valent and nine-valent vaccines, developed by the Netherlands Vaccine Institute (NVI) HexaMen (Peeters, Rumke

et al. 1996) and NonaMen (van den Dobbelsteen, van Dijken *et al.* 2007), respectively; and a bivalent vaccine (combined Cuban VA-MENGOC-BC & MeNZB) developed by the Finlay Institute and GSK (Boutriau, Poolman *et al.* 2007).

Table 5.8 – Potential coverage of PorA VR1,2 subtypes identified in meningococci in Scotland, 1974 – 2004 by experimental or licensed PorA-based OMV vaccines

Vaccine (Manufacturer)	PorA subtype(s)	Strain Coverage(%)[1]	
		Carriage[2] all strains	IMD[3] all strains
Cuban VA-MENGOC-BC (Finlay Institute)	P1.19,15	6.9%	7.5%
Norwegian (NIPH)[4]	P1.7,16	1.5%	2.5%
New Zealand MeNZB (NZMH[5] & Chiron)	P1.7-2,4	2.1%	10.1%
Bivalent (Finlay Institute & GSK)	P1.19,15 & P1.7-2, 4	9.0%	17.6%
HexaMen (NVI)[6]	P1.5-2,10; P1.12-1,13: P1.7-2,4; P1.19,15-1; P1.7,16 & P1.5-1,2-2	12.6%	31.9%
NonaMen (NVI)	HexaMen + P1.22,14; P1.7-1,1; P1.18-1,3	28.9%	38.1%

[1] Based on the prevalence of the homologous PorA subtype; [2] non-invasive strain collection; [3] invasive meningococcal disease strain collection; [4] NIPH, Norwegian Institute of Public Health; [5] NZMH, New Zealand Ministry of Health; [6] NVI, Netherlands Vaccine Institute

Against the whole carriage strain collection the potential coverage by any of the three monovalent vaccines or the bivalent vaccine does not reach 10%. Even the six-valent vaccine only covers around an eighth of the strain collection. The addition of three more PorA subtypes in the NonaMen vaccine considerably improves coverage to between one quarter and one third; however this level is still disappointing. The potential coverage of the subtypes amongst the IMD strain collection by licensed or experimental PorA-based OMV vaccines was also assessed (Table 5.8). Each of the vaccines in Table 5.8 would cover a higher proportion of the IMD strain collection than the carriage strain collection. The coverage of each strain collection by the monovalent and bivalent vaccines was poor. A modest coverage could be achieved by the nonavalent vaccine; however this was still below 30% and 40% for the carriage and IMD strain collections, respectively. At best a potential hexavalent PorA-based OMV vaccine using the

six most prevalent subtypes identified in the whole carriage strain collection in this study could cover 41.3% of strains, rising to 50.6% using the nine most prevalent. At best a potential hexavalent PorA-based OMV vaccine using the six or nine most prevalent subtypes identified in the whole IMD strain collection in this study could cover 57.2% and 66.6% strains, respectively.

5.9 Discussion

The data indicates that within most PorA VR1 and VR2 families, one or two variants tended to predominant and any other variants were only identified sporadically. For example, six variants belonging to the P1.15 Family were identified; variant 15 was predominant (n=66), followed by variant 15-1 (n=22), while the remaining four variants were identified two times or fewer. In a few PorA VR1 and VR2 Families several variants were predominant in the carriage strain collection; i.e. three variants of the P1.5 Family were identified at least 30 times. Furthermore, of the ten currently defined PorA VR1 families only five or six were identified in high numbers. Within PorA variable region families considerable variation has been documented, as evident on the PorA variable region database (http://pubmlst.org/neisseria/PorA/), yet only a comparatively small number of variants were identified in this strain collection. PorA subtyping of the carriage strain collection also identified several new VR sequences providing further evidence for the antigenic variability of the meningococcus. In twelve carried meningococci, and in the ATCC 13090 serogroup B isolate, DNA sequencing of PorA VRs1-3 was not determined because a PCR product could not be amplified using the *porA*-specific primers outlined in Chapter 2.

A handful of laboratories, including the Scottish *Haemophilus*, *Legionella*, Meningococcus & Pneumococcus Reference Laboratory (SHLMPRL), perform DNA sequencing of PorA VR3 in addition to VRs 1 & 2. The inclusion of VR3 data has enabled further discrimination of meningococcal strains by characterising a larger number of subtypes. Despite this, however, PorA VR3 is not one of the typing targets recently recommended by the EMGM. The recommended strain designation for meningococci includes the serogroup, PorA VRs 1 & 2, FetA VR,

MLST sequence type and clonal complex (Brehony, Jolley *et al.* 2007). An example being: C: P1.5, 2-1: F5-4: ST-11 (cc11). At the time this project commenced FetA VR sequencing was not employed by the reference laboratory in Scotland as a means to characterise strains of meningococci, and as such was not included as part of this study.

The data presented here provides evidence that a comparatively small number of dominant PorA VR1 variants, VR2 variants and PorA subtypes have been maintained over many years within carried meningococci from Scotland. In contrast, the majority of PorA subtypes were either short lived or occurred sporadically; supporting the findings of others (Buckee, Jolley *et al.* 2008; Buckee, Gupta *et al.* 2010). Despite the dominance of a few major subtypes, changes were observed in the prevalence of particular subtypes in carried meningococci and in IMD isolates in Scotland over the 31 year period. While none of the major PorA subtypes is exclusively present amongst only the IMD or only the carriage strain collection we have observed that particular subtypes are more prevalent in one strain collection versus the other, and subtypes are variable with respect to their temporal distribution.

Previous work by the reference laboratory using phenotypic methods indicated the prevalence of serotypes and serosubtypes in IMD isolates had changed in Scotland during a 15-20 year period (Clarke 1999b). Amongst serogroup C strains serotype 2b strains decreased while 2a:P1.5 strains increased. Serogroup B strains are more diverse in their antigenic combinations; however 2b:P1.10 and 15:P1.7,1.16 declined during the 1990s while 4:P1.4 increased (Clarke 1999b; Kyaw, Clarke *et al.* 2002). Similar observations were made in England and Wales around the same period; a decline in C:2b, B:2b and B:15:P1.7,16 strains and increases in B:4:P1.4 and B:NT:P1.9 strains during an eleven year period from 1993-1994 onwards (Gray, Trotter *et al.* 2006).

The carriage strain collection was genetically more diverse than the IMD strain collection based on Simpson's Index of diversity. The observed heterogeneity of PorA subtypes in carried meningococci over the thirty-one year period 1974 - 2004 indicates that monovalent PorA-based OMV vaccines would be of limited use against carried meningococci in Scotland. At the very least it would be necessary for a PorA-based OMV vaccine to include multiple subtypes to achieve

even moderate coverage. These moderate levels of coverage are to be expected given the observed diversity in PorA subtypes amongst the carriage strain collection. PorA-based OMV vaccines were developed to target predominant disease strains of serogroup B meningococci (Bjune, Hoiby *et al.* 1991; Sierra, Campa *et al.* 1991; Oster, Lennon *et al.* 2005) therefore one would expect that coverage would increase when assessing IMD strains from Scotland. Indeed, a previous study reported a higher level of coverage of invasive meningococcal isolates by PorA-based OMV vaccines; 65% of all isolates or 94.8% of serogroup B isolates across all age groups by the nine-valent vaccine (Sullivan 2008). Although the period assessed by Sullivan (1972-1998) was not identical to the period discussed in this report (1974-2004) there was considerable overlap and much of the IMD strain collection discussed herein uses data from the study by Sullivan. While one would expect some variation in coverage, the differences are too large to be explained by temporal variation alone. One can only assume differences must exist between the criteria chosen by Sullivan to define vaccine coverage compared with the criteria used herein. By relaxing coverage to include isolates possessing only one VR identical to the vaccine subtype(s), coverage of the two strain collections defined herein does increase (data not shown).

Due to the temporal variation displayed by PorA subtypes it would be pertinent to discuss the most contemporary subtyping data available at the SHLMPRL. During the period 2003 to June 2008, 69 subtypes were identified amongst 384 isolates from confirmed cases of meningococcal disease (R. Ure and B. Denham, personal communication). This PorA subtyping data from the SHLMPRL indicate that vaccine coverage has changed slightly providing further evidence of temporal changes in subtype prevalence. Based on these more recent data (data not shown), coverage across all strains from all ages was 1.3%, 3.4%, 18.0%, 19.3%, 23.2% and 39.1% for the different vaccine preparations as listed in the order outlined in Table 5.8. Thus indicating increases in coverage by the NIPH monovalent, MeNZB monovalent, GSK & Finlay Institute bivalent and NVI nonavalent vaccines. Contrastingly, coverage by the Cuban monovalent and NVI hexavalent vaccines has actually reduced compared with the coverage data for the IMD collection.

Undoubtedly, potential vaccine coverage is under-estimated as the data presented here only take into account strains with identical PorA subtypes. If PorA-related subtypes (based on one shared VR) or indeed heterologous strains (assuming cross-protection between subtypes of the same family) were taken into account perhaps the potential coverage may increase. Limited data exists on the precise bactericidal responses to the vast array of different VR1, VR2 or VR3 peptide epitopes within different PorA subtypes. Immunodominance of VR epitopes appears to be subtype-specific as immune responses due mainly to the VR1 or VR2 have been reported for different PorA subtypes; due to VR1 of subtypes P1.7,16 (van der Voort, van Dijken *et al.* 1997) and P1.12,13 (Wedege, Kolberg *et al.* 1995) or due to VR2 of subtypes P1.5,10 (van der Voort, van der Ley *et al.* 1996; van der Voort, van Dijken *et al.* 1997; Martin, Borrow *et al.* 2000) and P1.7-2,4 (Martin, Ruijne *et al.* 2006). The VR2 epitope has been identified in clinical trials as the main target of vaccine-induced serum bactericidal antibodies. Modification or deletion of the VR2 epitope allowed strains to resist, to varying degrees, complement-mediated lysis (Martin, Ruijne *et al.* 2006).

Due consideration must also be paid to the other antigenic components within these PorA-based OMV vaccines which could themselves be responsible for some part of the immune response generated within a potential recipient following vaccine administration. Other than PorA, OMVs contain multiple different membrane associated components which may elicit an immune response *in vivo*. Boutriau and colleagues reported some cross-protection against heterologous strains when using a bivalent OMV vaccine; however this cross-protection was variable and may be due to other antigens in the OMV preparation (Boutriau, Poolman *et al.* 2007). Investigations using various immunological assays has identified that multiple antigens gave rise to high levels of specific and cross-reacting antibodies in vaccinees receiving MeNZB (7-2,4) or MenBvac (7,16) with no significant difference in post-vaccine responses of the two vaccine groups with each of the PorA antigens (Wedege, Hoiby *et al.* 1998). Proteomic analysis of the VA-MENGOC-BC OMV vaccine preparations identified at least 31 different proteins, of which five or six (FrpB, PorA, PorB, RmpM, Opa and OpcA) contribute 58-65% of the protein content depending upon vaccine batch (Uli, Castellanos-Serra *et al.* 2006). Vipond and colleagues also identified around 40

proteins in their analyses of batches of the MeNZB OMV vaccine, including proteins thought to be important in the development of protective immunity such as Omp85, FetA, PorA, PorB, RmpM, OpcA and NspA (Vipond, Wheeler *et al.* 2005; Vipond, Suker *et al.* 2006).

Presently, meningococcal disease is declining across much of Europe and, where countries have introduced MCC vaccination, the majority of disease cases are due to serogroup B strains (Trotter, Chandra *et al.* 2007). The publication of the genome sequences of a few meningococcal strains of different capsular serogroups from disease (Parkhill, Achtman *et al.* 2000; Tettelin, Saunders *et al.* 2000; Bentley, Vernikos *et al.* 2007; Peng, Yang *et al.* 2008) or from carriage (Schoen, Blom *et al.* 2008) has paved the way for new approaches to vaccine antigen discovery (Pizza, Scarlato *et al.* 2000; Saunders and Butcher 2003; Kelly and Rappuoli 2005; Sardinas, Climent *et al.* 2009). Vaccine strategies targeting serogroup B strains are shifting focus towards a range of subcapsular antigens to improve strain coverage (Pizza, Donnelly *et al.* 2008; Bambini, Muzzi *et al.* 2009; Jacobsson, Hedberg *et al.* 2009). Several antigens, including factor H-binding protein (Masignani, Comanducci *et al.* 2003; Fletcher, Bernfield *et al.* 2004; Pizza, Donnelly *et al.* 2008), Neisseria adhesion A (NadA) or Genome-derived neisserial antigen (GNA) 1994 (Comanducci, Bambini *et al.* 2002), GNA2091, GNA2132 and GNA1030 are currently under investigation for use in various vaccine preparations (Rappuoli 2008; Snape 2008; Jacobsson, Hedberg *et al.* 2009). To keep pace with new developments in vaccine research the SHLMPRL must consider expanding their repertoire of molecular surveillance targets should the efforts of the scientific and research community in producing a serogroup-independent vaccine reach fruition.

6 Strain types identified amongst carried meningococci in Scotland, 1974 - 2004: compilation of serogrouping, MLST & PorA data

6.1 Introduction

The data on sero- & geno-grouping, MLST, and PorA VR sequencing of carried meningococci from the preceding three chapters was combined to enable a more detailed analysis of the circulating carriage strain types in Scotland during the 31-year period covered by the strain collection. The carriage strain collection was characterised into strain types on the basis of serogroup/genogroup: cc: ST: PorA subtype (VR1,VR2,VR3). Data on the most prevalent strain types amongst the carriage strain collection was studied for evidence of any temporal pattern in the distribution of clonal complexes; i.e. any pattern where the prevalence of a particular clonal complex may have altered throughout the period of time covered by the strain collection. The IMD strain collection was examined for the presence of the major strain types found in the carriage strain collection, and vice versa.

6.2 Correlation of serogroup or genogroup with multilocus sequence types and clonal complexes

Table 6.1 displays the prevalence of serogroup and clonal complex associations. Differences were evident in the diversity of lineages associated with particular serogroups, or vice versa. Some lineages were strictly associated to one serogroup while other lineages were associated with several different serogroups. Serogroup A was found to be associated with three clonal complexes; cc1/subgroup I/II, cc5/subgroup III and cc198. Four isolates of serogroup or genogroup A were not assigned to a known clonal complex. No other serogroup was associated with cc1 or cc5 strains. Serogroup B was associated with twenty-three distinct clonal complexes and with STs not assigned to any currently defined clonal complexes. No other serogroup or

genogroup was associated with cc32/ET-5, cc162, cc213, cc231, cc282, cc376 and cc865.

Table 6.1 – Correlation of the serogroup or genogroup with clonal complexes identified in carried meningococci in Scotland, 1974 – 2004

	serogroup[1]/genogroup[2]								NG			
cc	**A**	**B**	**C**	**W135**	**Y**	**X**	**Z**	**29E**	***cnl***	***ctrA***	**PCR NEG**	**ND**
cc1	5/-											
cc5	2/-											
cc8		10/1	71/-									
cc11		3/-	9/-	5/-	1/-							
cc18												
cc22		6/-	8/-	37/6	6/1							
cc23					35/6					1	1	
cc32		19/-										
cc35		45/10	7/-		1/-					1		
cc37												
cc41-44		68/22	18/2		1/-						1	
cc53									31			1
cc60		2/-						1/14				
cc92		-/1	1/-		-/1					1		
cc103							5/1				1	
cc162		1/-										
cc167		1/-			15/4					1		
cc174		2/-	2/-	1/-	2/-							
cc175			1/-									
cc178			-/1		1/-			-/12				
cc198	1/-			1/-					7			
cc212			2/3					-/2				
cc213		23/4										
cc231		1/-										
cc254		4/-	5/-				-/1	2/22			1	
cc269		24/7	-/1									1
cc282		1/-										
cc334		4/-	21/1									
cc364		6/-	2/1	1/-								
cc376		1/1										
cc461		5/-	2/1									
cc549												
cc750						6/1						
cc865		1/1										
cc1117		-/1							1			
cc1136									1			
cc1157		-/1				-/1		-/5				
UA	4/1	42/13	4/1	5/-		2/1	3/10	-/3		2	2	2

[1] Number of strains of a particular serogroup; [2] Number of strains of a particular genogroup; cc, clonal complex; UA, unassigned; NG (NEG), strains were negative by all genogrouping PCR; NG (ND), strains were not fully characterised by genogrouping PCR

Serogroup C was associated with fifteen distinct clonal complexes and with STs not assigned to any currently defined clonal complexes. Serogroup W135 was associated with just five distinct clonal complexes and with STs not assigned to any currently defined clonal complexes. Most specimens were predominantly of the cc22 lineage with a few of the cc11/ET-37 lineage. Serogroup Y was associated with nine distinct clonal complexes but was not associated with any STs not assigned to currently defined clonal complexes. Most specimens were predominantly of the cc23/Cluster A3 lineage, followed by the cc167 lineage. A

few specimens were associated with the cc22 lineage while the other six lineages were uncommon. Serogroup or genogroup X was associated with just two distinct clonal complexes, cc750 and cc1157, and with STs not assigned to any currently defined clonal complexes.

Clonal complexes composed of isolates of more than one sero- or genogroup were inspected further to assess the correlation with individual STs. For the majority of clonal complexes individual STs were found to be of just a single serogroup (data not shown); however Table 6.2 shows those STs found to be associated with more than one sero- or genogroup. Twenty-one STs were associated with more than one sero- or genogroup; twenty of which were assigned to sixteen ccs. In most instances these STs were only associated with two different sero- or genogroups; with the exception of ST11 (serogroups B, C, W135 or Y), ST103 (Z, Z/29E or NEG) and ST198 (A, W135 or *cnl*).

Table 6.2 – Sequence types identified in carried meningococci in Scotland, 1974 – 2004 which are associated with multiple sero- or genogroups

		serogroup[1]/genogroup[2]							NG			
cc	**ST**	**A**	**B**	**C**	**W135**	**Y**	**Z**	**29E**	**Z/29E**	***cnl***	***ctrA***	**(NEG)**
cc8	8		6/-	71/-								
cc11	11		2/-	7/-	3/-	1/-						
cc22	22		1/-		30/5							
	114			1/-		6/1						
	289		5/-	7/-								
cc23	23					26/2						1
cc35	457		14/5	3/-								
	472		3/1					-/1				
cc41-44	206		1/-	3/-								
	1097		2/-			1/-						
cc60	60		1/-					1/9				
cc103	103						5/1		1			1
cc167	167		1/-			8/-					1	
cc198	198	1/-			1/-					5		
cc212	212			2/2				-/1				
cc254	254			2/-				9/3				
cc269	283		1/-	-/1								
cc334	334		1/1	10/1								
cc364	413		1/-	1/1								
cc461	461		3/-	-/1								
UA	1162		5/-	2/-								

[1] Number of strains of a particular serogroup; [2] Number of strains of a particular genogroup; cc, clonal complex; ST, sequence type; NG, non-groupable; Z/29E, cross-reaction with *ctrA*-Z and *ctrA*-29E PCR; *cnl*, capsule null locus; *ctrA*, positive by non-serogroup specific *ctrA* PCR; NEG, no positive reaction with any genotypic serogrouping PCR; UA, unassigned

6.3 Correlation of serogroup or genogroup and of clonal complex with PorA subtype

Information relating to the serogroup or genogroup of carried meningococci was overlaid onto the phylogeny of the concatenated and aligned PorA VR1,2 and 3 amino acid sequences (Figure 6.1). Few strict associations between serogroup and genogroup with PorA subtype were observed. Perhaps the most notable exception was strains of subtype 7n,30n,38n, where n represents any variant within that VR family, which were all non-groupable and tested positive for the capsule null locus by PCR (Figure 6.2). Strains of a particular serogroup could posses a variety of different PorA subtypes, and vice versa. Considering the more prevalent subtypes, strains of three or more serogroups could possess identical subtypes.

With respect to PorA subtype, some clonal complexes were more diverse (Figure 6.3), i.e. cc35 and cc41-44; whereas other ccs were less diverse i.e. cc22 (mostly 18-1,3,38) (Figure 6.4) and cc8 (mostly 5,2,36-2) (Figure 6.5). Furthermore, the previously mentioned related subtypes 7n,30n,38n which were all non-groupable, *cnl*-positive (Figure 6.2) were all of cc53 (Figure 6.6). The subtypes 21,16,37-1 and 19,15,36 were associated with several distinct lineages; nine and seven respectively and with STs not assigned to currently-defined clonal complexes (Figure 6.7).

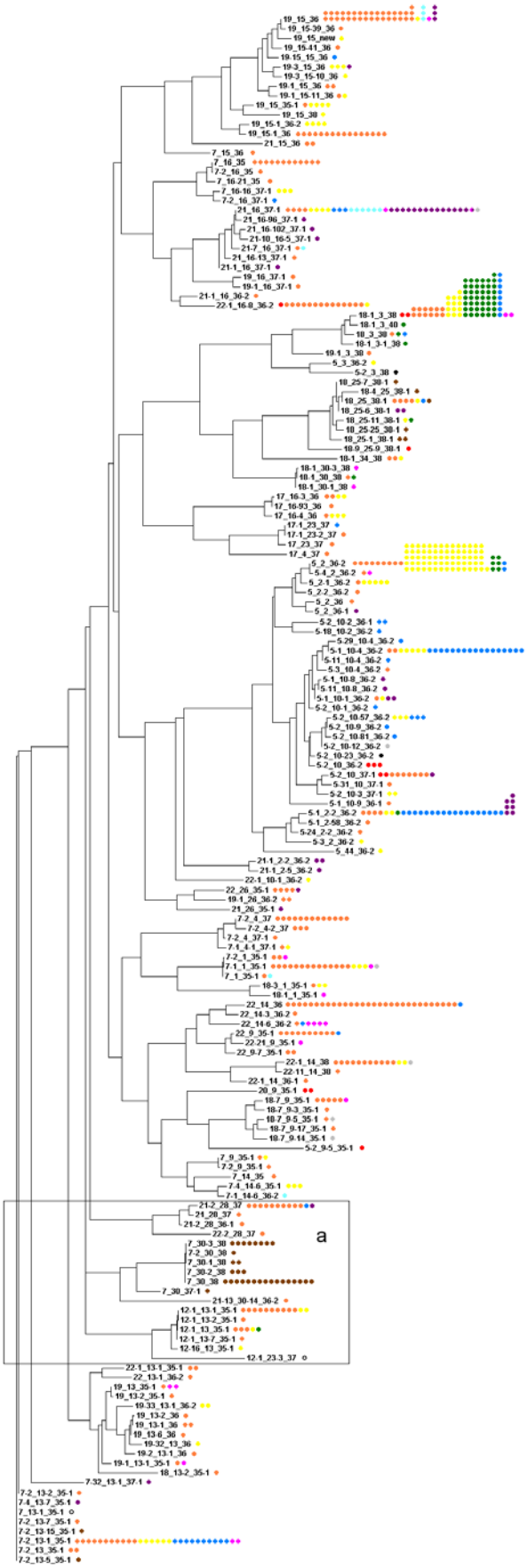

Figure 6.1 - Association of serogroup or genogroup with PorA subtypes identified in carried meningococci in Scotland, 1974 – 2004

For Legend see over page

See previous page

Figure 6.1 - Association of serogroup or genogroup with PorA subtypes identified in carried meningococci in Scotland, 1974 – 2004

The number of circles is equivalent to the number of times a particular subtype was identified amongst carried meningococci; Red solid circles, serogroup or genogroup A; Orange solid circles, serogroup or genogroup B; Yellow solid circles, serogroup or genogroup C; Green solid circles, serogroup or genogroup W135; Dark blue solid circles, serogroup or genogroup Y; Light blue solid circles, serogroup or genogroup X; Pink solid circles, serogroup or genogroup Z; Purple solid circles, serogroup or genogroup 29E; Brown solid circles, capsule null locus; Grey solid circles, *ctrA* non-specific-PCR positive; Half pink & half purple solid circle, cross-reaction between ctrA Z and ctrA 29E PCR; Black solid circle, phenotypically NG & genotypic PCR negative; Black open circle, genogroup not determined; boxed area marked 'a' is shown in Figure 6.2.

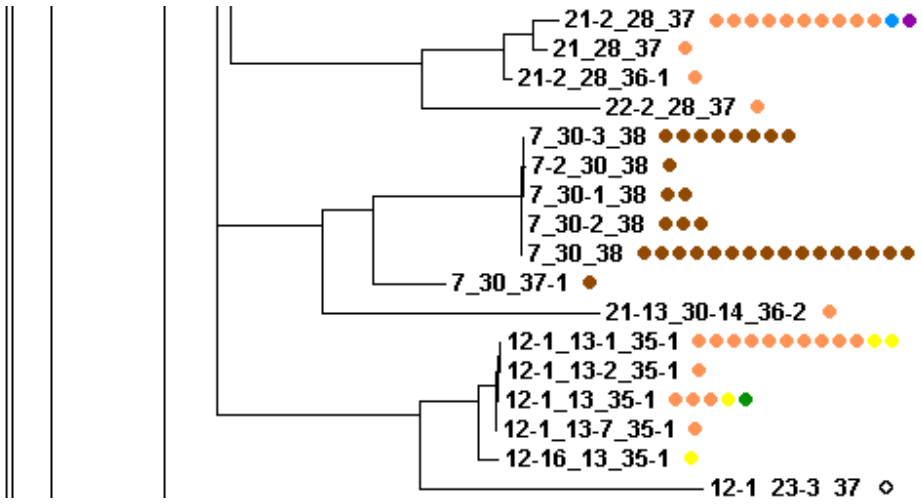

Figure 6.2 – Enlargement of section a of Figure 6.1

The number of circles is equivalent to the number of times a particular subtype was identified amongst carried meningococci; Orange solid circles, serogroup or genogroup B; Yellow solid circles, serogroup or genogroup C; Green solid circles, serogroup or genogroup W135; Dark blue solid circles, serogroup or genogroup Y; Purple solid circles, serogroup or genogroup 29E; Brown solid circles, capsule null locus; Black open circle, genogroup not determined

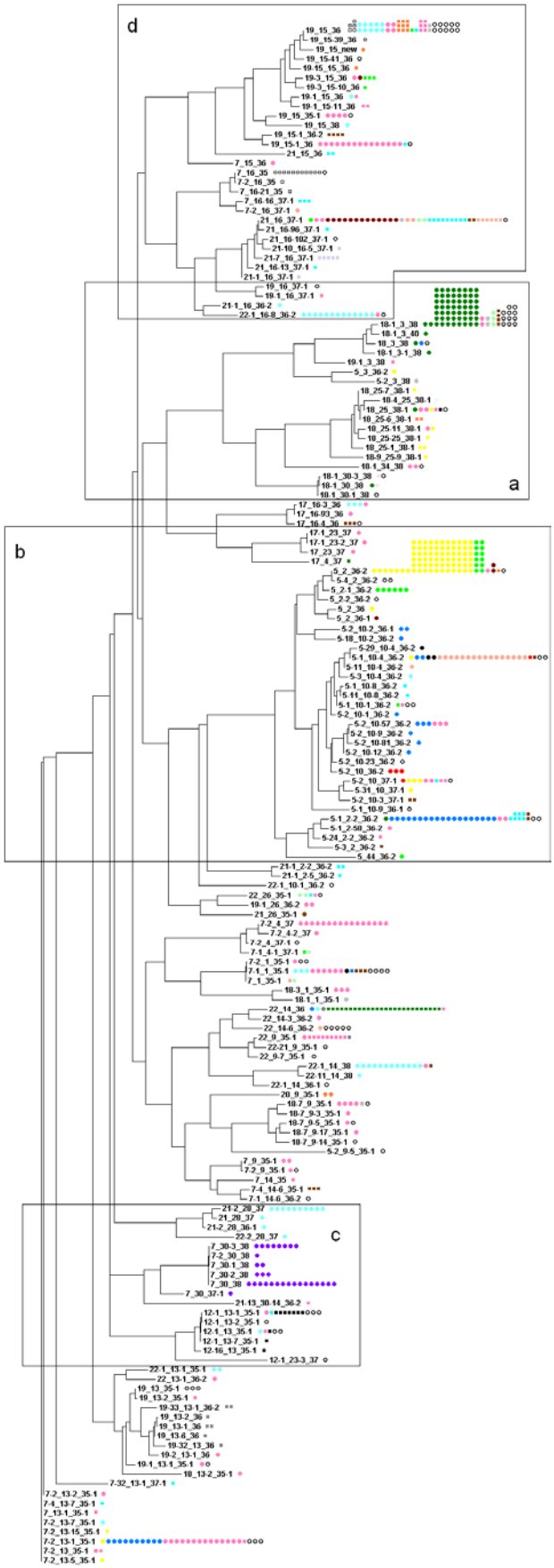

Figure 6.3 - Association of clonal complex with PorA subtypes identified in carried meningococci in Scotland, 1974 - 2004

For Legend see over page

See previous page

Figure 6.3 - Association of clonal complex with PorA subtypes identified in carried meningococci in Scotland, 1974 - 2004

The number of circles or squares is equivalent to the number of times a particular subtype was identified amongst carried meningococci; Red solid circle, cc1; Orange solid circle, cc5; Yellow solid circle, cc8; Bright green solid circle, cc11; Dark green solid circle, cc22; Dark blue solid circle, cc23; Black open squares, cc32; Light blue solid circle, cc35; Pink solid circle, cc41-44, Purple solid circle, cc53; Brown solid circle, cc60; Black solid circle, cc92; Light grey solid circle, cc103; Dark grey solid circle, cc162; Peach solid circle, cc167; Pale green solid circle, cc174; Red solid squares, cc175; Orange solid square, cc178; Yellow solid squares, cc198; Bright green solid square, cc212; Dark green solid square, cc213; Dark blue solid square, cc231; Light blue solid square, cc254; Pink solid square, cc269; Purple solid square, cc282; Brown solid square, cc334; Black solid square, cc364; Light grey solid square, cc376; Dark grey solid square, cc461; Peach solid square, cc750; Pale green solid square, cc865; Pale pink solid square, cc1117; Pale blue solid square, cc1136; Pale purple solid square, cc1157; Black open circle; Unassigned to a clonal complex; boxed area marked ‘a’ is shown in Figure 6.4; boxed area marked ‘b’ is shown in Figure 6.5; boxed area marked ‘c’ is shown in Figure 6.6; boxed area marked ‘d’ is shown in Figure 6.7.

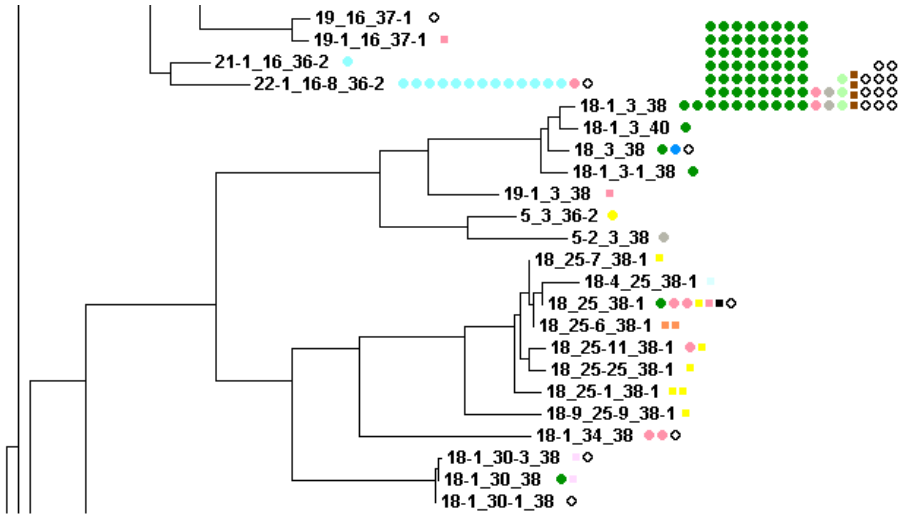

Figure 6.4 – Enlargement of section a of Figure 6.3

The number of circles or squares is equivalent to the number of times a particular subtype was identified amongst carried meningococci; Orange solid circle, cc5; Yellow solid circle, cc8; Bright green solid circle, cc11; Dark green solid circle, cc22; Dark blue solid circle, cc23; Light blue solid circle, cc35; Pink solid circle, cc41-44; Light grey solid circle, cc103; Dark grey solid circle, cc162; Peach solid circle, cc167; Pale green solid circle, cc174; Orange solid square, cc178; Yellow solid squares, cc198; Pink solid square, cc269; Brown solid square, cc334; Black solid square, cc364; Light grey solid square, cc376; Peach solid square, cc750; Pale green solid square, cc865; Pale pink solid square, cc1117; Pale blue solid square, cc1136; Pale purple solid square, cc1157; Black open circle; Unassigned to a clonal complex.

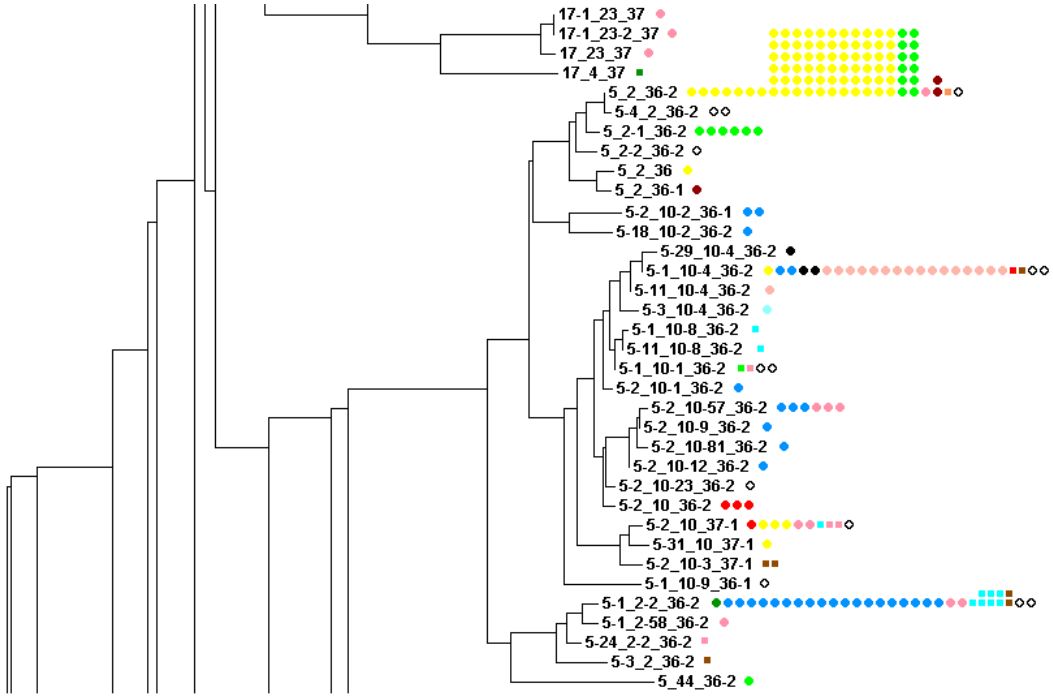

Figure 6.5 – Enlargement of section b of Figure 6.3

The number of circles or squares is equivalent to the number of times a particular subtype was identified amongst carried meningococci; Red solid circle, cc1; Yellow solid circle, cc8; Bright green solid circle, cc11; Dark green solid circle, cc22; Dark blue solid circle, cc23; Light blue solid circle, cc35; Pink solid circle, cc41-44; Brown solid circle, cc60; Black solid circle, cc92; Peach solid circle, cc167; Red solid square, cc1; Orange solid square, cc178; Bright green solid square, cc212; Light blue solid square, cc254; Pink solid square, cc269; Brown solid square, cc334; Pale blue solid square, cc1136; Black open circle; Unassigned to a clonal complex.

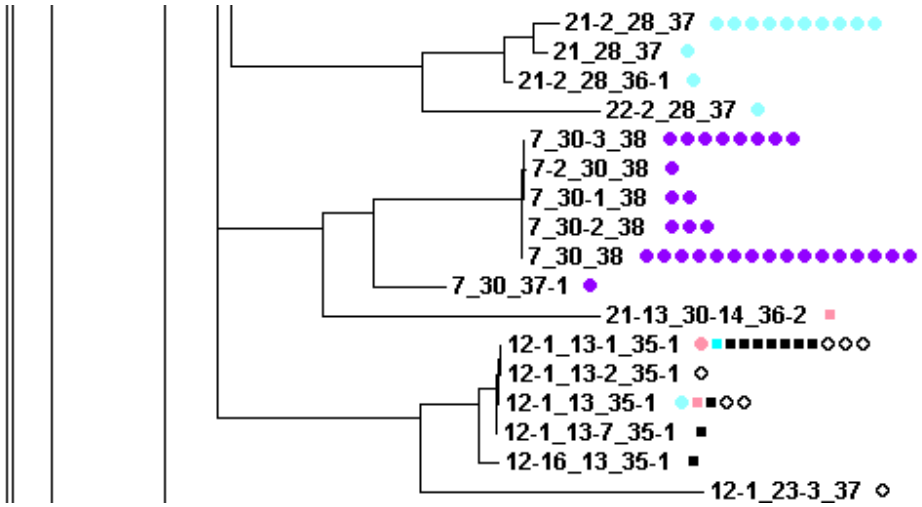

Figure 6.6 – Enlargement of section c of Figure 6.3

The number of circles or squares is equivalent to the number of times a particular subtype was identified amongst carried meningococci; Light blue solid circle, cc35; Pink solid circle, cc41-44, Purple solid circle, cc53; Light blue solid square, cc254; Pink solid square, cc269; Black solid square, cc364; Black open circle; Unassigned to a clonal complex.

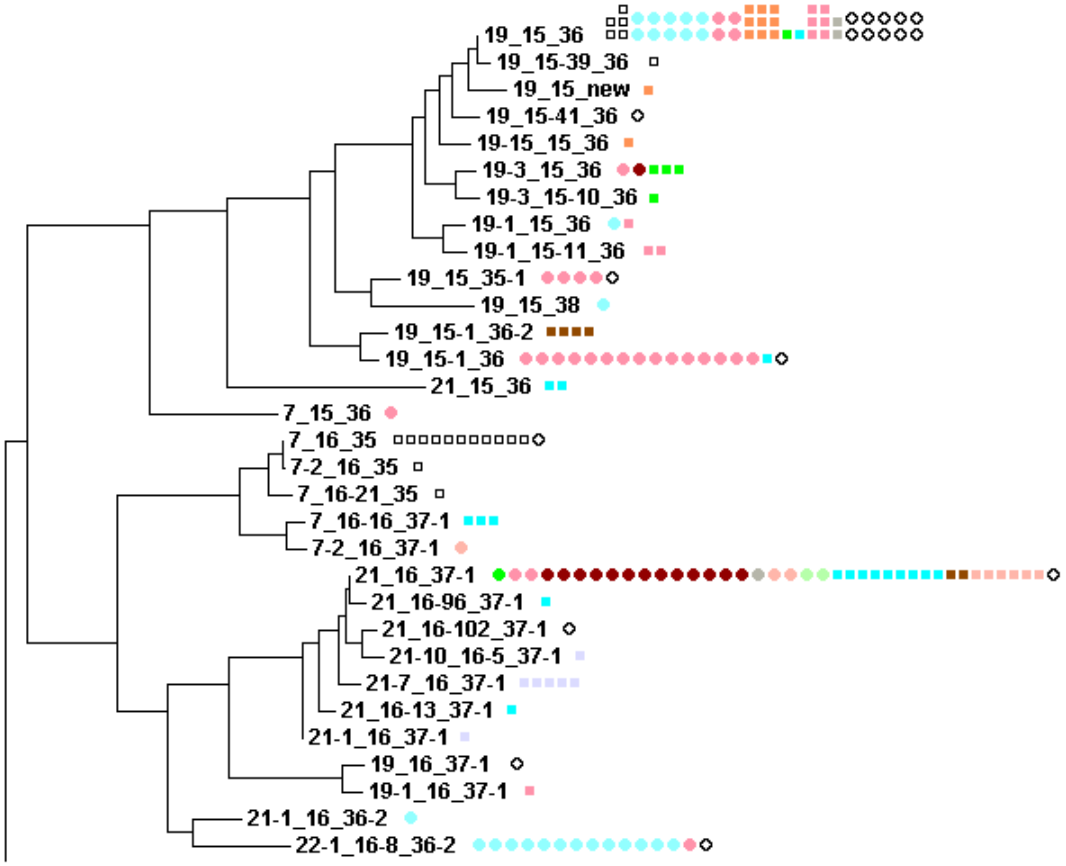

Figure 6.7 – Enlargement of section d of Figure 6.3

The number of circles or squares is equivalent to the number of times a particular subtype was identified amongst carried meningococci; Orange solid circle, cc5; Bright green solid circle, cc11; Black open squares, cc32; Light blue solid circle, cc35; Pink solid circle, cc41-44, Brown solid circle, cc60; Light grey solid circle, cc103; Peach solid circle, cc167; Pale green solid circle, cc174; Orange solid square, cc178; Bright green solid square, cc212; Light blue solid square, cc254; Pink solid square, cc269; Brown solid square, cc334; Light grey solid square, cc376; Peach solid square, cc750; Pale green solid square, cc865; Pale pink solid square, cc1117; Pale blue solid square, cc1136; Black open circle; Unassigned to a clonal complex.

6.4 Prevalence of strain types amongst carried meningococci in Scotland, 1974 – 2004

The carriage isolate collection was characterised into 438 strain types on the basis of serogroup/genogroup:cc:ST:PorA subtype (VR1,VR2,VR3) (Table 6.3). The vast majority of these strain types were identified in four carried meningococci or fewer while twenty-six strain types were identified in five or more carried meningococci, accounting for 290 specimens or 36.3% of the carriage isolate collection.

Table 6.3 – Prevalence of strain types identified in carried meningococci in Scotland, 1974 - 2004

Group	cc[1]	ST[2]	VR1[3]	VR2[4]	VR3[5]	Frequency[6] (Phenotypic/PCR)
A	cc1/subgroup I/II	1	5-2	10	36-2	3/-
			5-2	10	37-1	1/-
			ND	ND	ND	1/-
	cc5/subgroup III	5	20	9	35-1	2/-
	cc198	198	18-9	25-9	38-1	1/-
	Unassigned	3287	18-1	3	18	1/-
		7413	22-1	16-8	36-2	-/1
		8222	5-2	9-5	35-1	1/-
		8646	5-2	10	37-1	1/-
		8648	18-1	3	38	1/-
B	cc5/subgroup III	153	5-2	10	37-1	1/-
	cc11/ET-37	11	5	2	36-2	1/-
	cc8/Cluster A4	8	5	2	36-2	4/-
			5	2	36	1/-
			7-2	13-1	35-1	1/-
		153	5-2	10	37-1	2/-
			5	2-1	36-2	1/-
			5-31	10	37-1	-/1
		4037	5	2	36-2	1/-
	cc22	22	18-1	3	38	1/-
		289	18-1	3	38	5/-
	cc32/ET-5	32	7	16	35	4/-
			7	16-21	35	1/-
			7-2	16	35	1/-
		33	19	15	36	5/-
			19	15-39	36	1/-
		259	7	16	35	7/-
	cc35	35	22	14	36	1/-
			22-1	14	38	-/1
		278	7-2	13-7	35-1	1/-
		457	22-1	14	38	5/-
			22-1	16-8	36-2	4/2
			19	15	36	3/3
			19-1	15	36	1/-
			21-1	16	36-2	1/-
		472	21-2	28	37	3/-
		1197	21	28	37	1/-
		1292	22-2	28	37	1/-
		1651	22-11	14	38	-/1
		1710	22-1	13-1	35-1	2/-
		2380	21-2	28	37	-/1
		3295	22-1	14	38	1/-
		5945	21-2	28	37	1/-
		6033	21-2	28	37	1/-
		6402	19	15	36	1/-
		6658	22-1	14	38	1/-
		6660	22-1	16-8	36-2	2/-
		6904	22-1	14	38	-/1
		6905	5-3	10-4	36-2	1/-
		6907	22-1	16-8	36-2	1/-
		6911	22-1	16-8	36-2	2/-
		6913	19	15	36	2/-
		7271	21-2	28	37	1/-
		7404	12-1	13-2	35-1	1/-
		7408	19	15	36	1/-
		7410	7-1	1	35-1	1/-
		7797	21-2	28	37	1/-
		7798	17	16-3	36	-/1
		7903	22-1	16-8	36-2	1/-
		7969	22-1	16-8	36-2	1/-
		7997	21-2	28	36-1	1/-
		8001	21-2	28	37	1/-
		8232	22-1	14	38	1/-
	cc41-44/Lineage 3	41	7-2	4	37	4/1
			7-2	4-2	37	1/-
			ND	13-15	ND	1/-
			18-1	3	38	1/-
		43	19	15-1	36	9/5
			19	15	16	1/-
			19-2	13-1	36	1/-
		44	5-1	2-2	36-2	2/-
			7-1	1	35-1	2/-
			5-2	10	37-1	1/-

Group	cc[1]	ST[2]	VR1[3]	VR2[4]	VR3[5]	Frequency[6] (Phenotypic/PCR)
B (cont.)	cc41-44/Lineage 3 (cont.)	44 (cont.)	7-2	13	35-1	1/-
			19-1	26	36-2	-/2
			22	13-1	3-2	1/-
		136	5-1	2-58	36-2	1/-
			17	16-3	36	-/1
			17	16-93	36	1/-
		170	18-1	3	38	1/-
			21	16	37-1	1/-
		180	7-2	13-1	35-1	-/1
		206	18-3	1	35-1	1/-
		340	7-2	4	37	-/1
		437	5-2	10	37-1	1/-
			7-2	13-1	35-1	1/-
			22-1	14	38	-/1
		464	19	15	36	1/-
		482	21	16	37-1	-/1
		571	18-7	9	35-1	1/-
		577	7-2	4	37	8/-
		833	7	15	36	1/-
		835	19	35	36	-/1
		1097	17	23	37	1/-
			17	23-2	37	1/-
		1361	12-1	13-1	35-1	-/1
		1433	7-2	1	35-1	1/-
		1489	5	2	36-2	1/-
		1908	7-1	1	35-1	1/-
		1915	7-2	13-1	35-1	1/-
		1947	7-2	9	35-1	-/1
		2120	18-7	9	35-1	1/-
		2433	19	15-1	36	-/1
		2459	7-1	1	35-1	1/-
		2514	18-7	9-3	35-1	-/1
			19	15	36	1/-
		2532	18-7	19-5	35-1	1/-
			18-7	19-17	35-1	1/-
		2631	7	9	35-1	-/1
		3296	7-2	13-1	35-1	3/1
		3866	7-2	4	37	1/-
		5120	7-2	13-1	35-1	1/-
		5471	18-7	9	35-1	-/1
		6034	18-7	9	35-1	1/-
		6655	7-1	1	35-1	1/-
		7270	18	25	38-1	1/-
		7621	18	13-2	35-1	1/-
		7624	7-1	1	35-1	1/-
		7720	18-1	34	38	1/-
		7730	7-2	13-2	35-1	1/-
		7799	18	25	38-1	1/-
		8220	22	14-3	36-2	-/1
	cc60	60	5	2	36-2	1/-
		allele	ND	ND	ND	1/-
	cc96	94	5-1	10-4	36-2	-/1
	cc162	162	22	14	36	1/-
	cc167	167	5-1	10-4	36-2	1/-
	cc174	2547	22	26	35-1	1/-
		7939	22	26	35-1	1/-
	cc213	213	22	14	36	20/3
			17	4	37	-/1
		1218	22	14	36	1/-
		5951	22	14	36	1/-
	cc231	1011	7-1	1	35-1	1/-
	cc254	3294	21	15	36	2/-
		7991	21	16-13	37-1	1/-
		8221	12-1	13-1	35-1	1/-
	cc269	13	19	15	36	1/-
		269	5-24	2-2	36-2	-/1
			12-1	13	35-1	1/-
			18	25	38-1	1/-
			19	15	36	3/-
			19-1	15-11	36	1/-
			19-1	16	37-1	-/1
			22	9	35-1	1/-
		275	22	9	35-1	1/1
		283	7	14	35	1/-
		352	22	9	35-1	1/-
		479	19-1	15	36	-/1
		492	21-13	30-14	36-2	1/-

Group	cc[1]	ST[2]	VR1[3]	VR2[4]	VR3[5]	Frequency[6] (Phenotypic/PCR)
B	cc269	1161	22	9	35-1	1/-
(cont.)	(cont.)	1273	22	9	35-1	1/-
		1284	22	14	36	-/1
		1416	22	9	35-1	1/-
		2166	22	26	35-1	-/1
		2239	5-2	10	37-1	1/-
		2592	19	15	36	1/-
		2803	19-1	3	38	1/-
		3286	22	9	35-1	-/1
		5984	19	15	36	1/-
		6908	5-2	10	37-1	1/-
		7269	19	13-2	35-1	1/-
		7992	22	9	35-1	1/-
		8000	7-2	13	35-1	1/-
		8231	7-2	13-1	35-1	1/-
	cc282	1802	5-1	10-1	36-2	1/-
	cc334	334	5-1	2-2	36-2	1/-
		2552	7-1	1	35-1	2/-
		3285	21	16	27-1	1/-
	cc364	15	12-1	13-7	35-1	1/-
		413	12-1	13-1	35-1	1/-
		2534	12-1	13-1	35-1	1/-
		4650	12-1	13-1	35-1	2/-
		7965	12-1	13-1	35-1	1/-
	cc376	7405	19	15	36	-/1
		7265	19	15	36	1/-
	cc461	461	19	13-1	36	1/-
			19	13-2	36	1/-
			22	9	35-1	1/-
		6403	19	13-6	36	1/-
		7972	19	13-1	36	1/-
	cc865	460	7-1	4-1	37-1	1/-
		865	7	1	35-1	-/1
	cc1117	1117	18-1	3	38	-/1
	cc1157	1157	21-7	16	37-1	-/1
	Unassigned	284	19	15	36	1/-
			ND	ND	ND	1/-
		288	19	15-1	36	1/-
			18-1	3	38	1/-
		336	7-1	1	35-1	1/1
			19	13	35-1	1/-
		1162	NEG	NEG	NEG	5/-
		2152	5-1	10-9	36-1	1/-
		2528	18-7	9	35-1	-/1
		2875	neg	neg	neg	2/-
		3621	7-1	1	35-1	1/-
		4053	19	15	36	-/1
		4221	19	15	36	-/2
			19	15-41	36	-/1
			19	16	37-1	-/1
			22-1	14	36	-/1
		4597	5-1	2-2	36-2	1/-
		5952	21	16	37-1	1/-
		6654	18	3	38	1/-
			18-1	3	38	1/-
		6656	12-1	13	35-1	1/-
		6659	7	16	35	1/-
		6661	19	15	36	1/-
		6663	7-2	1	35-1	-/1
		7268	17	16-4	36	1/-
		7398	7-2	4	37-1	1/-
		7399	12-1	13	35-1	1/-
		7407	22	9-7	35-1	1/1
		7411	18-1	3	38	1/-
		7412	5-4	2	36-2	1/-
		7620	5	2	36-2	1/-
		7622	19	15	36	1/-
		7723	5-1	10-4	36-2	1/-
		7726	7-1	1	38-1	1/-
		7727	12-1	13-1	35-1	1/-
		7729	12-1	13-1	35-1	1/-
		7801	18	25	38-1	1/-
		7802	22	14-6	36-2	1/-
		7905	5	2-2	36-2	1/-
		7970	18-1	3	38	1/-
		7990	19	15	36	-/1
		7998	22	26	35-1	1/-
		7999	18-1	34	38	-/1

Group	cc[1]	ST[2]	VR1[3]	VR2[4]	VR3[5]	Frequency[6] (Phenotypic/PCR)
B	Unassigned	8003	12-1	13-1	35-1	1/-
(cont.)	(cont.)	8066	7-2	13-1	35-1	-/1
		abcZ neg	neg	neg	neg	1/-
C	cc8/Cluster A4	8	5	2	36-2	69/-
			5	3	36-2	1/-
			5-1	10-4	36-2	1/-
	cc11/ET-37	11	5	2	36-2	1/-
			5	2-1	36-2	5/-
			5	44	36-2	1/-
		67	5	2	36-2	1/-
		4677	21	16	37-1	1/-
	cc22	114	18-1	3	38	1/-
		289	18	25	38-1	1/-
			18-1	3	38	6/-
	cc35	457	12-1	13	35-1	1/-
			19	15	36	1/-
			22-1	14	38	1/-
		3676	17	16-3	36	1/-
		7264	7-1	1	35-1	1/-
	cc41-44/Lineage 3	42	22-1	16-8	36-2	1/-
		206	7	9	35-1	1/-
			18-3	1	35-1	1/-
			19	15	35-1	1/-
		337	18-3	1	35-1	1/-
		1362	19	15	36	1/-
		1574	7-2	13-1	35-1	6/-
		3278	18-1	34	38	-/1
		6657	5-2	10-57	36-2	3/-
			19	15	36	1/-
			19-3	15	36	1/-
		7266	18	25-11	38-1	-/1
		7971	19	15	35-1	1/-
	cc92	7722	5-1	10-4	36-2	1/-
	cc174	1057	18-1	3	38	1/-
		3293	18-1	3	38	1/-
	cc175	7800	5-1	10-4	36-2	1/-
	cc178	7987	19	15	new	-/1
	cc212	212	7-1	4-1	37-1	-/1
			19	15	36	1/-
			19-3	15	36	1/1
		1229	19-3	15-10	36	-/1
	cc254	254	21	16	37-1	2/-
		468	7	16-16	37-1	3/-
	cc269	283	19-1	15-11	36	-/1
	cc334	189	5-2	10-3	37-1	2/-
		334	5-1	2-2	36-2	1/-
			5-1	10-4	36-2	-/1
			18-1	3	38	1/-
			19	15-1	36-2	4/-
			21	16	37-1	1/-
		1031	7-4	14-6	35-1	2/-
		1034	5-3	2	36-2	1/-
			17	16-4	36	3/-
		2508	7-4	14-6	35-1	1/-
		2526	22-1	14	38	1/-
		3464	7-1	1	35-1	1/-
	cc364	413	12-1	13-1	35-1	1/1
		7733	12-16	13	35-1	1/-
	cc461	461	19-32	13	36	-/1
		6430	19-33	13-1	36	1/-
		6910	19-33	13-1	36	1/-
	Unassigned	344	5-1	10-1	36-2	-/1
			5-1	10-4	36-2	1/-
W135	cc11/ET-37	11	5	2	36-2	4/-
		473	5	2	36-2	1/-
		1246	5	2	36-2	1/-
	cc22	22	5-1	2-2	36-2	1/-
			18	3	38	-/1
			18-1	3	38	28/4
		1224	18-1	3	40	1/-
			18-1	3-1	38	1/-
		1226	18-1	3	38	1/-
		2878	18-1	3	38	1/-
		5948	18-1	30	38	-/1
		7409	18-1	3	38	2/-
		7904	18-1	3	38	1/-
	cc174	185	18-1	3	38	1/-
	cc198	198	18	25-11	38-1	1/-

Group	cc[1]	ST[2]	VR1[3]	VR2[4]	VR3[5]	Frequency[6] (Phenotypic/PCR)
W135	cc364	462	12-1	13	35-1	1/-
	Unassigned	1251	18-1	3	38	5/-
X	cc750	24	7	1	35-1	1/-
		750	21	16	37-1	5/1
	cc1157	1157	21-7	16	37-1	-/1
	Unassigned	961	19	15	36	1/-
		1220	19	15	36	1/-
		5947	7-1	14-6	36-2	-/1
		7989	19	15	36	1/-
Y	cc11/ET-37	11	5	2	36-2	2/-
	cc22	114	18-1	3	38	6/1
		7409	18-1	3	38	1/-
	cc23/Cluster A3	23	5-1	2-2	36-2	12/-
			5-2	10-2	36-1	2/-
			5-2	10-57	36-2	-/2
			5-2	10-81	36-2	1/-
			7-2	13-1	35-1	10/-
			22	14	36	1/-
		183	5-18	10-2	36-2	-/1
		1655	5-1	10-4	36-2	1/1
		1794	5-1	2-2	36-2	1/-
		1811	18	3	38	1/-
		5544	5-2	10-9	36-2	1/-
		6800	5-2	10-57	36-2	1/-
		7406	5-1	2-2	36-2	1/-
		7731	5-1	2-2	36-2	-/2
	cc41-44/Lineage 3	1097	17-1	23	37	1/-
		7728	22	9	35-1	1/-
	cc92	7721	5-29	10-4	36-2	-/1
	cc167	167	5-1	10-4	36-2	5/-
			5-11	10-4	36-2	1/-
			7-2	16	37-1	1/-
			21	16	37-1	1/-
		168	5-1	10-4	36-2	½
		766	5-1	10-4	36-2	3/-
			22	14-6	36-2	-/1
		2415	5-1	10-4	36-2	1/1
		6031	5-1	10-4	36-2	1/-
		7803	5-1	10-4	36-2	1/-
	cc174	1466	21	16	37-1	2/-
	cc178	6662	19-15	15	36	1/-
	cc364	7725	18	25	38-1	1/-
ctrA Z	cc103	103	18-1	1	35-1	-/1
			18-1	3	38	-/2
			18-7	9	35-1	-/1
			21	16	37-1	-/1
			ND	ND	ND	-/1
	cc254	1953	19	15	36	-/1
	Unassigned	1455	7-2	13-1	35-1	-/1
			19	13	35-1	-/2
			19-1	13-1	35-1	-/1
		2154	22	14-6	36-2	-/4
		4874	5-4	2	36-2	-/1
		5946	7-2	1	35-1	-/1
		5953	18-1	30-1	38	-/1
		7272	7-2	13-1	35-1	-/1
		7967	18-1	30-3	38	-/1
		7975	7-1	1	35-1	-/1
		8647	22-21	9	35-1	-/1
ctrA 29E	cc35	472	21-2	28	37	-/1
	cc60	60	21	16	37-1	-/9
		1650	5	2	36-2	-/1
		1780	21	16	37-1	-/1
		2104	21	16	37-1	-/1
		2155	5	2	36-1	-/1
		6903	19-3	15	36	-/1
		7724	21	16	37-1	-/1
	cc178	178	5	2	36-2	-/1
			18	25-6	38-1	-/1
			19	15	36	-/6
		5949	19	15	36	-/1
		6912	19	15	36	-/1
		new *fumC*	19	15	36	-/1
	cc212	212	19-3	15	36	-/1
		7618	5-1	10-1	36-2	-/1
	cc254	254	5-1	2-2	36-2	-/2
			5-2	10	37-1	-/1
			5-11	10-8	36-2	-/1

Group	cc[1]	ST[2]	VR1[3]	VR2[4]	VR3[5]	Frequency[6] (Phenotypic/PCR)
ctrA 29E	cc254	254	7-4	13-7	35-1	-/1
(cont.)	(cont.)	(cont.)	21	16	37-1	-/3
			21-1	2-2	36-2	-/2
			21-1	2-5	36-2	-/1
		1839	5-1	2-2	36-2	-/1
		2520	7-32	13-1	37-1	-/1
			21	16	37-1	-/1
			21	16-96	37-1	-/1
			22	26	35-1	-/1
		2701	21	16	37-1	-/1
		4881	5-1	2-2	36-2	-/1
		6914	5-1	2-2	36-2	-/1
		6915	5-1	2-2	36-2	-/1
		7203	19	15-1	36	-/1
		7968	5-1	10-8	36-2	-/2
		new *fumC* & *pgm*	21	16	37-1	-/1
	cc1157	1157	21-1	16	37-1	-/1
			21-7	16	37-1	-/3
		7619	21-10	16-5	37-1	-/1
	Unassigned	4385	5-1	10-1	36-2	-/1
		6032	7-2	9	35-1	-/1
ctrA 29E	Unassigned	7732	21	16-102	37-1	-/1
ctrA Z/29E	cc60	2002	21	16	37-1	-/1
cnl	cc53	53	7	30	37-1	-/1
			7	30	38	-/15
			7	30-1	38	-/2
			7	30-2	38	-/3
			7	30-3	38	-/8
			7-2	30	38	-/1
		5950	7	30	38	-/1
	cc198	198	18	25	38-1	-/1
			18	25-1	38-1	-/2
			18	25-7	38-1	-/1
			18	25-25	38-1	-/1
		823	7-2	13-15	35-1	-/1
		7782	7-2	13-15	35-1	-/1
	cc1117	1117	18-1	30-3	35-1	-/1
	cc1136	8635	18-4	25	38-1	-/1
ctrA	cc23/cluster A3	8002	5-2	10-12	36-2	-/1
	cc35	7988	22-1	14	38	-/1
	cc92	7966	7-1	1	35-1	-/1
	cc167	167	21	16	37-1	-/1
	Unassigned	3258	18-7	9-14	35-1	-/1
		7267	18-7	9-5	35-1	-/1
PCR NEG	cc23/cluster A3	23	5-1	2-2	36-2	-/1
	cc41-44/Lineage 3	577	7-2	4	37	-/1
	cc103	103	5-2	3	38	-/1
	cc254	7964	21	16	37-1	-/1
	Unassigned	4221	19	15	36	-/1
		8222	5-2	10-23	37-1	-/1
NG	cc53	53	-	30	38	1/-
	cc269	283	7	13-1	35-1	1/-
	Unassigned	2539	12-1	23-3	37	1/-
		4878	-	-	38	1/-

[1] cc, clonal complex; [2] ST, Sequence Type; [3] VR1, PorA Variable Region 1; [4] VR2, PorA Variable Region 2; [5] VR3, PorA Variable Region 3; [6] Frequency, indicates the number of strains of a particular strain type where the "group" was identified phenotypically (to the left of the stroke) or by PCR (to the right of the stroke); NG, nongroupable; ND, not determined; PCR NEG, specimens failed to give a positive result with any of the serogroup specific, *cnl* or *ctrA* PCR tests;

Eleven, four, four, two and two strain types associated with sero- or genogroups B, C, Y, W135, 29E, respectively. Two strain types were associated with capsule null locus-containing isolates and one strain type was associated with serogroup X. The most prevalent strain types, listed in Table 6.3, in rank order were, sixty-nine specimens of the strain type C:8:8:5,2,36-2 that were isolated during 1985 -

1987; thirty-two specimens of the strain type W135/w:22:22:18-1,3,38 (n=28/4) that were isolated during 1977 – 2001; twenty-three specimens of the strain type B/b:213:213:22,14,36 (n=20/3) that were isolated during 1996 – 2003; fifteen specimens of the strain type *cnI*:53:53:7,30,38 that were isolated during 1984 – 2003; fourteen specimens of the strain type B/b:41-44:43:19,15-1,36 (n=9/5) that were isolated during 1983 - 2002; twelve specimens of the strain type Y:23:23:5-1,2-2,36-2 that were isolated during 1979 - 2001; ten specimens of the strain type Y:23:23:7-2,13-1,35-1 that were isolated during 1985; nine specimens of the strain type *ctrA*29E:60:60:21,16,37-1 that were isolated during 1984 – 1988; eight specimens of the strain type B:41-44:577:7-2,4,37 that were isolated during 1977 - 1997; eight specimens of the strain type *cnI*:53:53:7,30-3,38 that were isolated during 1984 – 2002; seven specimens of the strain type B:32:259:7,16,35 that were isolated during 1987 and seven specimens of the strain type Y/y:22:114:18-1,3,38 (n=6/1) that were isolated during 1985 – 1988 (data not shown).

A further six specimens of the strain type B/b:35:457:22-1,16-8,36-2 (n=4/2) that were isolated during 1985 - 1988; six specimens of the strain type B/b:35:457:19,15,36 (n=3/3) that were isolated during 1982 - 1997; six specimens of the strain type C:22:289:18-1,3,38 that were isolated during 1985 – 1988; six specimens of the strain type C:41-44:1574:7-2,13-1,35-1 that were isolated during 1985 – 1987; six specimens of the strain type X/x:750:750:21,16,37-1 (n=5/1) that were isolated during 1998 – 2001 and six specimens of the strain type *ctrA*29E:178:178:19,15,36 that were isolated during 1984 – 1987; five specimens of the strain type B:22:289:18-1,3,38 that were isolated during 1977 - 1989; five specimens of the strain type B:32:33:19,15,36 that were isolated during 1982 – 1987; five specimens of the strain type B:35:457:22-1,14,38 that were isolated during 1979 - 1987; five specimens of the strain type B/b:41-44:41:7-2,4,37 (n=4/1) that were isolated during 1996 - 2001; five specimens of the strain type B:UA:1162:neg,neg,neg that were isolated during 1984 – 1993; five specimens of the strain type C:11:11:5,2-1,36-2 that were isolated during 1986 - 1987; five specimens of the strain type W135:UA:1251:18-1,3,38 that were isolated during 1985 – 1986 and five specimens of the strain type Y:167:167:5-1,10-4,36-2 that were isolated during 1985 – 1988 (data not shown).

Those STs identified to be associated with multiple sero- or genogroups listed in Table 6.2 were examined further by comparing the PorA subtypes to identify strains which may have undergone capsule switching. The data indicate that at least fourteen capsule switching events may have occurred within the carriage strain collection if we are to assume that strains that are of identical ST and PorA subtype but of a different sero- or genogroup provides us with evidence of a capsule switching event (Table 6.4).

Table 6.4 – Strain types of the meningococcal carriage strain collection that may have undergone capsule switching

cc	ST	PorA			Group				
		VR1	VR2	VR3	B	C	W135	Y	29E
8	8	5	2	36-2	4	70			
11	11	5	2	36-2	1	1	3	1	
		5	2-1	36-2	1	6			
22	22	18-1	3	38	1		32		
	114	18-1	3	38		1		7	
	289	18-1	3	38	4	7			
35	457	22-1	14	38	5	1			
	472	21-2	28	37	3				1
41-44	206	18-3	1	35-1	1	1			
167	167	5-1	10-4	36-2	1			6	
212	212	19-3	15	36		2			1
254	254	21	16	37-1		2			3
334	334	5-1	2-2	36-2	1	1			
	413	12-1	13-1	35-1	1	2			
1162	1162	-	-	-	5	1			

cc, clonal complex; ST, sequence type; VR, variable region; Group, sero- or genogroup; numbers in the columns below "Group" indicate the number of carried meningococci of a particular strain type that is associated with a particular serogroup.

Within the carriage strain collection nine capsule switching events may have occurred involving serogroups B and C; two capsule switching events may have occurred involving serogroups C and 29E; one capsule switching event may have occurred involving serogroups B and W135; one capsule switching event may have occurred involving serogroups B and Y; one capsule switching event may have occurred involving serogroups B and 29E and two capsule switching events may have occurred involving serogroups C and Y. Interestingly, strains of ST11:5,2,36-2 may have undergone more than one capsule switching event as three isolates were of serogroup W135 and one strain each of the serogroups B, C and Y were observed within the strain collection (Table 6.4).

6.5 Prevalence and temporal distribution of the major strain types amongst the carriage and IMD strain collections in Scotland, 1974 - 2004

The IMD strain collection was examined for the prevalence of the common strain types previously identified in the carriage strain collection (Table 6.5). Eight of the common strain types identified in the carriage strain collection were not present in the IMD strain collection. These were two related *cnl* strains that differed only in VR2 *cnl*:53:53:7,30,38 and *cnl*:53:53:7,30-3,38; as well as the strains Y:23:23:5-1,2-2,36-2; *ctrA*29E:60:60:21,16,37-1; Y:22:114:18-1,3,38; B:35:457:22-1,16-8,36-2; *ctrA*29E:178:178:19,15,36 and B:UA:1162:-:-:-.

Table 6.5 – Association of prevalent strain types identified in carried meningococci in Scotland, 1974 - 2004 & comparison with a carriage phenotype

Strain type						Prevalence (%)		OR (95% CI)
Group	cc	ST	VR1	VR2	VR3	NIV	IMD	
C	8	8	5	2	36-2	69 (8.64)	147 (5.31)	**1.68 (1.25, 2.27)**
W135/w	22	22	18-1	3	38	32 (4.00)	11 (0.40)	**10.45 (5.24, 20.83)**
B/b	213	213	22	14	36	23 (2.88)	34 (1.23)	**2.38 (1.40, 4.07)**
cnl	53	53	7	30	38	15 (1.87)	-	-
B/b	41-44	43	19	15-1	36	14 (1.75)	17 (0.61)	**2.88 (1.42, 5.88)**
Y	23	23	5-1	2-2	36-2	12 (1.50)	-	-
Y	23	23	7-2	13-1	35-1	10 (1.25)	4 (0.14)	**8.75 (2.74, 27.99)**
ctrA29E	60	60	21	16	37-1	9 (1.13)	35 (1.26)[1]	-
B	41-44	577	7-2	4	37	8 (1.00)	1 (0.04)	**27.97 (3.49, 224.01)**
cnl	53	53	7	30-3	38	8 (1.00)	-	-
B	32	259	7	16	35	7 (0.88)	27 (0.98)	0.90 (0.39, 2.07)
Y/y	22	114	18-1	3	38	7 (0.88)	-	-
B/b	35	457	22-1	16-8	36-2	6 (0.75)	-	-
B/b	35	457	19	15	36	6 (0.75)	19 (0.69)	1.09 (0.44, 2.75)
C	22	289	18-1	3	38	6 (0.75)	4 (0.14)	**5.23 (1.47, 18.57)**
C	41-44	1574	7-2	13-1	35-1	6 (0.75)	9 (0.33)	**2.32 (2.22, 17.75)**
X/x	750	750	21	16	37-1	6 (0.75)	3 (0.11)	**6.97 (1.74, 27.94)**
ctrA29E	178	178	19	15	36	6 (0.75)	-	-
B	22	289	18-1	3	38	5 (0.63)	5 (0.18)	3.48 (1.00, 12.05)
B	32	33	19	15	36	5 (0.63)	69 (2.49)	**0.25 (0.10, 0.61)**
B	35	457	22-1	14	38	5 (0.63)	2 (0.07)	**8.71 (1.69, 44.96)**
B	41-44	41	7-2	4	37	5 (0.63)	220 (7.95)	**0.07 (0.03, 0.18)**
B	UA	1162	-	-	-	5 (0.63)	19 (0.69)[2]	-
C	11	11	5	2-1	36-2	5 (0.63)	59 (2.13)[3]	**0.29 (0.12, 0.72)**
W135	UA	1251	18-1	3	38	5 (0.63)	4 (0.14)	**4.35 (1.17, 16.24)**
Y	167	167	5-1	10-4	36-2	5 (0.63)	2 (0.07)	**8.71 (1.69, 44.96)**

Group, serogroup or genogroup; cc, clonal complex; ST, sequence type; VR1, PorA variable region 1; VR2, PorA variable region 2; VR3, PorA variable region 3, IMD, invasive meningococcal disease isolates; NIV, non-invasive meningococcal isolates; OR, odds ratio; CI, confidence interval; ORs & 95% CIs in bold indicate statistical significance; [1] Serogroup B:60:60:21,16,37-1; [2] B:UA:1162:5-1,2-2,36-2; [3] C:11:11:any subtype = 400 (14.45%)

Fourteen strain types were more prevalent amongst the carriage strain collection than the IMD strain collection. Three strain types accounted for a greater proportion of the IMD strain collection than the carriage strain collection; these were B:32:33:19,15,36 (2.49% vs. 0.63%), B:41-44:41:7-2,4,37 (7.95% vs. 0.63%) and C:11:11:5,2-1,36-2 (2.13% vs. 0.63%). Estimation of the carried or invasive potential of strain types by odds ratios (ORs) indicated that twelve strain types were significantly associated with a carriage phenotype and a three further strain types were significantly associated with invasive disease (Table 6.5).

The carriage strain collection was examined for the prevalence of the common strain types previously identified in the IMD strain collection (Table 6.6). Five of the common strain types identified in the IMD strain collection were not present in the carriage strain collection; these were B:8:8:5-1,2-2,36-2; C:11:11:5-1,10-4,36-2; B:32:32:7-1,16,35; C:8:8:5-1,2-2,36-2 and C:11:11:5-1,10-8,36-2.

Table 6.6 – Association of prevalent strain types identified in invasive meningococcal disease isolates in Scotland, 1974 - 2004 with an invasive phenotype

Strain type						Prevalence (%)		OR (95% CI)
Group	cc	ST	VR1	VR2	VR3	IMD	NIV	
B	41-44	41	7-2	4	37	220 (7.95)	5 (0.63)	**13.72 (5.63, 33.40)**
C	11	11	5	2	36-2	188 (6.79)	1 (0.04)	**58.17 (8.14, 415.80)**
C	8	8	5	2	36-2	147 (5.31)	69 (8.64)	**0.59 (0.44, 0.79)**
A	1	1	5-2	10	37-1	117 (4.23)	1 (0.04)	**35.23 (4.91, 252.67)**
C	11	11	5-1	10-4	36-2	71 (2.57)	-	-
B	32	33	19	15	36	69 (2.49)	5 (0.63)	**4.06 (1.63, 10.10)**
C	11	11	5	2-1	36-2	59 (2.13)	5 (0.63)	**3.46 (1.38, 8.65)**
B	269	269	19-1	15-11	36	55 (2.00)	1 (0.04)	**16.18 (2.24, 117.13)**
B	32	32	7-1	16	35	43 (1.55)	-	-
C	41-44	206	any	any	any	41 (1.48)	3 (0.38)	**3.99 (1.23, 12.92)**
B	269	275	22	9	35-1	39 (1.41)	1 (0.04)	**11.41 (1.56, 83.17)**
B	32	32	7	16	35	36 (1.30)	4 (0.50)	2.62 (0.93, 7.38)
C	8	8	5-1	2-2	36-2	35 (1.26)	-	-
W135	11	11	5	2	36-2	35 (1.26)	4 (0.50)[1]	2.55 (0.90, 7.19)
C	11	11	5-1	10-8	36-2	34 (1.23)	-	-
B	213	213	22	14	36	34 (1.23)	23 (2.88)	**0.42 (0.25, 0.72)**
B	32	259	7	16	35	27 (0.98)	7 (0.88)	1.11 (0.48, 2.57)

Group, serogroup; cc, clonal complex; ST, sequence type; VR1, PorA variable region 1; VR2, PorA variable region 2; VR3, PorA variable region 3, IMD, invasive meningococcal disease isolates; NIV, non-invasive meningococcal isolates; OR, odds ratio; CI, confidence interval; [1] including NCTC 11203; ORs & 95% CIs in bold indicate statistical significance

Fifteen of the prevalent strain types were more common in the IMD strain collection compare to the carriage strain collection. The strain types

C:8:8:5,2,36-2 and B:213:213:22,14,36 accounted for a greater proportion of the carriage strain collection than the IMD strain collection (Table 6.6). Estimation of the carriage or invasive potential of strain types by odds ratios (ORs) indicated that eight strain types were significantly associated with invasive disease and a further two strain types were significantly associated with a carriage phenotype (Table 6.6). Temporal changes in the distribution of prevalent strain types amongst the IMD strain collection are evident from Figures 6.8 – 6.12. An apparent lack of prevalent strain types was observed during 1982 – 1984; however, this is likely a reflection in the reduced number of samples from these years in the strain collection, as seen in Figure 3.1.

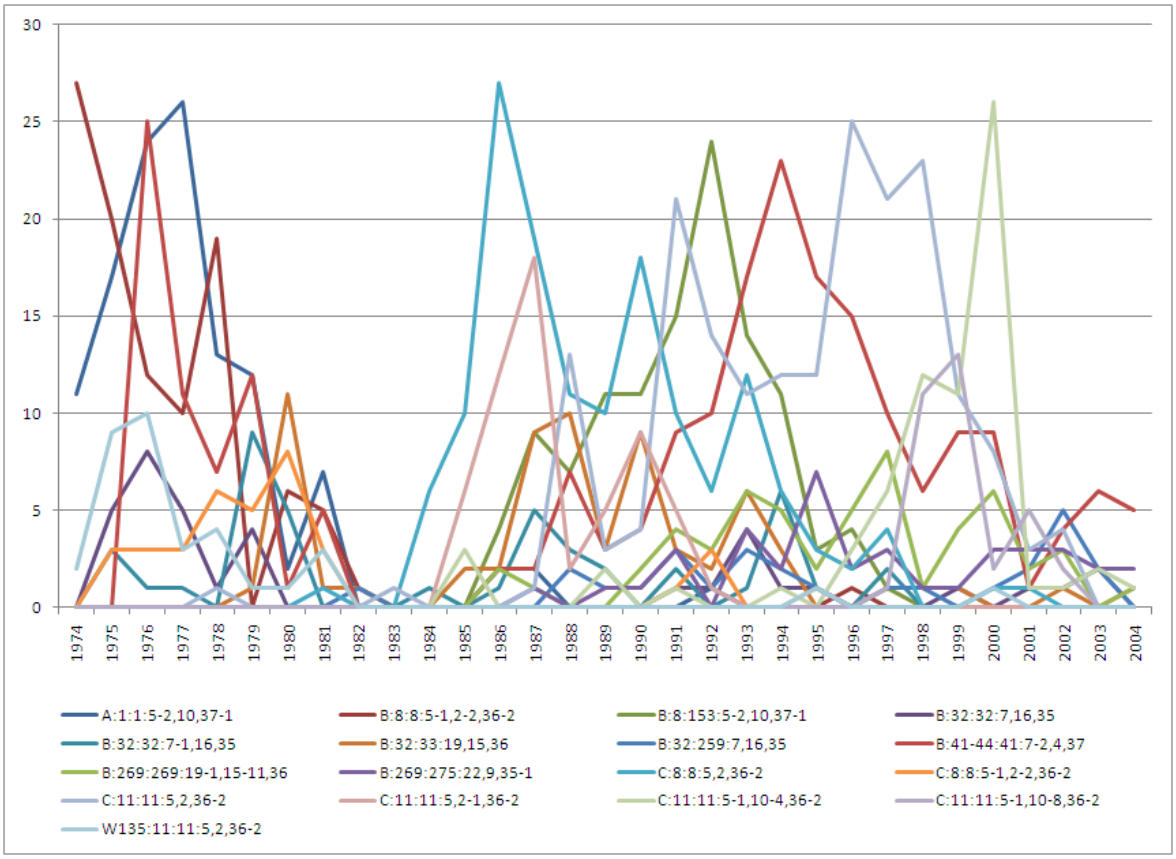

Figure 6.8 - Temporal distribution of prevalent strain types identified in the IMD strain collection in Scotland, 1974 - 2004

Y-axis, number of isolates; x-axis, year of isolation

The temporal distribution of strain types is characterised by a number of peaks in prevalence and for some strains this appears to be a cyclic occurrence every 3-5 years (Figures 6.8 – 6.12). No individual strain type has persisted throughout the full 31-year period however antigenic or capsular alterations in the

predominant lineages have been observed throughout the 31-year period (Figure 6.8 – 6.12). Despite the recent decline of cc8, it is clear that major changes in this lineage have occurred during the period covered by the strain collections (Figure 6.9). Sixty-five percent (356/547) of strains of cc8 were ST-8; predominantly of serogroups B (n=145) and C (n=206). Further, subtype 5,2,36-2 (n=167) and 5-1,2-2,36-2 (n=136) predominated. ST-8:5,2,36-2 isolates were predominantly serogroup C rather than B; 147 and 20 respectively. In contrast, ST-8:5-1,2-2,36-2 isolates were predominantly of serogroup B rather than C; 101 and 35 respectively.

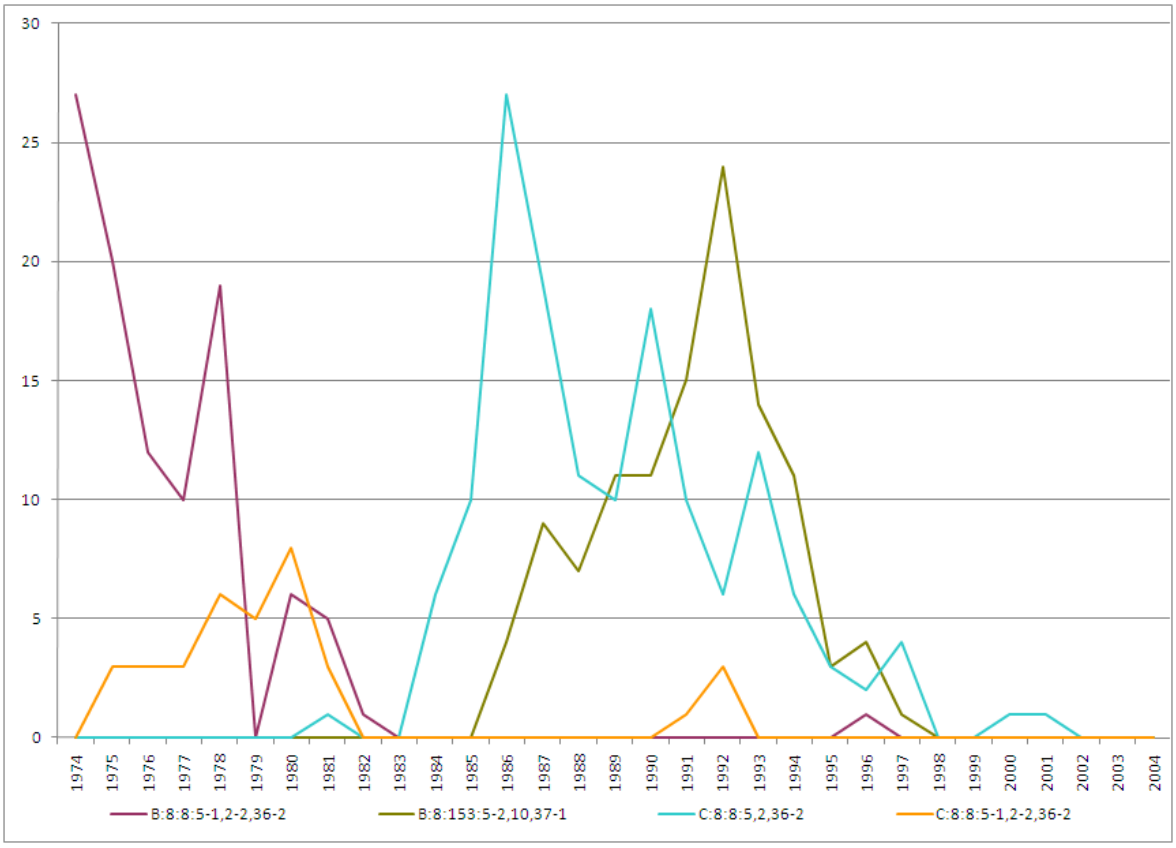

Figure 6.9 - Temporal distribution of the major cc8 strain types identified in the IMD strain collection in Scotland, 1974 - 2004

The temporal distribution of these strains indicates that during the first 10-year period B:8:8:5-1,2-2,36-2 strains declined while C:8:8:5-1,2-2,36-2 strains increased; although not to the same numbers. The strains appear to have been replaced, first by C:8:8:5,2,36-2 and then B:8:153:5-2,20,37-1 strains during the subsequent decade; however cc8 appears to have declined rapidly during the late 1990s and accounted for only a small number of the IMD strain collection

thereafter (Figure 6.9). ST-11 isolates were of serogroup W135 during the 1970s and early 1980s. Subsequently ST-11 isolates were of serogroup C (Figure 6.10).

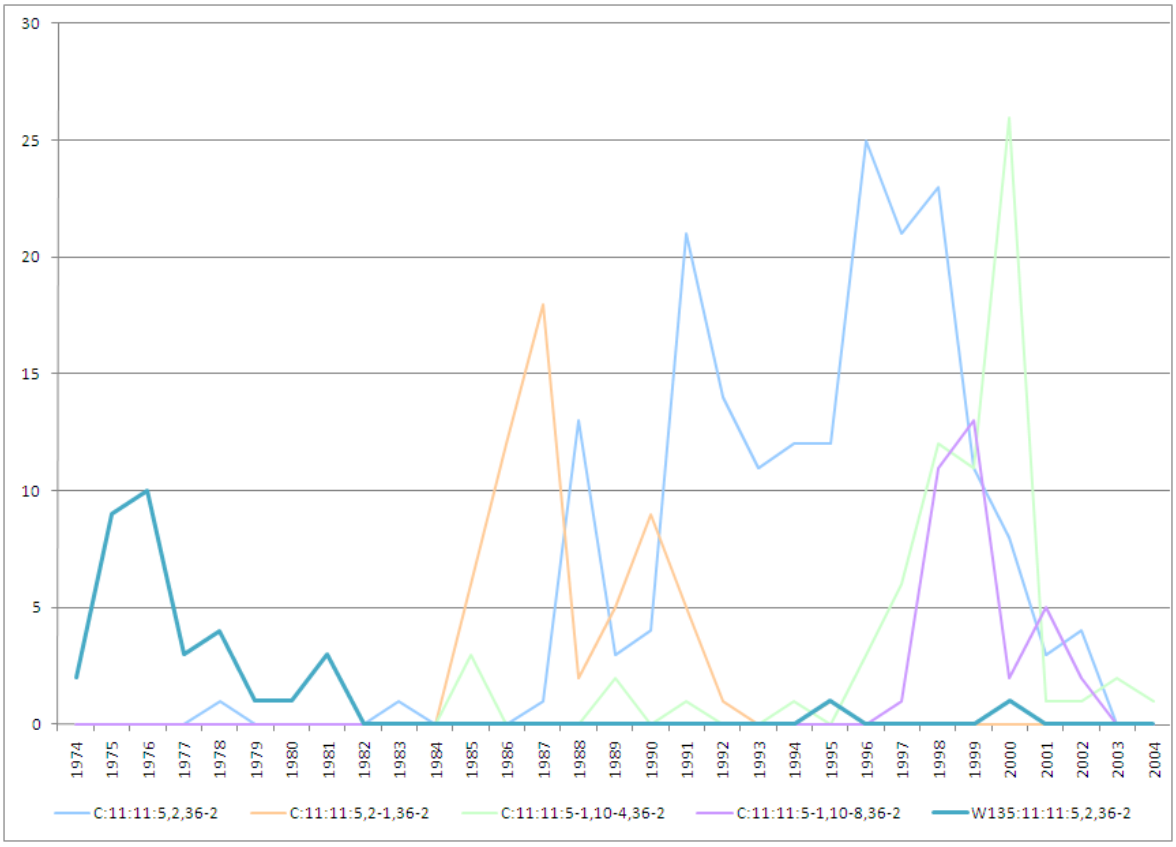

Figure 6.10 - Temporal distribution of the major cc11 strain types identified in the IMD strain collection in Scotland, 1974 - 2004

During the latter 20 year period a number of subtypes were associated with serogroup C ST-11 isolates; 5,2-1,36-2 predominate in the mid 1980s to early 1990s; succeeded by 5,2,36-2 from the late 1980s peaking in 1990 and again later that decade; succeeded by 5-1,10-4,36-2 and 5-1,10-8,36-2 in the late 1990s. However following the introduction of the MCC vaccine in 1999 there was a dramatic reduction in C:11:11 strains regardless of subtype (Figure 6.10). Serogroup B isolates are genetically more diverse and patterns in the temporal distribution of strain types are less apparent. As noted earlier, cc32 has been in decline throughout the 31 year period (Figure 6.11). However, within this cc, the B:32:259:7,16,35 strain would appear to have emerged more recently in Scotland than the three other major cc32 strains displayed in Figure 6.11.

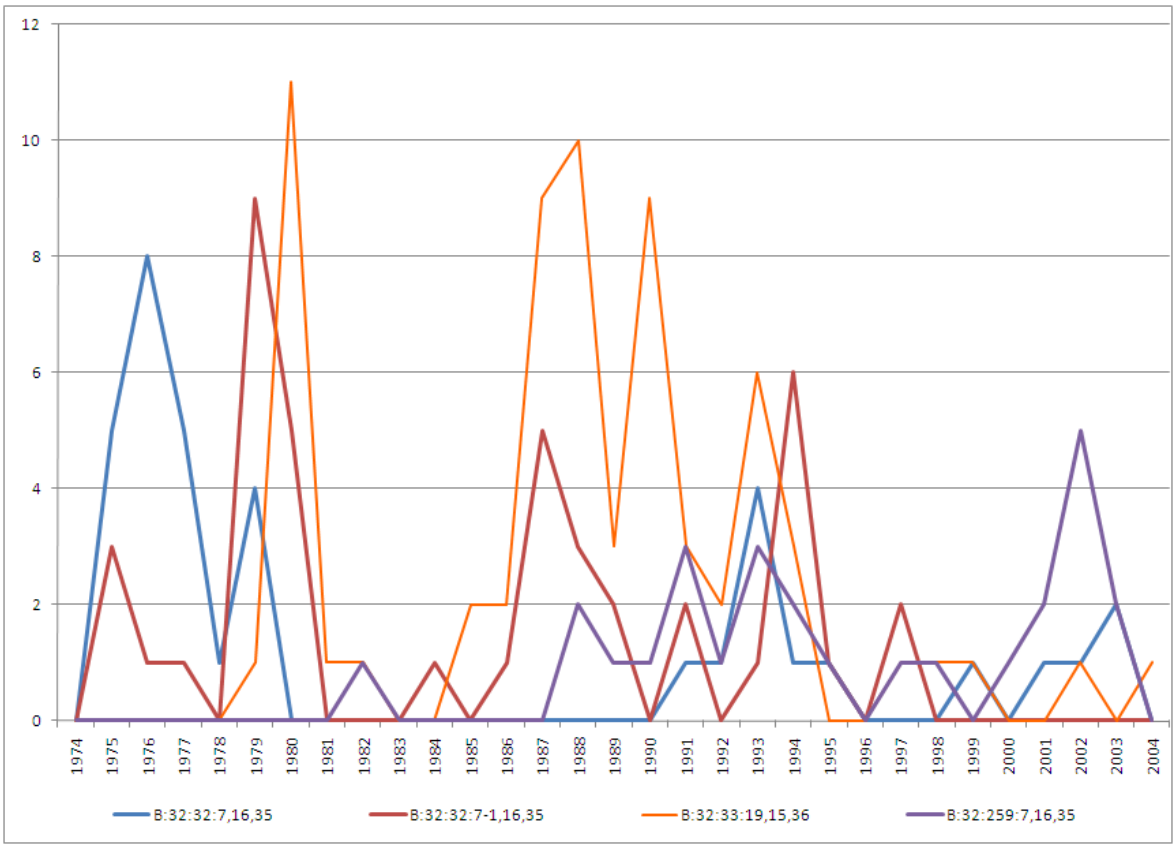

Figure 6.11 - Temporal distribution of the major cc32 strain types identified in the IMD strain collection in Scotland, 1974 - 2004

In contrast, cc269 has been increasing in prevalence due to two major strain types; B:269:269:19-1,15-11,36 and B:269:275:22,9,35-1 (Figure 6.12). Interestingly, the temporal distribution of strain type B:41-44:41:7-2,4,37 is characterised by two peaks; the first, in 1976 was, followed by a period of decline over the next 6 years. During the early 1990s the strain appears to have re-emerged, with a peak in 1994 which was again followed by a period of decline (Figure 6.12).

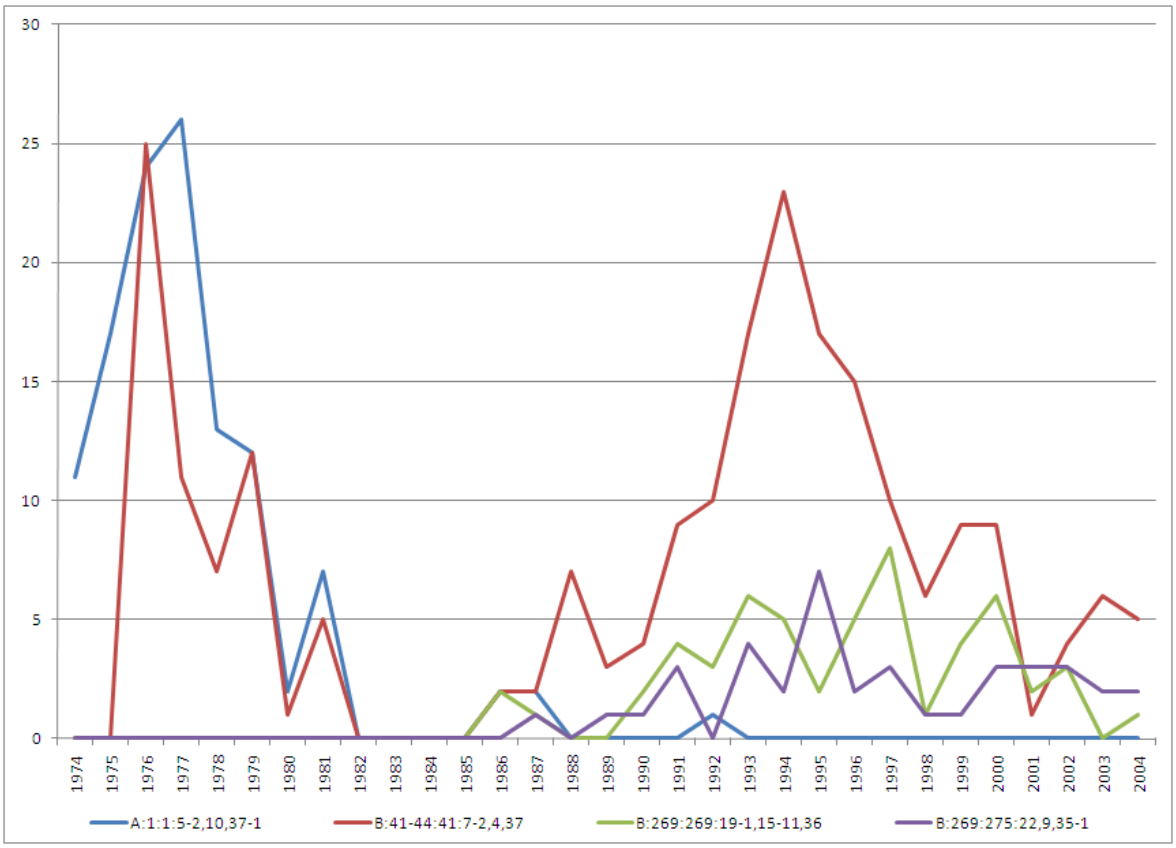

Figure 6.12 - Temporal distribution of the other major strain types identified in the IMD strain collection in Scotland, 1974 - 2004

Temporal patterns in the carriage strain collection are less apparent. However, as highlighted in Figure 6.13, the temporal distribution of the three most common strain types illustrates some interesting features. The distribution of the strain type C:8:8:5,2,36-2 in the carriage strain collection is punctuated by a large peak in prevalence that is short-lived (Figure 6.13) and occurs just prior to a peak in prevalence of this strain type amongst the IMD strain collection (Figures 6.8 & 6.9). The strain type W135:22:22:18-1,3,38 has been observed throughout each decade of the period covered by the carriage strain collection (Figure 6.13). However, this strain was not commonly identified amongst the IMD strain collection. Contrastingly, the strain type B:213:213:22,14,36 was only found in the latter period of the carriage strain collection (Figure 6.13) and strains belonging to cc213, as a whole, were not identified prior to 1994 and 1996 in the IMD and carriage strain collections, respectively. Moreover this cc was only recently assigned as a recognised cc during 2004 (http://pubmlst.org/neisseria).

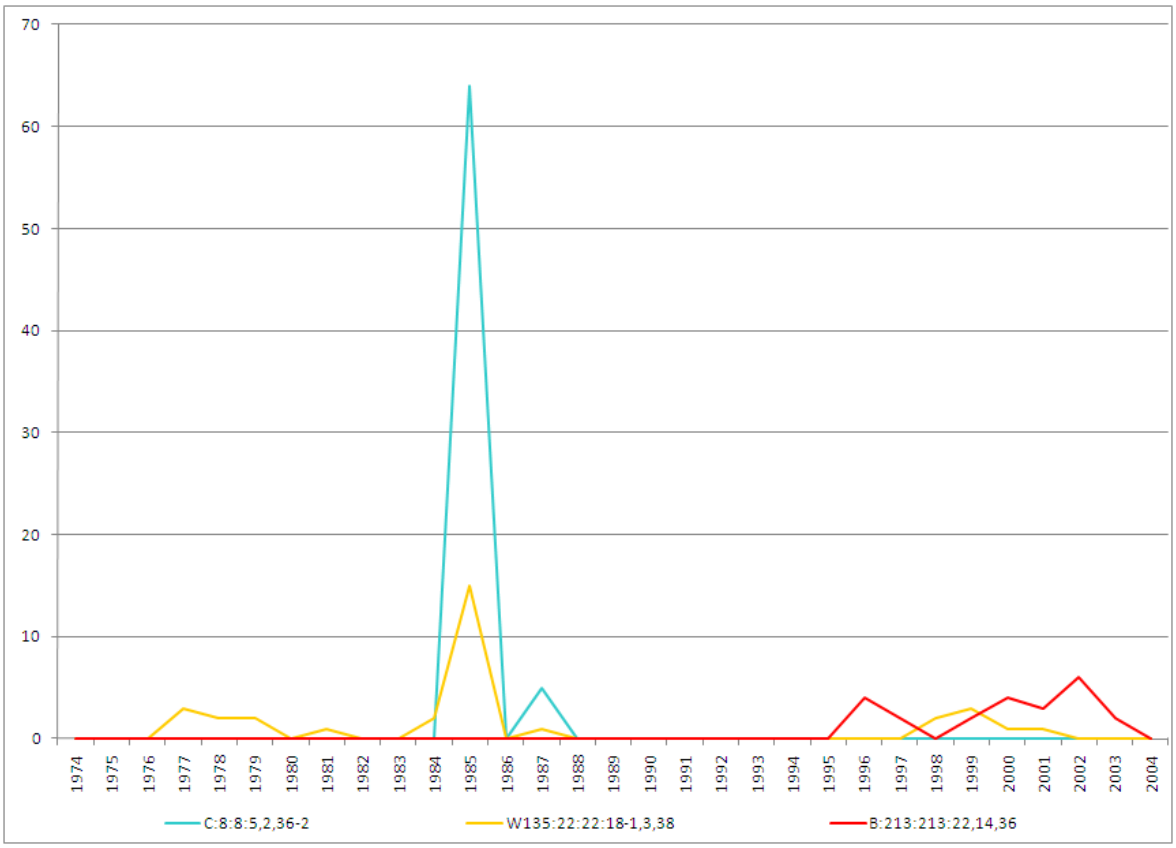

Figure 6.13 - Temporal distribution of the major strain types identified in the carriage strain collection in Scotland, 1974 – 2004

6.6 Potential coverage of serogroup B meningococci by licensed or experimental PorA-based outer membrane vesicle vaccines

The potential coverage by licensed or experimental PorA-based OMV vaccines of the strains of the carriage and IMD strain collections was assessed in the previous chapter. However, the PorA-based vaccines were developed to target predominant serogroup B strains causing disease in different geographic areas, therefore only serogroup B or NG *siaD* B PCR-positive strains from each of the strain collections was reassessed for coverage by these vaccines (Table 6.7). An increase in coverage by all vaccines was observed when each strain collection was limited to serogroup B and NG *siaD* B PCR-positive strains; coverage was highest with the NonaMen vaccine however this still only accounted for just over

one third or just under half of sero- or genogroup B isolates for the carriage and IMD strain collections, respectively. Regarding serogroup or genogroup B strains only; a potential PorA-based OMV vaccine using the six or nine most prevalent subtypes would achieve 36.4% and 46.4% coverage, respectively, of the carriage strain collection.

Table 6.7 – Differences in potential coverage of PorA VR1,2 subtypes identified in meningococci in Scotland, 1974 – 2004 by experimental or licensed PorA-based OMV vaccines: with emphasis on serogroup B meningococci

Vaccine (Manufacturer)	PorA subtype(s)	Strain Coverage(%)[1]			
		carriage[2]		IMD[3]	
		all strains	B only	all strains	B only
Cuban VA-MENGOC-BC (Finlay Institute)	P1.19,15	6.9%	10.2%	7.5%	10.4%
Norwegian (NIPH)[4]	P1.7,16	1.5%	3.6%	2.5%	4.3%
New Zealand MeNZB (NZMH[5] & Chiron)	P1.7-2,4	2.1%	4.8%	10.1%	17.0%
Bivalent (Finlay Institute & GSK)	P1.19,15 & P1.7-2, 4	9.0%	15.1%	17.6%	27.5%
HexaMen (NVI)[6]	P1.5-2,10; P1.12-1,13: P1.7-2,4; P1.19,15-1; P1.7,16 & P1.5-1,2-2	12.6%	17.5%	31.9%	41.0%
NonaMen (NVI)	HexaMen + P1.22,14; P1.7-1,1; P1.18-1,3	28.9%	34.0%	38.1%	48.3%

[1] Based on the prevalence of the homologous PorA subtype; [2] carriage strain collection; [3] invasive meningococcal disease strain collection; [4] NIPH, Norwegian Institute of Public Health; [5] NZMH, New Zealand Ministry of Health; [6] NVI, Netherlands Vaccine Institute

6.7 Discussion

The observation that several distinct meningococcal lineages were composed of isolates of more than one sero- or genogroup initially suggested the widespread occurrence of capsule switching events within these lineages. However, closer inspection of the data did not support this possibility as the association of serogroup was, in most cases, restricted to individual STs within these clonal complexes. Whilst carried meningococci were characterised into 297 STs (see

Chapter 4) during this study, only twenty-one (7.07%) STs were associated with more than one serogroup. Further analysis of individual strain types provided evidence that capsule switching may have occurred within a small number of strains of the carriage strain collection.

Statistically significant associations with IMD or with a carriage phenotype were observed for prevalent strain types amongst carried meningococci and IMD isolates isolated in Scotland during the period 1974 - 2004. Unexpectedly, the strain type C:8:8:5,2,36-2 was significantly associated with a carriage phenotype despite its high prevalence and its longevity over a period of 15 years from 1983 - 1998 in the IMD strain collection; contrastingly this strain type was only present in the carriage strain collection over a three-year period, 1984 – 1987. This may indicate bias in the carriage strain collection, perhaps as a result of the increased swabbing of individuals in the community during a period of increased disease in the Lanarkshire area.

In their updated report, Jolley *et al.* speculated that the observed high prevalence of serogroup C isolates may have been due to the recent circulation of the ST-11/ET-37 lineage within the Czech Republic following a period of absence (Jolley, Kalmusova *et al.* 2002). This explanation may also be applicable to the high prevalence of serogroup C isolates amongst the carriage strain collection in Scotland. MLST and subtyping data indicates the strain C:8:8:5,2,36-2 was responsible for the majority of carried serogroup C isolates. Temporal analysis of this strain type indicates this strain rapidly increased in prevalence in the period following the first observation of this strain from a case of IMD in 1981. Subsequently this strain peaked in prevalence during 1985 amongst the carriage strain collection and then during 1986 amongst the IMD strains collection, before declining over the following 20-year period. As previously noted in Chapter 3, a large proportion of the carriage strain collection was received during the period 1984 – 1993; many of which were isolated in the Lanarkshire region. During 1984 – 1986 an unexpectedly high number of cases of meningococcal disease occurred (Mathew and Chaudhuri 1986; Thomson and Jackson 1987).

It is possible that some of the isolates from this period of the carriage strain collection were obtained following further detailed investigations by Lanarkshire

health board, and these isolates were subsequently forwarded to the reference laboratory. Investigations into the carriage of meningococci among teenagers aged 15-18 years in one region of Germany found that carriage rates were typical of that age group. However, considerable variation in carriage was observed between different schools, towns or counties and between collection periods (Glitza, Ehrhard *et al.* 2008). It may be the case that the perceived increase in cases of meningococcal disease in Lanarkshire during 1984 - 1986 was a regional phenomenon and was not experienced throughout the country. Similarly, in the USA during the 1990s, the state of Oregon experienced an unusually high incidence of cases of serogroup B ET-5; particularly in teenagers (Diermayer, Hedberg *et al.* 1999; Tondella, Popovic *et al.* 2000).

Changes in the prevalence of different lineages in Scotland appear to have occurred over relatively short time periods and this feature may not be fully observed by the short time-frame covered in the study by Yazdankhah and colleagues. Two thirds of the Norwegian carriage isolates were obtained in response to a disease outbreak due to the cc32/ET-5 (Yazdankhah, Kriz *et al.* 2004). Carriage of meningococci is known to be influenced by epidemics and is often higher in contacts of disease cases (Cardenosa, Dominguez *et al.* 2001). The outbreak lineage was not detected in significantly high numbers in carriage by Yazdankhah and colleagues (Yazdankhah, Kriz *et al.* 2004). However isolates obtained from close family contacts of cases of IMD are often identical (Cardenosa, Dominguez *et al.* 2001; Tzanakaki, Urwin *et al.* 2001). Other lineages associated with increased disease potential have been found not to be prevalent in high numbers in carriage studies; i.e. serogroup C and/or cc11/ET-37 (Jolley, Kalmusova *et al.* 2000; Yazdankhah, Kriz *et al.* 2004) suggesting the prevalence of individual lineages in carriage is not mirrored in the circulating disease lineages over equivalent periods in time. However, the updated report by Jolley *et al*. suggests otherwise (Jolley, Kalmusova *et al.* 2002).

Different lineages may be distinct in their capacity for and duration of carriage and in their pathogenic potential. Glitza and colleagues found over a quarter of colonised teenagers were carriers over at least 23 weeks, of which greater than 20% carried the same strain. Furthermore, carriage was observed in over a third of colonised teenagers for at least 15 weeks. Acquisition of a new strain was observed in over a third of colonised teenagers (Glitza, Ehrhard *et al.* 2008).

Variations in the duration of carriage of strains and the replacement by a different lineage have also been observed amongst military recruits (Jones, Christodoulides *et al.* 1998; Caugant, Tzanakaki *et al.* 2007). Trotter and colleagues concluded that phenotype (i.e. C:2a) and increasing age of the host to be associated with death in cases of IMD (Trotter, Fox *et al.* 2002). It is possible the predominant disease-causing lineage in Norway (Yazdankhah, Kriz *et al.* 2004) may have been lost or replaced by other meningococci in these individuals. As previously discussed the serogroup C ST-11/ET-37 lineage is not often carried. This lineage has been described as having a higher attack-rate than the serogroup B ST-41-44/Lineage 3 meningococci; indeed, even within the ST-41-44/Lineage 3, which was named as a result of the joining of two individual clonal complexes, ST-41 is more often associated with disease, while ST-43 is more often associated with a carriage phenotype (see Chapter 4). A naturally shortened period of carriage by C:ST-11/ET-37 strains could explain why this lineage is not detected, to the same extent as other lineages, during carriage studies or in carriage isolate strain collections. In Scotland during the 1990s, when C:ST-11/ET-37 strains were increasingly causing IMD, case-fatality rates for serogroup C strains in general were higher than for serogroup B strains (Mooney, Christie *et al.* 2004).

Previous work by the reference laboratory using phenotypic methods indicated the prevalence of serotypes and serosubtypes had changed in Scotland during a 15-20 year period (Clarke 1999b). Amongst serogroup C strains serotype 2b strains decreased while 2a:P1.5 strains increased. Serogroup B strains are more diverse in their antigenic combinations; however 2b:P1.10 and 15:P1.7,P1.16 declined during the 1990s while 4:P1.4 increased (Clarke 1999b; Kyaw, Clarke *et al.* 2002). Similar observations were made in England and Wales around the same period. There was a decline in C:2b, B:2b and B:15:P1.7,16 strains and increases in B:4:P1.4 and B:NT:P1.9 strains during an eleven year period from 1993-1994 onwards (Gray, Trotter *et al.* 2006).

Published and unpublished studies conducted at the SHLMPRL since then have identified a number of correlations between the phenotypic serotyping and serosubtyping OMP ELISA data and MLST lineages and *porA* variable region sequencing data (Clarke, Diggle *et al.* 2002; Diggle 2004; Sullivan 2008). Serotype 2a was predominantly associated with cc11/ET-37. Further, 2a:P1.5

strains were predominantly 5-1,10-4,36-2 or 5-1,10-8,36-2; whereas 2a:P1.2,P1.5 strains were predominantly 5,2,36-2 by *porA* VR sequencing. Serotype 2b was predominantly associated with cc8/Cluster A4; specifically 2b:P1.10 strains were predominantly ST-153. In addition, B:15:P1.7,P1.16 strains were predominantly cc32/ET-5; importantly meningococci of this cc have since been recognised as the cause of increased periods of disease in England during the 1980s (Bygraves, Urwin *et al.* 1999). B:4:P1.4 strains were predominantly ST-41:7-2,4,37 and B:NT:P1.9 strains were predominantly of cc269. PorA variable region sequencing of *N. meningitidis* isolates by the SHLMPRL commenced around 1999. For a short time the serotyping and serosubtyping OMP ELISA and PorA variable region sequencing was performed concurrently, however during October 2002 the decision was taken to cease the use of the OMP ELISA. There are currently no plans by the SHLMPRL to undertake DNA sequencing of *porB* alleles; previous evaluations concluded that this would provide little extra information in strain discrimination in return for the time and cost invested in such analyses (Dr. M. Diggle, personal communication).

PorA-based OMV vaccines were tailor-made to be effective against predominant disease-causing serogroup B strains. Furthermore, vaccination campaigns will likely target those most at risk from disease; i.e. children and young adolescents. As such, one must also consider the target age group and the association of PorA subtype with serogroup when discussing potential coverage by vaccines as the assessment of strain coverage may be altered by the inclusion of strains of other serogroups or strains isolated from individuals outside the target age groups. The coverage data presented herein is based only upon the prevalence of the homologous subtypes; coverage would increase if one was to account for strains possessing only one identical VR compared with those vaccine subtypes. Similarly, if antibodies against particular variants were cross-reactive with other variants within PorA families then coverage may be expected to increase. However, since strains of meningococci are frequently characterised as NST due to reduced expression of PorA or because they do not react with the available MAbs, this would require knowledge of the cross-reactivity of antibodies directed against individual variable loop epitopes and should also consider the level of PorA expression within individual strains.

7 Conclusions and future prospects

Prior knowledge of the circulating meningococcal lineages in Scotland has focused on the characterisation of isolates obtained from persons with meningococcal disease. Whilst this has provided valuable information, disease isolates represent only the tip of the iceberg of the overall population biology of the meningococcus. For the meningococcus the progression from commensalism to the disease-state merely results in the interruption of transmission between hosts. Only recently has there been an acceptance by the wider scientific community that more attention should be paid to the study of meningococcal isolates not obtained from cases of IMD. This study is the first of its kind in Scotland, to my knowledge, to characterise a large collection of carried meningococci covering a 31-year period by MLST and *porA* VR subtyping and to subsequently perform a comparison of the generated data with that of an IMD strain collection over the same period in Scotland.

Serological classification methods are phenotypically based and suffer from the deficiency that groupings based on them may contain strains that are genotypically dissimilar. Furthermore the panel of MAbs utilised by serological classification methods is also not comprehensive and strains frequently do not react; this was evident during this study since 23.8% of isolates of the carriage strain collection were NG (Chapter 3) whilst 63.8% of the carriage strain collection were NST (by OMP ELISA that was previously performed by the reference laboratory; data not shown). Serogroup-specific and serogroup non-specific PCR tests were successfully utilised to characterise the majority of phenotypically NG carried meningococci. In addition, molecular subtyping of the *porA* VRs 1, 2 and 3 was able to re-characterise the majority of the carriage strain collection into distinct *porA* subtypes. Non-groupable isolates [OR 17.66; 95% CI (12.71 to 24.54)] and those of sero- or genogroups W135 [OR 2.49; 95% CI (1.72 to 3.60)], Y [OR 6.26; 95% CI (4.18 to 9.39)], X [OR 3.13; 95% CI (1.20 to 8.14)], Z [OR 131.89; 95% CI (18.00 to 960.76)] and 29E [OR 21.30; 95% CI (4.76 to 95.36)] were significantly associated with a carriage phenotype. In contrast, serogroups A [OR 3.64; 95% CI (1.96 to 6.76)], B [OR 2.65; 95% CI (2.25 to 3.12)] and C [OR 1.92; 95% CI (1.58 to 2.33)] were significantly associated with invasive disease.

The prevalence of different serogroups amongst carried meningococci in Scotland has changed over time. For example, no isolates of serogroup A were isolated during the last 15 years of the period covered by the carriage strain collection in this study. This is consistent with the observations of the reference laboratory in Scotland that have documented a decline in the prevalence of serogroup A meningococci from cases of IMD (Fallon, Brown *et al.* 1984; Kyaw, Christie *et al.* 2002; Kyaw, Clarke *et al.* 2002; Clarke, Reid *et al.* 2002a; Clarke 2005; Sullivan 2008). Furthermore, this trend has been observed elsewhere in the UK and in other industrialised countries where serogroup A meningococci are seldom the cause of invasive disease (Jones 1995; Rosenstein, Perkins *et al.* 2001; Gray, Trotter *et al.* 2006; Russell, Urwin *et al.* 2008). Despite this, serogroup A meningococci are still prevalent in other regions of the world, causing significant levels of disease in Asia (Zhang, Shao *et al.* 2008; Yang, Zhang *et al.* 2009), parts of Russia (Koroleva, Beloshitskij *et al.* 2009) and in Africa (Nicolas, Norheim *et al.* 2005). Recently, a new serogroup A conjugate vaccine has been developed for use in Africa (Lee, Kuo *et al.* 2009) in the hope that it can achieve reductions in disease comparable with those previously observed in countries using MenC conjugate vaccine (Balmer, Borrow *et al.* 2002; Maiden and Stuart 2002; Mooney, Christie *et al.* 2004; Larrauri, Cano *et al.* 2005; de Greeff, de Melker *et al.* 2006; Booy, Jelfs *et al.* 2007).

A large number of STs identified during this study were characterised for the first time, some of which contained novel allele sequences. The carriage strain collection reported herein was also observed to be highly diverse with the majority of STs identified only once. This diversity observed within the carriage strain collection [0.981; 95% CI (0.955, 1.006)] was significantly greater than the diversity within an IMD strain collection [0.938; 95%CI (0.934, 0.942)] from the same period. Although no significant change in diversity throughout the 31-year period was observed within the carriage strain collection, this was observed within the IMD strain collection; 0.900 (95% CI 0.892, 0.907) for the period 1974-1983; compared to 0.938 (95% CI 0.929, 0.946) for the period 1984-1993; and 0.892 (95% CI 0.881, 0.903) for the period 1994-2004. Hence, the level of diversity, *D*, of the IMD strain collection for the periods 1974-1983 and 1994-2004 can be said to be significantly less compared with that for the period 1984-1993.

Temporal changes in the most prevalent clonal complexes were observed throughout the 31-year period; increases in cc22, cc41/44 and cc269 and decreases in cc1, cc5, cc8, cc11, cc32, cc35, cc37, cc254, cc334 and cc364 were observed. Whilst cc213 was formally assigned during 2004 (Ibarz-Pavon, Brehony *et al.* 2004) the data presented herein indicate cc213 emerged in Scotland during the early 1990s. This cc is now prevalent in Scotland in meningococci isolated from both carriage and in cases of IMD. Furthermore, for several clonal complexes a significant association with a carriage phenotype (cc22, cc23, cc35, cc92, cc167, cc174, cc212, cc213, cc254, cc461, cc750, cc1157 and meningococci unassigned to a clonal complex) or with invasive disease (cc1, cc8, cc11, cc32, cc41-44 and cc269) was observed. Despite significant associations at the level of cc, distinct differences in those associations were apparent for individual STs within a given clonal complex; most notably the significant association of ST41 and both ST43 and ST44 with invasive disease and a carriage phenotype, respectively.

Merging sero- or genogrouping data, MLST data and *porA* subtyping data allowed observation of the temporal changes that have occurred within the most prevalent carriage strain types and IMD strain types in Scotland over the 31-year period 1974 - 2004. Significant associations with invasive disease or with a carriage phenotype remained down to the level of strain type for eight and twelve of the most prevalent strain types, respectively. In addition several other strain types prevalent in one strain collection were absent from the other and could not be compared using the same method. The strain types B:213:22,14,36 [OR 2.38 (95%CI 1.40, 4.07)] and B:43:19,15-1,36 [OR 2.88 (1.42, 5.88)] were significantly associated with a carriage phenotype.

A feature of the carriage strain collection was the concentration of cc8 isolates during the period 1984 – 1986 and the high proportion of isolates obtained from individuals identified as resident in Lanarkshire at a time when an episode of increased disease was experienced within that region (Mathew and Chaudhuri 1986; Thomson and Jackson 1987). This was first noted in Lanarkshire in 1984 (Thomson and Jackson 1987) and was also noted in both east and west Scotland. However, unlike in England and Wales there were no reported local outbreaks of disease. Furthermore, serogroup C notifications/isolations increased during this

time period and subsequently predominated for the first time in Scotland since laboratory surveillance of infections commenced.

In this study, cc8 was found to be significantly associated with invasive disease, in agreement with the findings of others (Ure, Gray *et al.* 2004). Furthermore, whilst ST8 was also significantly associated with invasive disease, the most common strain type within cc8, C:8:8:5,2,36-2, [OR 1.68; 95% CI (1.25, 2.27)] was however, significantly associated with a carriage phenotype. Moreover, other prevalent cc8 strain types, C:8:8:5-1,2-2,36-2 and B:8:8:5-1:2-2:36-2, were absent from the carriage strain collection and could not be tested by the same method. Carriage of the meningococcus is reportedly higher among subjects during disease outbreaks or epidemics and this may explain the overrepresentation of this cc amongst the carriage strain collection. However, other lineages possessing the serogroup C capsular polysaccharide have been described as being carried for only a short duration. The emergence of an ET-15 C2a strain in Canada during the late 1980s reportedly had a short carriage period prior to infection given that these ET-15 isolates could not be found in group C meningococcal carriers during the outbreak in Victoria County in 1989 (Ashton, Ryan *et al.* 1991). Similarly during an outbreak of serogroup C disease in British Columbia, a carriage study of the local population revealed carriage of serogroup C was only 4% (Patrick, Champagne *et al.* 2003).

Cc269 was also reported to have emerged in Canada (Law, Lorange *et al.* 2006). MLST revealed its presence in a variety of EU countries in during 2000 – 2002 (Brehony, Jolley *et al.* 2007). This lineage has been identified in IMD and in the carriage strain collection in Scotland over 30 years ago suggesting that the emergence of this lineage is not a recent event. Nevertheless, the prevalence of this cc amongst IMD strains has increased in recent years and along with cc41-44 constitutes the majority of IMD cases in Scotland (Ure 2008). Interestingly, as observed during this study, cc269 is highly diverse with respect to PorA subtype. Similarly, in England and Wales, Russell and colleagues identified that this lineage had been prevalent at least as early as 1975 (Russell, Urwin *et al.* 2008). More recently, one third of serogroup B meningococci isolated in England and Wales are of cc269 (Lucidarme, Comanducci *et al.* 2009).

During the 1990s, serogroup C meningococci of the ST-11/ET-37 complex were an increasing problem in Europe, particularly in the UK (Clarke, Reid *et al.* 2002a). The emergence and transmission of serogroup W135 meningococci also of this cc was associated with the Hajj pilgrimage in 2000 (Taha, Achtman *et al.* 2000; Aguilera, Perrocheau *et al.* 2002). Although carriage of this lineage is low, analysis of the SHLMPRL meningococcal isolate collection during this study has revealed the presence of this lineage in Scotland since at least as early as 1974; initially associated with IMD due to serogroup W135 until the early 1980s followed by serogroup B or predominantly C from the early 1980s. Russell and colleagues also identified this lineage in isolates from England and Wales as far back as 1975, predominantly of serogroup W135, and then increasingly of serogroup C (Russell, Urwin *et al.* 2008). Furthermore, the temporal analysis of ST11 meningococci isolated from cases of IMD in Scotland also indicates several changes have occurred in the subtypes associated with this lineage (Chapter 6); ST11 strains were initially W135:5,2,36-2 during the 1970s to early 1980s, subsequently the C:5,2-1,36-2 strain was prevalent during the period 1984 – 1993. This strain was then succeeded by the C:5,2,36-2 strain that began increasing from around 1987 and subsequently dominated during the 1990s. More recently, two more ST11 strain types emerged; C:5-1,10-4,36-2 and C:5-1,10-8,36-1 which both peaked in around 1999 – 2000. Interestingly these latter three C:11 strains were all circulating in Scotland at the same time.

The exchange of DNA coding for the sialyltransferase by meningococci enables clones to express different capsular polysaccharide (Swartley, Marfin *et al.* 1997). Fortunately, capsule switching has not been reported as yet in the UK (Maiden and Stuart 2002; Mooney, Christie *et al.* 2004; Diggle and Clarke 2005). However, reports from continental Europe and North America suggests that capsule switching in clinical isolates of *N. meningitidis* may be a common occurrence (Stefanelli, Fazio *et al.* 2003; Tsang, Law *et al.* 2005; Lancellotti, Guiyoule *et al.* 2006; Harrison 2008; Beddek, Li *et al.* 2009; Castilla, Vazquez *et al.* 2009; Simoes, Cunha *et al.* 2009; Harrison, Shutt *et al.* 2010). Evidence for capsule switching in an isolate was defined by Harrison and colleagues "as the presence of an unusual ST-serogroup combination; specifically a genetic sequence type (ST)/clonal complex in an isolate of a serogroup more generally associated with another serogroup". The authors presented data indicating 35 B-

to-C, seven C-to-B, two W135-to-Y and one W135-to-B capsule switching events (Harrison 2008; Harrison, Shutt *et al.* 2010). However, it is not clear what observations directed Harrison and colleagues, or others, when deciding "normal" serogroup-ST associations. Are they using local surveillance data (i.e. ABCs data, in the case of Harrison *et al*.) only or are they using global data? Are they using the MLST website?

The assumption that an uncommon ST:serogroup association must be the result of some recent recombination event is perhaps misleading, particularly in countries using capsular-based vaccines. In countries using MCC vaccines, for example, one would reasonably expect to observe a reduction in disease cases due to serogroup C strains. In turn, this may allow for the emergence of other less virulent strains which would have previously been out-competed by more virulent serogroup C strains. Furthermore, geographic and temporal variations in the prevalence of meningococcal serogroups are well documented. Consequently, a rare ST-serogroup association in one geographic location may not necessarily be rare in another location. Thus, a rare ST:serogroup association could indicate the emergence of an existing strain into a geographic niche where the strain had previously been at undetectable levels or absent.

The association of sero- and genogrouping data with cc in this study initially indicated the potential existence of multiple cases of capsule switching within the carriage strain collection. However, closer inspection of the association of sero- or genogroup with individual STs revealed that the majority of STs were of a single serogroup. There were a small number of exceptions; for twenty-one STs at least two different serogroups were observed. Moreover, once subtyping data were included into the analysis this reduced the potential number of capsule switching events amongst the carriage strain collection. From the data, it is not possible to predict the direction (i.e. C-to-B or B-to-C) of these potential capsule switching events and in some instances the data suggest that the strain types were not mutually exclusive. It may be beneficial to re-test the isolates implicated in possible capsule switching by agglutination and serogroup-specific PCR to confirm these results, given that some isolates initially recorded as serogroup Z were later found to be genogroup 29E by PCR. Only nongroupable isolates from the carriage strain collection were systematically tested using the serogroup-specific PCR tests to characterise these strains by genogroup.

Simultaneous carriage of more than one strain of *N. meningitidis* has been documented (Caugant, Tzanakaki *et al.* 2007); however the extent to which this occurs is not fully apparent. On the basis that meningococcal strains of identical genetic lineages may possess different capsular polysaccharides (Swartley, Marfin *et al.* 1997) or antigenic profiles presumably, carriage of more than one strain must occur to allow horizontal genetic exchange of the capsule locus. Nasopharyngeal carriage of more than one strain of pneumococci, in some cases three, has been observed on the basis of serotyping (Kaltoft, Skov Sorensen *et al.* 2008; Brugger, Hathaway *et al.* 2009; Brugger, Frey *et al.* 2010) although it is not clear whether these strains were of the same genetic type.

Uncertainty remains regarding the optimal sampling method to identify meningococcal carriage. Different carriage studies often use different methods and, as such, are difficult to compare. The choice of sampling site, transport medium or direct plating of swabs, single or multiple swabs and length of time between swabbing and plating can influence the level of carriage. A recent review by Roberts and colleagues (Roberts, Greenwood *et al.* 2009) of the published literature concluded that meningococcal carriage would be best assessed by swabbing the posterior pharyngeal wall through the mouth with direct plating or keeping transport time to below five hours. Additionally the use of charcoal swabs and selective antibiotics to inhibit the growth of non-meningococcal bacteria has also been recommended to improve the yield of meningococci in the sample (Caugant, Hoiby *et al.* 1992; Roberts, Greenwood *et al.* 2009).

Characterisation of the meningococcal capsule-synthesis cluster in carried meningococci isolated from healthy subjects in Bavaria, Germany has shown that a significant proportion of isolates harbour a non-coding intergenic region of either 113 or 114bp in length, termed the capsule null locus (*cnl*) (Claus, Maiden *et al.* 2002). Four lineages containing the *cnl* were identified amongst the carriage strain collection during this study. Interestingly, two of these lineages contained a novel *cnl* allele distinct from that sequenced in Bavarian carriage isolates of the same lineages. Recent reports of *cnl*-possessing meningococci causing disease in immunocompromised and, more remarkably, immunocompetent individuals (Vogel, Claus *et al.* 2004b; Hoang, Thomas *et al.* 2005; Findlow, Vogel *et al.* 2007) has challenged our understanding of the

necessity for the meningococcus to be encapsulated in order to survive in the bloodstream and evade the host immune system (Klein, Ison *et al.* 1996; van Deuren, Brandtzaeg *et al.* 2000; Spinosa, Progida *et al.* 2007).

PorA is a candidate for inclusion in meningococcal vaccines (Devoy, Dyet *et al.* 2005; Oster, Lennon *et al.* 2005; Martin, Ruijne *et al.* 2006); therefore it is important to monitor the variation within this protein in the meningococcal population both during and following the introduction of strain-specific vaccines. A small number of laboratories, including the SHLMPRL, characterise the VR3 (Loop V) of *porA* (Molling, Unemo *et al.* 2000; Molling, Backman *et al.* 2001; Clarke, Diggle *et al.* 2003b; de Filippis, de Andrade *et al.* 2007). Although variation is less diverse, sequencing has shown that carriage strains possessing identical VR1 and VR2 may have a different VR3. Recently, recommendations for the typing of *N. meningitidis* isolates were proposed. The strain designation for meningococci, recommended by the European Meningococcal Disease Society (EMGM), is to include the serogroup, PorA VRs, FetA VR, MLST sequence type and clonal complex. An example is C: P1.5,2-1: F5-4: ST-11 (cc11) (Jolley, Brehony *et al.* 2007).

Due to the heterogeneity of PorA subtypes in meningococci in Scotland the potential coverage by experimental or licensed PorA-based OMV vaccines would be limited. Therefore the introduction of monovalent or multivalent PorA-based vaccines in Scotland may be of little benefit. Improved strain coverage as a whole, not just against those of serogroup B, may require the addition of other vaccine antigens.

Meningococcal vaccines targeting particular polysaccharide capsules have been available in Scotland for a number of years (Danzig 2004). Knowledge of vaccine uptake and vaccine efficiency would aid in the interpretation of any effect of vaccination on the distribution of particular meningococcal serogroups. The deficiencies of polysaccharide vaccines are well documented so it may be difficult to speculate that these vaccines could have contributed significantly to changes in meningococcal population structure, particularly since these plain polysaccharide vaccines were made available or were recommended for distinct subsets of the population i.e. immunosuppressed individuals, contacts of IMD cases or to travellers (DoH 2006b).

Vaccination remains the best form of disease prevention, however currently licensed vaccines do not offer broad protection against all serogroups, or are not immunogenic in those individuals most at risk of IMD (i.e. infants <2 years). The serogroup B capsule is poorly immunogenic and is known to be structurally homologous with human cell lines (NCAM, neural cell adhesion molecule) (Finne, Leinonen *et al.* 1983; Finne, Bitter-Suermann *et al.* 1987). Consequently, alternative means must be sought when developing vaccines to target serogroup B meningococcal disease. The genomes of a small number of strains of *N. meningitidis* have been fully sequenced; the serogroup B strain MC58, the serogroup A strain Z2491, the serogroup C strain FAM18 and the serogroup C strain 053442 (Parkhill, Achtman *et al.* 2000; Tettelin, Saunders *et al.* 2000; Bentley, Vernikos *et al.* 2007; Peng, Yang *et al.* 2008). Genome-sequencing projects have facilitated the discovery of new vaccine antigen targets (Pizza, Scarlato *et al.* 2000).

A number of candidate antigens are currently being investigated, including factor H-binding protein (fHbp). Extensive prevalence and sequence analyses of fHbp from a variety of serogroup B *N. meningitidis* lineages indicates that all isolates have a fHbp gene and that variants so far identified can be classified into two or three distinct groups; subfamilies A and B (Fletcher, Bernfield *et al.* 2004; Murphy, Andrew *et al.* 2009) or groups 1, 2 and 3 (Masignani, Comanducci *et al.* 2003; Brehony, Wilson *et al.* 2009) respectively, in all countries surveyed. Pfizer and Novartis are currently investigating a bivalent fHbp vaccine (based on one family A and one family B variant) in clinical trials and the 5 Component Vaccine against Meningococcus B (5CVMB) respectively, in clinical trials. The 5CVMB is composed of the New Zealand OMV (Oster, Lennon *et al.* 2005) plus five other antigens; Factor H-binding protein (fHbp; GNA 1870: LP2086) variant 1; Neisserial adhesion A (NadA; GNA 1994), GNA 2091, GNA 2132 (Neisseria Heparin-binding antigen; NHBA) and GNA 1030 (Rappuoli 2008; Snape 2008).

Accurate and timely surveillance is essential in order to assess the impact that future vaccination campaigns will have on the circulating meningococcal lineages. I would recommend that, dependent upon the progress of the investigational vaccines, the SHLMPRL considers the introduction of prospective and retrospective molecular surveillance of the candidate vaccine antigens in meningococcal carriage and disease isolates from Scotland. Enhanced

surveillance of meningococcal disease in Scotland, through the Meningococcal Infectious Disease Augmented Surveillance (MIDAS) scheme, was implemented in November 1999 (Clarke 1999a) to coincide with the MenC immunisation campaign, whilst during the same year the Enhanced Surveillance of Meningococcal Disease (ESMD) was established in England, Wales and Northern Ireland (Shigematsu, Davison *et al.* 2002) to obtain accurate incidence data and monitor the impact of the MenC vaccine during and following its introduction in the UK from November 1999. Worldwide, many countries undertake active meningococcal surveillance programmes, however they are biased in favour of disease isolates. Furthermore, individual surveillance programmes may vary from country to country. Robust and accurate surveillance systems will facilitate the observation of circulating meningococcal lineages and also enable the identification of new clonal complexes, such as cc213 (Ibarz-Pavon, Brehony *et al.* 2004). Despite the ease of access to the MLST website and the data it holds, the scientific community is not duty-bound to deposit all their surveillance data on this publicly-accessible database. Moreover, data on the MLST website may be further biased towards novel sequence types. Strain data is an absolute requirement of the submission procedure for new allele sequences prior to assignment of a new ST by the database curator. However, the scientific community is not duty-bound to submit strain data for those strains of previously characterised STs and as such the data on the neisseria MLST database cannot replace the surveillance systems already in place in many countries.

A downward trend in cases of IMD is occurring across much of Europe and North America, irrespective of serogroup and with or without the introduction of conjugate vaccination campaigns. Undoubtedly conjugate vaccination campaigns have reduced the burden of disease due to the serogroup(s) targeted in the countries where the vaccines have been implemented. For instance, around 80% or more of disease cases in Scotland are now due to serogroup B following over a decade of using the MCC vaccine. Since 2003 few cases of serogroup C IMD have been reported; the recent surveillance data in Scotland puts the rate for all disease at 2.69 cases per 100,000 population and for the last two calendar years, 2008-2009, no case of serogroup C disease has been reported to HPS (McDonald 2010a; 2010b). In other countries where the actual numbers of disease cases due to serogroup C are low, there are no plans to introduce the MenC vaccine.

Cases of meningococcal disease are increasingly being confirmed by non-culture methods. This is due in part to improvements in disease recognition and subsequent early intervention with antibiotic therapy. Consequently fewer meningococcal isolates are being cultured and ultimately submitted to the SHLMPRL by regional bacteriology laboratories. The SHLMPRL currently uses *ctrA* as a target for the identification of meningococcal DNA in clinical samples from individuals suspected of having meningococcal disease. However, as previously mentioned, this gene is absent from meningococci possessing the *cnl*. Although reports of *cnl*-containing meningococci causing invasive disease are limited, if these strains were to become a more prominent cause of IMD then the *ctrA* PCR would not detect the strains in clinical samples. Indeed, these strains have only recently been recognised and they may be more prevalent in IMD cases than previously thought; especially in immunocompromised individuals or in individuals suffering from less severe symptoms. Research conducted by the CDC has identified a species-specific PCR target (*sodC*) that accurately identifies meningococcal DNA, irrespective of capsular status, in clinical specimens (Dolan 2008; Dolan, Hatcher *et al.* 2009) and in a carriage study (J. Dolan, personal communication). This assay could be readily introduced into the repertoire of molecular tests utilised by the SHLMPRL.

Whilst one must concede there are imperfections with the carriage strain collection characterised during this study, in the absence of a structured sampling programme to fully (and regularly) investigate meningococcal carriage irrespective of age, sex, geographic location, socio-economic status etc, the analysis of a carriage strain collection assembled from the isolate archive of the SHLMPRL constitutes a step in the right direction. Existing culture collections are often not representative of biological populations as they are composed of mostly virulent isolates. This is a circumstance which is reflected in Scotland by the disparity between the characterisation of disease versus carriage isolates by the SHLMPRL. Furthermore, this disparity is also apparent in the global data deposited on the Neisseria MLST website.

More recently, efforts have been made to redress the balance. However, carriage studies are technically demanding, expensive and time consuming and agreement has yet be reached on the methods of best practice; although recommendations have been proposed (Roberts, Greenwood *et al.* 2009).

Moreover, carriage studies are difficult to compare as they often assess different human populations of restricted age, ethnic origin or socio-economic status and are often performed following different methods. To my knowledge, a carriage study has not been performed since that which coincided with the implementation of the MenC polysaccharide-conjugate vaccine in the UK in 1999 (Balmer, Borrow *et al.* 2002; Maiden and Stuart 2002). However, the MenC-associated carriage study in 1999 only focused on the carriage of *N. meningitidis* in teenagers aged 15 - 17 years at the start of the first year of the study (Balmer, Borrow *et al.* 2002; Maiden and Stuart 2002). A carriage study in the UK involving Novartis is due to commence at the start of the academic year in 2010 (personal communication, Dr Mathew Diggle) however, the details regarding this were not known to the author at the time of writing.

The SHLMPRL is a fully accredited via the Clinical Pathology Accreditation (UK) Ltd. (CPA) scheme and the high standards by which the SHLMPRL currently operate may not have been in place during the reference laboratory's infancy. The meningococcal reference laboratory was born out of the special interest, exceptional dedication and labours of a few key individuals (chiefly Dr. R. Fallon and subsequently Dr. L. Smart) at a time when funding for such a laboratory, if at all in place, was very limited (Dr M. Diggle, personal communication). Moreover, without their endeavours the reference laboratory would likely never have existed.

Nevertheless, a number of matters regarding the SHLMPRL strain archive and database require attention. For the isolates obtained during the earlier years of the period covered by this study the availability of samples was an issue. An entry recorded on the meningococcal isolate database did not guarantee that a corresponding isolate was present amongst the laboratory isolate archive. Several hundred isolates listed in the meningococcal isolate database were absent from the laboratory isolate archive; presumably they were either never archived at the time the samples were processed or they have since been expended or discarded. Furthermore, during isolate recovery it was noted that isolates missing from the strain collection were frequently those recorded as NG. Were these isolates considered of lesser importance and not archived or were they discarded during a previous re-location of the reference laboratory? It is my opinion that the *N. meningitidis* isolate database is in need of improvement

and I would recommend that the database be updated in order to match the archived freeze-dried/lyophilised samples with their corresponding database record such that the precise location and condition of each specimen are recorded.

Related to this issue was that of the number of specimens that were not viable following attempts to sub-culture; particularly those isolates stored as freeze-dried/lyophilised specimens (those dating from 1974 – 1995). One of the functions of the reference laboratory is to maintain the meningococcal isolate archive. During the course of this study the quality and quantity of the currently archived lyophilised/freeze-dried specimens varied enormously and specimens were often not viable following resuscitation and sub-culturing. Therefore, based on the difficulties encountered with the resuscitation and recovery of *N. meningitidis* isolates, I would also recommend to the SHLMPRL to plan and implement the resuscitation and recovery of stored lyophilised/freeze-dried specimens (perhaps also older isolates stored on 5% glycerol or on Protect™ beads) and prepare fresh stocks to re-archive. Moreover, future research by the reference laboratory or its collaborators may benefit in using this extensive isolate archive and it would be regrettable if this valuable resource was put in jeopardy.

Long-term storage of *N. meningitidis* is best achieved by either lyophilisation or freezing. Lyophilised bacteria can be stored for long periods at 4ºC or at -20ºC (Popovic, Ajello *et al.* 1999). Contrastingly, however, freeze-dried (lyophilised) specimens have been securely stored at ambient temperature at the SHLMPRL for at least the previous nine years, if not longer. Consequently, this may have been detrimental to the viability of stored *N. meningitidis* cultures. Although a number of factors may all influence viability; from growth media, growth phase and cell concentration, the use of protective agents, the drying methodology, re-hydration of the specimen, as well as specimen storage and packaging (Morgan, Herman *et al.* 2006). Maintenance of the isolate archive and accurate documentation describing it is of particular importance given the proposed re-location of the Stobhill Microbiology Reference laboratories (including the SHLMPRL) to another site in Glasgow (Glasgow Royal Infirmary) in the next 2-3 years. At the time of writing no definite decision regarding re-location had been approved, nevertheless moving all the laboratory resources, not just the isolate

archive, and tracking its constituent parts would be aided by a more detailed laboratory inventory.

The exemplary surveillance system (MIDAS) in place in Scotland in the modern era does not extend to the earlier years of the period covered by this study. For some samples a comprehensive epidemiological record was not available; age-group data was not recorded for around 40% and 15% of the carriage and IMD strain collections, respectively. Although attempts were made to contact Health Protection Scotland and NHS health boards to seek their assistance, ascertainment of missing information could not be achieved.

This study cannot be directly compared with those more traditional carriage studies because the methodology employed is slightly different. Carriage of the meningococcus is known to vary in relation to the age-group of the population sampled (Cartwright, Stuart *et al.* 1987; Caugant, Kristiansen *et al.* 1988; Caugant, Hoiby *et al.* 1994; Pavlopoulou, Daikos *et al.* 2004; Bogaert, Hermans *et al.* 2005; Claus, Maiden *et al.* 2005) and the isolates are frequently non-serogroupable (Cartwright, Stuart *et al.* 1987; Caugant, Kristiansen *et al.* 1988; Caugant, Hoiby *et al.* 1994; Jolley, Kalmusova *et al.* 2000). The carriage strain collection in this study also displays these typical features. The isolates of the reference laboratory strain archive were assembled over the course of the past four or five decades. Isolates in this study were selected from the strain archive on the basis of the criteria set out in chapter two, and as such, isolates were not actively obtained by swabbing from a defined community during a defined time period. Therefore the determination of carriage rates, both regional and national, in Scotland of different serogroups, STs or ccs is not possible.

In this study, the criteria used to define a carried meningococcal isolate was set in order to exclude any bias that may occur due to the influence of any disease outbreaks. Furthermore, although this study has compared carriage strains with IMD strains the distinction between the two is, in situ, surely less clear-cut. Following acquisition of the meningococcus by a host the next step in the sequence of events leading to disease is adhesion (i.e. carriage) of the meningococcus to host cells; therefore all isolates causing disease would initially have been carried by the host.

Around a decade has passed since the UK meningococcal carriage study was undertaken and, while much has been gained from this study, the time may be right to implement a new carriage study given that changes in the meningococcal population can be observed to occur over a relatively short time frame. Carriage rates vary considerably according to the age-group studied (Cartwright, Stuart *et al.* 1987; Yazdankhah and Caugant 2004), however if we assume an average rate of carriage in Scotland equivalent to that reported during the Stonehouse survey of 10.9% (Cartwright, Stuart *et al.* 1987) then this would be equivalent to swabbing in excess of 7,250 individuals to obtain a strain collection of the same size as the carriage strain collection reported herein. Furthermore, to obtain a carriage strain collection of comparable size to the IMD strain collection would require swabbing nearly 25,400 individuals.

It is presumed that most cases of meningococcal disease occur due to the acquisition of the bacterium from a healthy carrier since contact with an individual with IMD will be rare; certainly in more industrialised countries where disease tends to be more sporadic and outbreaks occur infrequently. In the meningitis belt in sub-Saharan Africa, where disease incidence rates are typically higher and outbreaks are often large, the importance of transmission via an individual with IMD may be more significant. Furthermore, disease incidence in temperate countries peaks during the winter or early spring, whereas in sub-Saharan Africa disease incidence is usually highest during the dry season. This raises the question how does seasonality influence transmission? Are there variations in the transmission or duration of carriage of different meningococcal lineages?

Following exposure to the bacterium, adhesion to host cells is the critical first stage in the sequence of events necessary for colonisation and the possible subsequent dissemination throughout the body. Damage to the ciliated epithelial layer is known to be a contributing risk factor towards meningococcal infection. This may be due to preceding viral infection or active or passive smoking (Cartwright 1995b; Fischer, Hedberg *et al.* 1997; Yusuf, Rochat *et al.* 1999; Kriz, Bobak *et al.* 2000). Since 2006 the Smoking, Health and Social Care (Scotland) Bill has been in force making it illegal to smoke in enclosed public spaces. One wonders how this legislation will impact on the transmission and carriage of *N. meningitidis* and the incidence of meningococcal disease in Scotland. Whilst

exposure to cigarette smoke in enclosed public spaces should be reduced how would exposure in a household setting change and, in turn, would this impact on transmission amongst household members?

As observed in the SHLMPRL isolate database, meningococci can be obtained from various sites on the human host, not just from a variety of sites within the upper respiratory tract, but also from other mucosal surfaces such as those found in the ano-genital tract and from the conjunctiva. It would be of interest to characterise the non-respiratory tract isolates as it would add to our understanding on the circulating meningococcal lineages. Acapsulate, capsule null locus-containing strains of meningococci have been identified in individuals with infections of the conjunctiva (SHLMPRL; unpublished data). Moreover, distinct acapsulate pneumococcal lineages have also been found to cause infections of the conjunctiva (Hanage, Kaijalainen *et al.* 2006). The study by Young and colleagues described 1156 strains of meningococci isolated from carriers forwarded to the reference laboratory during 1972 - 1982; many from a study of meningococcal carriage in patients attending a department for sexually transmitted diseases. Most strains were isolated from the respiratory tract, and many were isolated from genital sources (Young, Harris *et al.* 1983).

The study by Young *et al.* concluded that among patients attending a clinic for sexually transmitted diseases, women without gonorrhoea were significantly less likely to be colonised with meningococci than were women with gonorrhoea and men with or without gonorrhoea. Women without gonorrhoea were also significantly less likely to be colonised with groupable meningococci but there were no significant differences in carriage rates of non-groupable meningococci. The authors discussed the effect of behavioural differences and therefore relative-risk on the different groups based on their results. Many of the women from which throat swabs were taken were asymptomatic contacts (of men with gonorrhoea) while the vast majority of males from whom throat swabs were taken attended the clinic with symptomatic urethritis. It was suggested that a higher incidence of meningococcal colonisation of the pharynx appears to be associated with the greater rate of recent mouth-to-mouth contact in the more sexually active and hence ‘high-risk’ patients rather than directly correlated with gonococcal infection. Furthermore, the authors suggested that these results may be explained if predominantly different transmission routes for groupable

and non-groupable isolates were to exist. If non-groupable isolates were primarily spread via droplet spray then most individuals would be exposed to a similar extent. In contrast, if groupable meningococci were primarily spread from direct salivary spread then they would be expected to be of higher incidence in their 'high risk' patients by virtue of their greater degree of mouth-to-mouth contact.

The progression of a strain from commensalism to disease is intimately linked to the host-pathogen interactions and it is becoming increasingly apparent that to fully understand why the meningococcus causes disease in one individual but not in another we must learn more about the influence of host genetics on immunity to bacterial pathogens. A number of genetic polymorphisms have been described which are associated with increased risk of meningococcal disease. For example, diversity in CEACAM genes (Callaghan, Rockett *et al.* 2008); Caucasian paediatric meningococcal disease patients carrying the functional Plasminogen-activator-inhibitor (PAI)-1 gene 4G/4G genotype are at an increased risk of developing vascular complications and dying from meningococcal disease (Haralambous, Hibberd *et al.* 2003); a polymorphism in the Factor H (fH) gene have been described in UK Caucasians that can result in reduced bactericidal activity against meningococci and an increased risk of contracting meningococcal disease (Haralambous, Dolly *et al.* 2006). Genetic polymorphism of the binding domain of surfactant protein-A2 has been described that increases the risk of meningococcal disease and risk of death (Jack, Cole *et al.* 2006). Interleukin-1 (IL-1) genotype also can influence the severity of meningococcal disease (Read, Camp *et al.* 2000). Other risk factors include those previously mentioned which are associated with increased risk of carriage; i.e. contact with an IMD case, overcrowding, ethnicity (e.g. Maori or Pacific Islanders), preceding viral infection, or age-group. Interestingly, bacterial load has been linked to disease severity (Darton, Guiver *et al.* 2009); possibly an indication of over-stimulation of immune system. Retrospective analyses of IMD patients and matched controls may reveal the extent of disease-associated host genetic polymorphisms in the Scottish population.

Microarray technology has been used to assess gene expression during meningeal-meningococcal interaction providing evidence for the release of host factors including cytokines and chemokines in response to bacterial challenge

(Wells, Tighe *et al.* 2001). Neisserial genome comparisons based on microarray hybridisation experiments has also prompted attempts to identify virulence gene candidates that were restricted to invasive isolates. Differences in the pathogenic potential of different meningococci (carried or invasive), gonococci or other Neisseria *spp.* were measured by the mere presence/absence of a particular genetic determinant. Conflicting results regarding the association of a filamentous phage Nf1 (MDA) with invasive isolates suggests this phage is not a characteristic of all invasive meningococcal strains (Bille et al 2005; Hotopp et al 2006). Nevertheless, it is currently not known what MDA encodes.

Characterisation of the individual *porA* VRs and the precise determination of the level of expression of the constituent PorA subtypes are warranted particularly since a number of prospective vaccines are at various stages of development. Furthermore, an assessment of the dominant epitopes would also be required to aid in the assessment of the immune response generated towards vaccine-associated subtypes. Serum bactericidal assays (SBAs) in vaccinees or using convalescent sera from patients (such as is performed by the Vaccine Evaluation Unit, Meningococcal Reference Laboratory, Manchester, UK) combined with DNA sequencing of the *porA* promoter region could be utilised to assess this.

Currently, the SHLMPRL performs Minimum Inhibitory Concentration (M.I.C.) assays on all *N. meningitidis* isolates for the following antibiotics; Sulphamethoxazole, Penicillin G, Chloramphenicol, Ciprofloxacin, Cefotaxime and Rifampicin. However, as previously mentioned, progressive laboratory practice meant that isolates obtained during the earlier period of the strain collection were not exposed to such rigorous protocols as those of a more contemporary period. Consequently not every isolate in the archive has been tested for its susceptibility to all the aforementioned antibiotics. This is one avenue worth further exploration, particularly since several of these antibiotics can be used to treat primary cases of meningococcal disease and oral Rifampicin is recommended in the UK for the prevention of secondary cases of meningococcal meningitis (and to facilitate eradication of the meningococcus from the nasopharynx). However, there is no widely adopted definition across Europe for breakpoints for antimicrobial resistance in *N. meningitidis* despite the efforts of the EMGM (Vázquez 2007; Vázquez, Enriquez *et al.* 2007). In addition, previous studies by the SHLMPRL on the prevalence of antibiotic

resistance has only focused on isolates of *Streptococcus pneumoniae* (Clarke, Scott *et al.* 2004; Clarke, Reid *et al.* 2005; Smith, Jefferies *et al.* 2006; Cooke 2010).

The Meningococcus remains an important pathogen of man despite the best efforts of the medical and scientific community over the preceding century and longer. Further study regarding carriage of the bacterium is warranted as there is much we have yet to learn. Diagnosis of IMD is problematic as initial symptoms are often non-specific and resemble other respiratory illnesses. The progression of disease is rapid, case-fatality rates exceed 10% and permanent sequelae are even more common. Isolation of the causative organism is not always possible, particularly if antibiotics have been administered prior to hospital admission, and laboratory-confirmed cases of IMD are increasingly being diagnosed by non-culture methods. As the complexity of this bacterium is unravelled, perhaps a more potent method for the eradication of meningococcal disease can be discovered. Meanwhile, current strategies to control the burden of *N. meningitidis* should not be taken for granted; vaccination and antibiotic use has been shown to be effective in high-risk populations. However, continued surveillance is required to establish the long-term effect on individual lineages should they undergo genetic changes that render a particular vaccine or antibiotic obsolete and we must remain vigilant despite a downward trend in cases of meningococcal disease in more industrialised countries.

8 List of References

Abad, R., B. Alcala, C. Salcedo, R. Enriquez, M. J. Uria, P. Diez and J. A. Vazquez (2006). "Sequencing of the *porB* gene: a step toward a true characterization of *Neisseria meningitidis*." Clin Vaccine Immunol **13**(10): 1087-91.

Abdillahi, H. and J. T. Poolman (1988). "Definition of meningococcal class 1 OMP subtyping antigens by monoclonal antibodies." FEMS Microbiol Immunol **1**(3): 139-44.

Achtman, M. (1995). Chapter 7 - Global Epidemiology of Meningococcal Disease. In, Meningococcal Disease Edited by Keith Cartwright, John Wiley & Sons.

Aguilera, J. F., A. Perrocheau, C. Meffre and S. Hahne (2002). "Outbreak of serogroup W135 meningococcal disease after the Hajj pilgrimage, Europe, 2000." Emerg Infect Dis **8**(8): 761-7.

Ala'Aldeen, D. A., K. R. Neal, K. Ait-Tahar, J. S. Nguyen-Van-Tam, A. English, T. J. Falla, P. M. Hawkey and R. C. Slack (2000). "Dynamics of meningococcal long-term carriage among university students and their implications for mass vaccination." J Clin Microbiol **38**(6): 2311-6.

Alcala, B., L. Arreaza, C. Salcedo, M. J. Uria, L. De La Fuente and J. A. Vazquez (2002a). "Capsule switching among C:2b:P1.2,5 meningococcal epidemic strains after mass immunization campaign, Spain." Emerg Infect Dis **8**(12): 1512-4.

Alcala, B., C. Salcedo, L. Arreaza, S. Berron, L. De La Fuente and J. A. Vazquez (2002b). "The epidemic wave of meningococcal disease in Spain in 1996-1997: probably a consequence of strain displacement." J Med Microbiol **51**(12): 1102-6.

Andersen, J., L. Berthelsen, B. Bech Jensen and I. Lind (1998). "Dynamics of the meningococcal carrier state and characteristics of the carrier strains: a longitudinal study within three cohorts of military recruits." Epidemiol Infect **121**(1): 85-94.

Anderson, A., T. Jones, A. Arora, G. Zlotnick, S. Hoiseth, E. Murphy, L. McNeil, H. Harris, D. Z. Jiang and K. Jansen (2009). Development of a Factor H binding protein vaccine for broad protection against invasive *Neisseria meningitidis* serogroup B (MNB) Disease. 10th Meeting of the European Meningococcal Disease Society (EMGM). Manchester, U.K.

Anonymous (2003). Enhanced Surveillance of Meningococcal Disease National Annual Report: July 2002 - June 2003, Health Protection Agency.

Anonymous (2005). The Smoking, Health and Social Care (Scotland) Bill. The Scottish Parliament.

Arreaza, L., B. Alcala, C. Salcedo and J. A. Vazquez (2001). "Interruption of *siaD* in a meningococcal carrier isolate mediated by an insertion sequence." Clin Diagn Lab Immunol **8**(2): 465-6.

Ashton, F. E., A. Ryan, B. Diena and H. J. Jennings (1983). "A new serogroup (L) of *Neisseria meningitidis*." J Clin Microbiol **17**(5): 722-7.

Ashton, F. E., J. A. Ryan, A. Borczyk, D. A. Caugant, L. Mancino and D. Huang (1991). "Emergence of a virulent clone of *Neisseria meningitidis* serotype 2a that is associated with meningococcal group C disease in Canada." J Clin Microbiol **29**(11): 2489-93.

Baker, M. G., D. R. Martin, C. E. Kieft and D. Lennon (2001). "A 10-year serogroup B meningococcal disease epidemic in New Zealand: descriptive epidemiology, 1991-2000." J Paediatr Child Health **37**(5): S13-9.

Bakir, M., A. Yagci, N. Ulger, C. Akbenlioglu, A. Ilki and G. Soyletir (2001). "Asymtomatic carriage of *Neisseria meningitidis* and *Neisseria lactamica* in relation to *Streptococcus pneumoniae* and *Haemophilus influenzae* colonization in healthy children: apropos of 1400 children sampled." Eur J Epidemiol **17**(11): 1015-8.

Balkhy, H. H., Z. A. Memish, M. A. Almuneef and A. O. Osoba (2004). "*Neisseria meningitidis* W-135 carriage during the Hajj season 2003." Scand J Infect Dis **36**(4): 264-8.

Balkhy, H. H., Z. A. Memish and A. O. Osoba (2003). "Meningococcal carriage among local inhabitants during the pilgrimage 2000-2001." Int J Antimicrob Agents **21**(2): 107-11.

Balmer, P., R. Borrow and E. Miller (2002). "Impact of meningococcal C conjugate vaccine in the UK." J Med Microbiol **51**(9): 717-22.

Bambini, S., A. Muzzi, P. Olcen, R. Rappuoli, M. Pizza and M. Comanducci (2009). "Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus." Vaccine **27**(21): 2794-803.

Beddek, A. J., M. S. Li, J. S. Kroll, T. W. Jordan and D. R. Martin (2009). "Evidence for capsule switching between carried and disease-causing *Neisseria meningitidis* strains." Infect Immun **77**(7): 2989-94.

Bennett, D. E., R. M. Mulhall and M. T. Cafferkey (2004). "PCR-based assay for detection of *Neisseria meningitidis* capsular serogroups 29E, X, and Z." J Clin Microbiol **42**(4): 1764-5.

Bennett, J. S., D. T. Griffiths, N. D. McCarthy, K. L. Sleeman, K. A. Jolley, D. W. Crook and M. C. Maiden (2005). "Genetic diversity and carriage dynamics of *Neisseria lactamica* in infants." Infect Immun **73**(4): 2424-32.

Bentley, S. D., G. S. Vernikos, L. A. Snyder, C. Churcher, C. Arrowsmith, T. Chillingworth, A. Cronin, P. H. Davis, N. E. Holroyd, K. Jagels, M. Maddison, S. Moule, E. Rabbinowitsch, S. Sharp, L. Unwin, S. Whitehead, M. A. Quail, M. Achtman, B. Barrell, N. J. Saunders and J. Parkhill (2007). "Meningococcal genetic variation mechanisms viewed through comparative analysis of serogroup C strain FAM18." PLoS Genet **3**(2): e23.

Bettinger, A., N. Le Saux, D. Scheifele, S. Halperin, W. Vaudry, B. Bortolussi and R. Tsang (2008). Poster P136 - The changing incidence of meningococcal invasive infections across Canada, IMPACT 2002 - 2007. 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Bevanger, L., K. Bergh, G. Gisnas, D. A. Caugant and L. O. Froholm (1998). "Identification of nasopharyngeal carriage of an outbreak strain of *Neisseria meningitidis* by pulsed-field gel electrophoresis versus phenotypic methods." J Med Microbiol **47**(11): 993-8.

Bhattacharjee, A. K., H. J. Jennings and C. P. Kenny (1978). "Structural elucidation of the 3-deoxy-D-manno-octulosonic acid containing meningococcal 29-e capsular polysaccharide antigen using carbon-13 nuclear magnetic resonance." Biochemistry **17**(4): 645-51.

Bhattacharjee, A. K., H. J. Jennings, C. P. Kenny, A. Martin and I. C. Smith (1975). "Structural determination of the sialic acid polysaccharide antigens of *Neisseria meningitidis* serogroups B and C with carbon 13 nuclear magnetic resonance." J Biol Chem **250**(5): 1926-32.

Bhattacharjee, A. K., H. J. Jennings, C. P. Kenny, A. Martin and I. C. Smith (1976). "Structural determination of the polysaccharide antigens of *Neisseria meningitidis* serogroups Y, W-135, and BO1." Can J Biochem **54**(1): 1-8.

Bille, E., J. R. Zahar, A. Perrin, S. Morelle, P. Kriz, K. A. Jolley, M. C. Maiden, C. Dervin, X. Nassif and C. R. Tinsley (2005). "A chromosomally integrated bacteriophage in invasive meningococci." J Exp Med **201**(12): 1905-13.

Birtles, A., K. Hardy, S. J. Gray, S. Handford, E. B. Kaczmarski, V. Edwards-Jones and A. J. Fox (2005). "Multilocus sequence typing of *Neisseria meningitidis* directly from clinical samples and application of the method to the investigation of meningococcal disease case clusters." J Clin Microbiol **43**(12): 6007-14.

Bjune, G., E. A. Hoiby, J. K. Gronnesby, O. Arnesen, J. H. Fredriksen, A. Halstensen, E. Holten, A. K. Lindbak, H. Nokleby, E. Rosenqvist and et al. (1991). "Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway." Lancet **338**(8775): 1093-6.

Block, C., M. Gdalevich, R. Buber, I. Ashkenazi, S. Ashkenazi and N. Keller (1999). "Factors associated with pharyngeal carriage of *Neisseria meningitidis* among Israel Defense Force personnel at the end of their compulsory service." Epidemiol Infect **122**(1): 51-7.

BNF (2010). Chapter 5 - Infections. British National Formulary 59, BMJ Group and Pharmacutical Press.

Bogaert, D., P. W. Hermans, H. Boelens, M. Sluijter, A. Luijendijk, H. C. Rumke, S. Koppen, A. van Belkum, R. de Groot and H. A. Verbrugh (2005). "Epidemiology of nasopharyngeal carriage of *Neisseria meningitidis* in healthy Dutch children." Clin Infect Dis **40**(6): 899-902.

Booy, R., J. Jelfs, H. El Bashir and M. D. Nissen (2007). "Impact of meningococcal C conjugate vaccine use in Australia." Med J Aust **186**(3): 108-9.

Borrow, R., H. Claus, U. Chaudhry, M. Guiver, E. B. Kaczmarski, M. Frosch and A. J. Fox (1998). "siaD PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal infections." FEMS Microbiol Lett **159**(2): 209-14.

Boutriau, D., J. Poolman, R. Borrow, J. Findlow, J. D. Domingo, J. Puig-Barbera, J. M. Baldo, V. Planelles, A. Jubert, J. Colomer, A. Gil, K. Levie, A. D. Kervyn, V. Weynants, F. Dominguez, R. Barbera and F. Sotolongo (2007). "Immunogenicity and safety of three doses of a bivalent (B:4:p1.19,15 and B:4:p1.7-2,4) meningococcal outer membrane vesicle vaccine in healthy adolescents." Clin Vaccine Immunol **14**(1): 65-73.

Brandtzaeg, P. (1995). Chapter 4 - Pathogenesis of Meningococcal Infections. In, Meningococcal Disease Edited by Keith Cartwright, John Wiley & Sons Ltd.

Brehony, C., K. A. Jolley and M. C. Maiden (2007). "Multilocus sequence typing for global surveillance of meningococcal disease." FEMS Microbiol Rev **31**(1): 15-26.

Brehony, C., D. J. Wilson and M. C. Maiden (2009). "Variation of the factor H-binding protein of Neisseria meningitidis." Microbiology **155**(Pt 12): 4155-69.

Brooks, J. L., R. J. Fallon and J. E. Heckels (1995). "Sequence variation in class 1 outer membrane protein in *Neisseria meningitidis* isolated from patients with meningococcal infection and close household contacts." FEMS Microbiol Lett **128**(2): 145-50.

Brugger, S. D., P. Frey, S. Aebi, J. Hinds and K. Muhlemann (2010). "Multiple colonization with *S. pneumoniae* before and after introduction of the seven-valent conjugated pneumococcal polysaccharide vaccine." PLoS One **5**(7): e11638.

Brugger, S. D., L. J. Hathaway and K. Muhlemann (2009). "Detection of *Streptococcus pneumoniae* strain cocolonization in the nasopharynx." J Clin Microbiol **47**(6): 1750-6.

Buckee, C. O., S. Gupta, P. Kriz, M. C. Maiden and K. A. Jolley (2010). "Long-term evolution of antigen repertoires among carried meningococci." Proc Biol Sci **277**(1688): 1635-41.

Buckee, C. O., K. A. Jolley, M. Recker, B. Penman, P. Kriz, S. Gupta and M. C. Maiden (2008). "Role of selection in the emergence of lineages and the evolution of virulence in *Neisseria meningitidis*." Proc Natl Acad Sci U S A **105**(39): 15082-7.

Bundle, D. R., H. J. Jennings and C. P. Kenny (1973). "An improved procedure for the isolation of meningococcal, polysaccharide antigens, and the structural determination of the antigen from serogroup X." Carbohydr Res **26**(1): 268-70.

Bundle, D. R., I. C. Smith and H. J. Jennings (1974). "Determination of the structure and conformation of bacterial polysaccharides by carbon 13 nuclear magnetic resonance. Studies on the group-specific antigens of *Neisseria meningitidis* serogroups A and X." J Biol Chem **249**(7): 2275-81.

Bygraves, J. A. and M. C. Maiden (1992). "Analysis of the clonal relationships between strains of *Neisseria meningitidis* by pulsed field gel electrophoresis." J Gen Microbiol **138**(3): 523-31.

Bygraves, J. A., R. Urwin, A. J. Fox, S. J. Gray, J. E. Russell, I. M. Feavers and M. C. Maiden (1999). "Population genetic and evolutionary approaches to analysis of *Neisseria meningitidis* isolates belonging to the ET-5 complex." J Bacteriol **181**(18): 5551-6.

Callaghan, M. J., K. Rockett, C. Banner, E. Haralambous, H. Betts, S. Faust, M. C. Maiden, J. S. Kroll, M. Levin, D. P. Kwiatkowski and A. J. Pollard (2008). "Haplotypic diversity in human CEACAM genes: effects on susceptibility to meningococcal disease." Genes Immun **9**(1): 30-7.

Capecchi, B., J. Adu-Bobie, F. Di Marcello, L. Ciucchi, V. Masignani, A. Taddei, R. Rappuoli, M. Pizza and B. Arico (2005). "*Neisseria meningitidis* NadA is a new invasin which promotes bacterial adhesion to and penetration into human epithelial cells." Mol Microbiol **55**(3): 687-98.

Carbonnelle, E., S. Helaine, X. Nassif and V. Pelicic (2006). "A systematic genetic analysis in *Neisseria meningitidis* defines the Pil proteins required for assembly, functionality, stabilization and export of type IV pili." Mol Microbiol **61**(6): 1510-22.

Cardenosa, N., A. Dominguez, A. Orcau, H. Panella, P. Godoy, S. Minguell, N. Camps and J. A. Vazquez (2001). "Carriers of *Neisseria meningitidis* in household contacts of meningococcal disease cases in Catalonia (Spain)." Eur J Epidemiol **17**(9): 877-84.

Cartwright, K. (1995a). Chapter 1 - Introduction and Historial perspectives. In, Meningococcal Disease Edited by Keith Cartwright, John Wiley & Sons.

Cartwright, K. (1995b). Chapter 5 - Meningococcal Carriage and Disease. In, Meningococcal Disease Edited by Keith Cartwright, John Wiley & Sons.

Cartwright, K. A., J. M. Stuart, D. M. Jones and N. D. Noah (1987). "The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*." Epidemiol Infect **99**(3): 591-601.

Cartwright, K. A., J. M. Stuart and P. M. Robinson (1991). "Meningococcal carriage in close contacts of cases." Epidemiol Infect **106**(1): 133-41.

Castilla, J., J. A. Vazquez, C. Salcedo, M. Garcia Cenoz, J. J. Garcia Irure, L. Torroba, X. Beristain, R. Abad and A. Barricarte (2009). "B:2a:P1.5 meningococcal strains likely arisen from capsular switching event still spreading in Spain." J Clin Microbiol **47**(2): 463-5.

Caugant, D. A., K. Bovre, P. Gaustad, K. Bryn, E. Holten, E. A. Hoiby and L. O. Froholm (1986). "Multilocus genotypes determined by enzyme electrophoresis of *Neisseria meningitidis* isolated from patients with systemic disease and from healthy carriers." J Gen Microbiol **132**(3): 641-52.

Caugant, D. A., C. Fogg, F. Bajunirwe, P. Piola, R. Twesigye, F. Mutebi, L. O. Froholm, E. Rosenqvist, V. Batwala, I. S. Aaberge, J. A. Rottingen and P. J. Guerin (2006). "Pharyngeal carriage of *Neisseria meningitidis* in 2-19-year-old individuals in Uganda." Trans R Soc Trop Med Hyg **100**(12): 1159-63.

Caugant, D. A., L. O. Froholm, K. Bovre, E. Holten, C. E. Frasch, L. F. Mocca, W. D. Zollinger and R. K. Selander (1986). "Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease." Proc Natl Acad Sci U S A **83**(13): 4927-31.

Caugant, D. A., E. A. Hoiby, P. Magnus, O. Scheel, T. Hoel, G. Bjune, E. Wedege, J. Eng and L. O. Froholm (1994). "Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population." J Clin Microbiol **32**(2): 323-30.

Caugant, D. A., E. A. Hoiby, E. Rosenqvist, L. O. Froholm and R. K. Selander (1992). "Transmission of *Neisseria meningitidis* among asymptomatic military recruits and antibody analysis." Epidemiol Infect **109**(2): 241-53.

Caugant, D. A., B. E. Kristiansen, L. O. Froholm, K. Bovre and R. K. Selander (1988). "Clonal diversity of *Neisseria meningitidis* from a population of asymptomatic carriers." Infect Immun **56**(8): 2060-8.

Caugant, D. A., L. F. Mocca, C. E. Frasch, L. O. Froholm, W. D. Zollinger and R. K. Selander (1987). "Genetic structure of *Neisseria meningitidis* populations in relation to serogroup, serotype, and outer membrane protein pattern." J Bacteriol **169**(6): 2781-92.

Caugant, D. A. and P. Nicolas (2007). "Molecular surveillance of meningococcal meningitis in Africa." Vaccine **25 Suppl 1**: A8-11.

Caugant, D. A., G. Tzanakaki and P. Kriz (2007). "Lessons from meningococcal carriage studies." FEMS Microbiol Rev **31**(1): 52-63.

CDC (2008). Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Neisseria meningitidis*, 2007. Atlanta, Center for Disease Control and Prevention (CDC).

CDC (2010a). "Licensure of a meningococcal conjugate vaccine (Menveo) and guidance for use - Advisory Committee on Immunization Practices (ACIP), 2010." Morb Mortal Wkly Rep **59**(9): 273.

CDC (2010b). Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Neisseria meningitidis*, provisional-2009. Atlanta, Centers for Disease Control and Prevention.

Chiou, C. S., J. C. Liao, T. L. Liao, C. C. Li, C. Y. Chou, H. L. Chang, S. M. Yao and Y. S. Lee (2006). "Molecular epidemiology and emergence of worldwide epidemic clones of Neisseria meningitidis in Taiwan." BMC Infect Dis **6**: 25.

Clarke, S. C., Brownlie, S., Denham, B. C., Mooney, J., Christie, P., and G. F. S. Edwards (2002c). "Meningococcal Disease in Scotland 2000." SCIEH Weekly Report **36**(40): 258-61.

Clarke, S. C., Brownlie, S., Denham, B. C., Reid, J. A., Thom, L., Christie, P., and G. F. S. Edwards (2001). "Meningococcal Disease in Scotland 1999." SCIEH Weekly Report **35**(21): 130-3.

Clarke, S. C., Christie, P., Edwards, G. F. S. (1999a). "Enhanced Surveillance of meningococcal disease in Scotland." SCIEH Weekly Report **33**: 235-6.

Clarke, S. C., Christie, P., Reid, J. A., Thom, L., Denham, B. C. and G. F. S. Edwards (1999b). "Meningococcal Disease in Scotland 1998." SCIEH Weekly Report **33**(45): 286-8.

Clarke, S. C., Denham, B. C., Mooney, J. D., Christie, P., and G.F.S. Edwards (2003a). "Meningococcal Disease in Scotland 2001." SCIEH Weekly Report **37**(12): 66-8.

Clarke, S. C., Denham, B. C., Mooney, J. D., Donaghy, M. and G.F.S. Edwards (2003c). "Meningococcal Disease in Scotland 2002." SCIEH Weekly Report **37**(36): 222-4.

Clarke, S. C., Denham, B. C., Mooney, J. D., McMenamin, J., and G.F.S. Edwards (2005). "Meningococcal disease in Scotland 2003." HPS Weekly Report **39**(04): 26-29.

Clarke, S. C., M. A. Diggle and G. F. Edwards (2002). "Multilocus sequence typing and porA gene sequencing differentiates strains of *Neisseria meningitidis* during case clusters." Br J Biomed Sci **59**(3): 160-2.

Clarke, S. C., M. A. Diggle, P. Molling, M. Unemo and P. Olcen (2003b). "Analysis of PorA variable region 3 in meningococci: implications for vaccine policy?" Vaccine **21**(19-20): 2468-73.

Clarke, S. C., J. Reid, L. Thom, B. C. Denham and G. F. Edwards (2002a). "Laboratory confirmation of meningococcal disease in Scotland, 1993-9." J Clin Pathol **55**(1): 32-6.

Clarke, S. C., J. A. Reid, L. Thom, M. A. Diggle, S. McChlery, K. Scott and G. F. Edwards (2005). "Erythromycin resistance among invasive pneumococci in Scotland, 1994-2003." Br J Biomed Sci **62**(1): 28-30.

Clarke, S. C., K. J. Scott and S. M. McChlery (2004). "Erythromycin resistance in invasive serotype 14 pneumococci is highly related to clonal type." J Med Microbiol **53**(Pt 11): 1101-3.

Claus, H., M. C. Maiden, R. Maag, M. Frosch and U. Vogel (2002). "Many carried meningococci lack the genes required for capsule synthesis and transport." Microbiology **148**(Pt 6): 1813-9.

Claus, H., M. C. Maiden, D. J. Wilson, N. D. McCarthy, K. A. Jolley, R. Urwin, F. Hessler, M. Frosch and U. Vogel (2005). "Genetic analysis of meningococci carried by children and young adults." J Infect Dis **191**(8): 1263-71.

Claus, H., U. Vogel, M. Muhlenhoff, R. Gerardy-Schahn and M. Frosch (1997). "Molecular divergence of the *sia* locus in different serogroups of *Neisseria meningitidis* expressing polysialic acid capsules." Mol Gen Genet **257**(1): 28-34.

Claus, H., U. Vogel, H. Swiderek, M. Frosch and C. Schoen (2007). "Microarray analyses of meningococcal genome composition and gene regulation: a review of the recent literature." FEMS Microbiol Rev **31**(1): 43-51.

Climent, Y., R. Urwin, D. Yero, I. Martinez, A. Martin, F. Sotolongo, M. C. Maiden and R. Pajon (2010b). "The genetic structure of *Neisseria meningitidis* populations in Cuba before and after the introduction of a serogroup BC vaccine." Infect Genet Evol **10**(4): 546-54.

Climent, Y., D. Yero, I. Martinez, A. Martin, K. A. Jolley, F. Sotolongo, M. C. Maiden, R. Urwin and R. Pajon (2010a). "Clonal distribution of disease-associated and healthy carrier isolates of *Neisseria meningitidis* between 1983 and 2005 in Cuba." J Clin Microbiol **48**(3): 802-10.

Cohen, N. J. (2003). "Introduction of the National Meningococcal C Vaccination Program." Communicable Diseases Intelligence **27**(2): 161-2.

Cohn, A. C., J. R. MacNeil, L. H. Harrison, C. Hatcher, J. Theodore, M. Schmidt, T. Pondo, K. E. Arnold, J. Baumbach, N. Bennett, A. S. Craig, M. Farley, K. Gershman, S. Petit, R. Lynfield, A. Reingold, W. Schaffner, K. A. Shutt, E. R. Zell, L. W. Mayer, T. Clark, D. Stephens and N. E. Messonnier (2010). "Changes in *Neisseria meningitidis* disease epidemiology in the United States, 1998-2007: implications for prevention of meningococcal disease." Clin Infect Dis **50**(2): 184-91.

Comanducci, M., S. Bambini, B. Brunelli, J. Adu-Bobie, B. Arico, B. Capecchi, M. M. Giuliani, V. Masignani, L. Santini, S. Savino, D. M. Granoff, D. A. Caugant, M. Pizza, R. Rappuoli and M. Mora (2002). "NadA, a novel vaccine candidate of *Neisseria meningitidis*." J Exp Med **195**(11): 1445-54.

Comanducci, M., S. Bambini, D. A. Caugant, M. Mora, B. Brunelli, B. Capecchi, L. Ciucchi, R. Rappuoli and M. Pizza (2004). "NadA diversity and carriage in *Neisseria meningitidis*." Infect Immun **72**(7): 4217-23.

Conyn-van Spaendonck, M. A., R. Reintjes, L. Spanjaard, E. van Kregten, A. G. Kraaijeveld and P. H. Jacobs (1999). "Meningococcal carriage in relation to an outbreak of invasive disease due to *Neisseria meningitidis* serogroup C in the Netherlands." J Infect **39**(1): 42-8.

Cooke, B. J., Smith, A. J., Diggle, M., Lamb, K., Robertson, C., Inverarity, D., Jefferies, J, Edwards, G., Mitchell, T., Clarke, S., and J. McMenamin (2010). "Antibiotic Resistance in Invasive *Streptococcus pneumoniae* Isolates, Identified in Scotland Between 1999 and 2007." J Med Microbiol **in press**.

Cookson, S. T., J. L. Corrales, J. O. Lotero, M. Regueira, N. Binsztein, M. W. Reeves, G. Ajello and W. R. Jarvis (1998). "Disco fever: epidemic meningococcal disease in northeastern Argentina associated with disco patronage." J Infect Dis **178**(1): 266-9.

Corless, C. E., M. Guiver, R. Borrow, V. Edwards-Jones, A. J. Fox and E. B. Kaczmarski (2001). "Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR." J Clin Microbiol **39**(4): 1553-8.

Cotter, S., P. O'Lorcain, M. Fitzgerald, D. O'Flannigan, M. Cafferkey, K. Murphy and N. O'Sullivan (2009). Poster P034 - Epidemiology and surveillance of invasive meningococcal disease (IMD) in Ireland, 2008. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Danzig, L. (2004). "Meningococcal vaccines." Pediatr Infect Dis J **23**(12 Suppl): S285-92.

Darton, T., M. Guiver, S. Naylor, D. L. Jack, E. B. Kaczmarski, R. Borrow and R. C. Read (2009). "Severity of meningococcal disease associated with genomic bacterial load." Clin Infect Dis **48**(5): 587-94.

Davies, A. L., D. O'Flanagan, R. L. Salmon and T. J. Coleman (1996). "Risk factors for *Neisseria meningitidis* carriage in a school during a community outbreak of meningococcal infection." Epidemiol Infect **117**(2): 259-66.

de Filippis, I., C. F. de Andrade, L. Silva, D. R. Prevots and A. C. Vicente (2007). "PorA variable antigenic regions VR1, VR2, and VR3 of *Neisseria meningitidis* serogroups B and C isolated in Brazil from 1999 to 2004." Infect Immun **75**(7): 3683-5.

de Greeff, S. C., H. E. de Melker, L. Spanjaard, L. M. Schouls and A. van Derende (2006). "Protection from routine vaccination at the age of 14 months with meningococcal serogroup C conjugate vaccine in the Netherlands." Pediatr Infect Dis J **25**(1): 79-80.

de Wals, P., G. Deceuninck, N. Boulianne and G. de Serres (2004). "Effectiveness of a mass immunization campaign using serogroup C meningococcal conjugate vaccine." Jama **292**(20): 2491-4.

Debard, A. L., B. Lamy, G. Monneret, J. P. Mira, A. Pachot, M. Kleijer, M. F. Aillaud, A. Boibieux, J. Bienvenu, G. Carret, G. Fournier and J. Bohe (2005). "FcgammaRIIIb and complement component C7 codeficiency in a patient with recurrence of fulminant meningococcal septic shock." Clin Infect Dis **40**(11): 1679-83.

Derrick, J. P., R. Urwin, J. Suker, I. M. Feavers and M. C. Maiden (1999). "Structural and evolutionary inference from molecular variation in Neisseria porins." Infect Immun **67**(5): 2406-13.

Devoy, A. F., K. H. Dyet and D. R. Martin (2005). "Stability of PorA during a meningococcal disease epidemic." J Clin Microbiol **43**(2): 832-7.

Diermayer, M., K. Hedberg, F. Hoesly, M. Fischer, B. Perkins, M. Reeves and D. Fleming (1999). "Epidemic serogroup B meningococcal disease in Oregon: the evolving epidemiology of the ET-5 strain." Jama **281**(16): 1493-7.

Diggle, M. A. (2004). The molecular characterisation of *Neisseria meningitidis* in Scotland before, during and after the initial introduction of the meningococcal serogroup C conjugate (MenC) vaccines Faculty of Science. Glasgow, University of Glasgow. **Ph.D.:** 286.

Diggle, M. A., C. M. Bell and S. C. Clarke (2003). "Nucleotide sequence-based typing of meningococci directly from clinical samples." J Med Microbiol **52**(Pt 6): 505-8.

Diggle, M. A. and S. C. Clarke (2002). "Rapid assignment of nucleotide sequence data to allele types for multi-locus sequence analysis (MLSA) of bacteria using an adapted database and modified alignment program." J Mol Microbiol Biotechnol **4**(6): 515-7.

Diggle, M. A. and S. C. Clarke (2004). "Genotypic characterization of *Neisseria meningitidis* using Pyrosequencing." Mol Biotechnol **28**(2): 139-45.

Diggle, M. A. and S. C. Clarke (2005). "Increased genetic diversity of *Neisseria meningitidis* isolates after the introduction of meningococcal serogroup C polysaccharide conjugate vaccines." J Clin Microbiol **43**(9): 4649-53.

Diggle, M. A. and S. C. Clarke (2006). "Molecular methods for the detection and characterization of *Neisseria meningitidis*." Expert Rev Mol Diagn **6**(1): 79-87.

Diggle, M. A., K. Smith, E. K. Girvan and S. C. Clarke (2003). "Evaluation of a fluorescence-based PCR method for identification of serogroup a meningococci." J Clin Microbiol **41**(4): 1766-8.

Djibo, S., P. Nicolas, J. M. Alonso, A. Djibo, D. Couret, J. Y. Riou and J. P. Chippaux (2003). "Outbreaks of serogroup X meningococcal meningitis in Niger 1995-2000." Trop Med Int Health **8**(12): 1118-23.

DoH (2006a). Chapter 11 - Immunisation schedule. Immunization against Infectious Diseases - "The Green Book"
Crown Publishing.

DoH (2006b). Chapter 22 - Meningococcal. Immunization against Infectious Diseases - "The Green Book", Crown Publishing.

Dolan-Livengood, J. M., Y. K. Miller, L. E. Martin, R. Urwin and D. S. Stephens (2003). "Genetic basis for nongroupable Neisseria meningitidis." J Infect Dis **187**(10): 1616-28.

Dolan, J. (2008). Poster P012 - "*sodC* as a species identifier for real-time PCR detection of *N. meningitidis*". 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Dolan, J., C. Hatcher, M. Bach, D. Satterfild, K. E. Arnold, D. S. Stephens, L. H. Harrison, R. A. Hollick, N. R. Messonnier, L. W. Mayr and a. T. A. B. C. S. A. Team (2009). Validation of *sodC*-based Real-Time PCR Assay for the detection of *N. meningitidis*. 10th Meeting of the European Meningococcal Disease Society (EMGM). Manchester, U.K.

Dominguez, A., N. Cardenosa, C. Izquierdo, F. Sanchez, N. Margall, J. A. Vazquez and L. Salleras (2001). "Prevalence of *Neisseria meningitidis* carriers in the school population of Catalonia, Spain." Epidemiol Infect **127**(3): 425-33.

Dull, P., C. Gill and K. Veitch (2009). MenACYW-CRM, a quadrivalent meningococcal conjugate vaccine suitable from infancy through adulthood. 10th Meeting of the European Meningococcal Disease Society (EMGM). Manchester, U.K.

ECDC (2009). Annual Epidemiological Report on Communicable Diseases in Europe, 2009. Stockholm, European Centre for Disease Prevention and Control (ECDC).

Efron, A., C. Sorhouet, C. Salcedo, J. Vazquez and M. Regueira (2008). Poster P169 - Significant increase of serogroup W135 invasive *Neisseria meningitidis* strains in Argentina: A new epidemiological feature on the region. 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Emonts, M., J. A. Hazelzet, R. de Groot and P. W. Hermans (2003). "Host genetic determinants of Neisseria meningitidis infections." Lancet Infect Dis **3**(9): 565-77.

Enright, M. C. and B. G. Spratt (1998). "A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease." Microbiology **144 (Pt 11)**: 3049-60.

Erwin, A. L., K. L. Nelson, T. Mhlanga-Mutangadura, P. J. Bonthuis, J. L. Geelhood, G. Morlin, W. C. Unrath, J. Campos, D. W. Crook, M. M. Farley, F. W. Henderson, R. F. Jacobs, K. Muhlemann, S. W. Satola, L. van Alphen, M. Golomb and A. L. Smith (2005). "Characterization of genetic and phenotypic diversity of invasive nontypeable *Haemophilus influenzae*." Infect Immun **73**(9): 5853-63.

Erwin, A. L. and A. L. Smith (2007). "Nontypeable *Haemophilus influenzae*: understanding virulence and commensal behavior." Trends Microbiol **15**(8): 355-62.

Fallon, R. J. (1986a). "Meningococcal infections in Scotland, 1985 " Communicable Diseases Scotland Weekly Report(17): 7-15.

Fallon, R. J. (1986b). "Meningococcal infections in Scotland, first and second quarters 1986." Communicable Diseases Scotland Weekly Report(43): 5-10.

Fallon, R. J. (1986c). "Meningococcal serotypes and serogroup B disease in north-west Europe." Lancet **2**(8510): 807-8.

Fallon, R. J., W. M. Brown and W. Lore (1984). "Meningococcal infections in Scotland 1972-82." J Hyg (Lond) **93**(2): 167-80.

Fallon, R. J. and W. M. Morris (1986). "Serotypes of Group B *Neisseria meningitidis* isolated from meningococcal disease in Scotland." Antonie Van Leeuwenhoek **52**(3): 2.

Feavers, I. M., A. J. Fox, S. Gray, D. M. Jones and M. C. Maiden (1996). "Antigenic diversity of meningococcal outer membrane protein PorA has implications for epidemiological analysis and vaccine design." Clin Diagn Lab Immunol **3**(4): 444-50.

Feavers, I. M., S. J. Gray, R. Urwin, J. E. Russell, J. A. Bygraves, E. B. Kaczmarski and M. C. Maiden (1999). "Multilocus sequence typing and antigen gene sequencing in the investigation of a meningococcal disease outbreak." J Clin Microbiol **37**(12): 3883-7.

Feavers, I. M. and M. C. Maiden (1998). "A gonococcal *porA* pseudogene: implications for understanding the evolution and pathogenicity of *Neisseria gonorrhoeae*." Mol Microbiol **30**(3): 647-56.

Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage and B. G. Spratt (2004). "eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data." J Bacteriol **186**(5): 1518-30.

Figueroa, J. E. and P. Densen (1991). "Infectious diseases associated with complement deficiencies." Clin Microbiol Rev **4**(3): 359-95.

Findlow, H., U. Vogel, J. E. Mueller, A. Curry, B. M. Njanpop-Lafourcade, H. Claus, S. J. Gray, S. Yaro, Y. Traore, L. Sangare, P. Nicolas, B. D. Gessner and R. Borrow (2007). "Three cases of invasive meningococcal disease caused by a capsule null locus strain circulating among healthy carriers in Burkina Faso." J Infect Dis **195**(7): 1071-7.

Finne, J., D. Bitter-Suermann, C. Goridis and U. Finne (1987). "An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues." The Journal of Immunology **138**(12): 4402-4407.

Finne, J., M. Leinonen and P. H. Mäkelä (1983). "ANTIGENIC SIMILARITIES BETWEEN BRAIN COMPONENTS AND BACTERIA CAUSING MENINGITIS." The Lancet **322**(8346): 355-357.

Fischer, M., K. Hedberg, P. Cardosi, B. D. Plikaytis, F. C. Hoesly, K. R. Steingart, T. A. Bell, D. W. Fleming, J. D. Wenger and B. A. Perkins (1997). "Tobacco smoke as a risk factor for meningococcal disease." Pediatr Infect Dis J **16**(10): 979-83.

Fitzpatrick, P. E., R. L. Salmon, P. R. Hunter, R. J. Roberts and S. R. Palmer (2000). "Risk factors for carriage of *Neisseria meningitidis* during an outbreak in Wales." Emerg Infect Dis **6**(1): 65-9.

Fletcher, L. D., L. Bernfield, V. Barniak, J. E. Farley, A. Howell, M. Knauf, P. Ooi, R. P. Smith, P. Weise, M. Wetherell, X. Xie, R. Zagursky, Y. Zhang and G. W. Zlotnick (2004). "Vaccine potential of the *Neisseria meningitidis* 2086 lipoprotein." Infect Immun **72**(4): 2088-100.

Frasch, C. E. and L. F. Mocca (1982). "Strains of *Neisseria meningitidis* isolated from patients and their close contacts." Infect Immun **37**(1): 155-9.

Frasch, C. E., W. D. Zollinger and J. T. Poolman (1985). "Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes." Rev Infect Dis **7**(4): 504-10.

Frosch, M., U. Edwards, K. Bousset, B. Krausse and C. Weisgerber (1991). "Evidence for a common molecular origin of the capsule gene loci in gram-negative bacteria expressing group II capsular polysaccharides." Mol Microbiol **5**(5): 1251-63.

Frosch, M. and A. Muller (1993). "Phospholipid substitution of capsular polysaccharides and mechanisms of capsule formation in Neisseria meningitidis." Mol Microbiol **8**(3): 483-93.

Frosch, M., D. Muller, K. Bousset and A. Muller (1992). "Conserved outer membrane protein of *Neisseria meningitidis* involved in capsule expression." Infect Immun **60**(3): 798-803.

Frosch, M., C. Weisgerber and T. F. Meyer (1989). "Molecular characterization and expression in *Escherichia coli* of the gene complex encoding the polysaccharide capsule of *Neisseria meningitidis* group B." Proc Natl Acad Sci U S A **86**(5): 1669-73.

Fuchs, T. M., H. Deppisch, V. Scarlato and R. Gross (1996). "A new gene locus of *Bordetella pertussis* defines a novel family of prokaryotic transcriptional accessory proteins." J Bacteriol **178**(15): 4445-52.

Ganguli, S., G. Zapata, T. Wallis, C. Reid, G. Boulnois, W. F. Vann and I. S. Roberts (1994). "Molecular cloning and analysis of genes for sialic acid synthesis in Neisseria meningitidis group B and purification of the meningococcal CMP-NeuNAc synthetase enzyme." J Bacteriol **176**(15): 4583-9.

Gilmore, A., G. Jones, M. Barker, N. Soltanpoor and J. M. Stuart (1999). "Meningococcal disease at the University of Southampton: outbreak investigation." Epidemiol Infect **123**(2): 185-92.

Glitza, I. C., I. Ehrhard, B. Muller-Pebody, R. Reintjes, T. Breuer, A. Ammon and H. G. Sonntag (2008). "Longitudinal study of meningococcal carrier rates in teenagers." Int J Hyg Environ Health **211**(3-4): 263-72.

Gold, R., I. Goldschneider, M. L. Lepow, T. F. Draper and M. Randolph (1978). "Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children." J Infect Dis **137**(2): 112-21.

Gorla, M. C., A. P. Lemos, C. T. Sacchi, J. C. de Moraes and L. G. Milagres (2003). "Comparison of PorA VR types and porA promoter sequence from *Neisseria meningitidis* B isolated from non-immunised children and vaccine failures immunised with a serogroup B outer membrane protein vaccine." Vaccine **21**(21-22): 2871-6.

Granoff, D. M. (2010). "Review of meningococcal group B vaccines." Clin Infect Dis **50 Suppl 2**: S54-65.

Gray-Owen, S. D. (2003). "Neisserial Opa proteins: impact on colonization, dissemination and immunity." Scand J Infect Dis **35**(9): 614-8.

Gray, S., H. Campbell, M. Chandra, A. Fox, A. D. Carr, W. J. Marsh, L. S. Newbold, R. H. Mallard, M. Ramsay and E. B. Kaczmarski (2009). Poster P027 - The epidemiology and surveillance of meningococcal disease in England and Wales. 10th Meeting of the European Meningococcal Disease Society (EMGM). Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Gray, S. J., C. L. Trotter, M. E. Ramsay, M. Guiver, A. J. Fox, R. Borrow, R. H. Mallard and E. B. Kaczmarski (2006). "Epidemiology of meningococcal disease in England and Wales 1993/94 to 2003/04: contribution and experiences of the Meningococcal Reference Unit." J Med Microbiol **55**(Pt 7): 887-96.

Grecki, M. and M. Bienias (2006). "Outbreak of invasive meningococcal disease among soldiers in Skwierzyna, Poland, March 2006." Euro Surveill **11**(7): E060706 4.

Grundmann, H., S. Hori and G. Tanner (2001). "Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms." J Clin Microbiol **39**(11): 4190-2.

Guiver, M., R. Borrow, J. Marsh, S. J. Gray, E. B. Kaczmarski, D. Howells, P. Boseley and A. J. Fox (2000). "Evaluation of the Applied Biosystems automated Taqman polymerase chain reaction system for the detection of meningococcal DNA." FEMS Immunol Med Microbiol **28**(2): 173-9.

Hahne, S. J., S. J. Gray, F. Jean, Aguilera, N. S. Crowcroft, T. Nichols, E. B. Kaczmarski and M. E. Ramsay (2002). "W135 meningococcal disease in England and Wales associated with Hajj 2000 and 2001." Lancet **359**(9306): 582-3.

Hammerschmidt, S., R. Hilse, J. P. van Putten, R. Gerardy-Schahn, A. Unkmeir and M. Frosch (1996). "Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element." Embo J **15**(1): 192-8.

Hammerschmidt, S., A. Muller, H. Sillmann, M. Muhlenhoff, R. Borrow, A. Fox, J. van Putten, W. D. Zollinger, R. Gerardy-Schahn and M. Frosch (1996). "Capsule phase variation in *Neisseria meningitidis* serogroup B by slipped-strand mispairing in the polysialyltransferase gene (siaD): correlation with bacterial invasion and the outbreak of meningococcal disease." Mol Microbiol **20**(6): 1211-20.

Hanage, W. P., T. Kaijalainen, A. Saukkoriipi, J. L. Rickcord and B. G. Spratt (2006). "A successful, diverse disease-associated lineage of nontypeable pneumococci that has lost the capsular biosynthesis locus." J Clin Microbiol **44**(3): 743-9.

Haralambous, E., S. O. Dolly, M. L. Hibberd, D. J. Litt, I. A. Udalova, C. O'Dwyer, P. R. Langford, J. Simon Kroll and M. Levin (2006). "Factor H, a regulator of complement activity, is a major determinant of meningococcal disease susceptibility in UK Caucasian patients." Scand J Infect Dis **38**(9): 764-71.

Haralambous, E., M. L. Hibberd, P. W. Hermans, N. Ninis, S. Nadel and M. Levin (2003). "Role of functional plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism in susceptibility, severity, and outcome of meningococcal disease in Caucasian children." Crit Care Med **31**(12): 2788-93.

Harrison, L. H. (2008). Evidence of capsular switching in invasive *Neisseria meningitidis* isolates in the pre-meningococcal conjugate vaccine era, United States, 2000 - 2005. 16th International Pathogenic Neisseria Conference (IPNC). Beurs World Trade Center, Rotterdam, The Netherlands.

Harrison, L. H. (2010). "Epidemiological profile of meningococcal disease in the United States." Clin Infect Dis **50 Suppl 2**: S37-44.

Harrison, L. H., K. A. Shutt, S. E. Schmink, J. W. Marsh, B. H. Harcourt, X. Wang, A. M. Whitney, D. S. Stephens, A. A. Cohn, N. E. Messonnier and L. W. Mayer (2010). "Population Structure and Capsular Switching of Invasive *Neisseria meningitidis* Isolates in the Pre-Meningococcal Conjugate Vaccine Era-United States, 2000-2005." J Infect Dis **201**(8): 1208-1224.

Harrison, L. H., C. L. Trotter and M. E. Ramsay (2009). "Global epidemiology of meningococcal disease." Vaccine **27 Suppl 2**: B51-63.

Health, D. o. (2006a). "Chapter 11 - Immunisation schedule." Immunization against Infectious Diseases - "The Green Book" from www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook.

Health, D. o. (2006b). "Chapter 22 - Meningococcal." Immunization against Infectious Diseases - "The Green Book", from www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook.

Heuberger, S., C. Mikula and B. Springer (2009). Poster P040 - Austria 2008, Epidemiology and surveillance of meningococcal disease. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Hilse, R., S. Hammerschmidt, W. Bautsch and M. Frosch (1996). "Site-specific insertion of IS1301 and distribution in *Neisseria meningitidis* strains." J Bacteriol **178**(9): 2527-32.

Hilse, R., J. Stoevesandt, D. A. Caugant, H. Claus, M. Frosch and U. Vogel (2000). "Distribution of the meningococcal insertion sequence IS1301 in clonal lineages of *Neisseria meningitidis*." Epidemiol Infect **124**(2): 337-40.

Hoang, L. M., E. Thomas, S. Tyler, A. J. Pollard, G. Stephens, L. Gustafson, A. McNabb, I. Pocock, R. Tsang and R. Tan (2005). "Rapid and fatal meningococcal disease due to a strain of *Neisseria meningitidis* containing the capsule null locus." Clin Infect Dis **40**(5): e38-42.

Hodgson, A., A. A. Forgor, D. Chandramohan, Z. Reed, F. Binka, C. Bevilacqua, D. Boutriau and B. Greenwood (2008). "A phase II, randomized study on an investigational DTPw-HBV/Hib-MenAC conjugate vaccine administered to infants in Northern Ghana." PLoS One **3**(5): e2159.

Holst, J., B. Feiring, J. E. Fuglesang, E. A. Hoiby, H. Nokleby, I. S. Aaberge and E. Rosenqvist (2003). "Serum bactericidal activity correlates with the vaccine efficacy of outer membrane vesicle vaccines against *Neisseria meningitidis* serogroup B disease." Vaccine **21**(7-8): 734-7.

Honisch, C., Y. Chen, C. Mortimer, C. Arnold, O. Schmidt, D. van den Boom, C. R. Cantor, H. N. Shah and S. E. Gharbia (2007). "Automated comparative sequence analysis by base-specific cleavage and mass spectrometry for nucleic acid-based microbial typing." Proc Natl Acad Sci U S A **104**(25): 10649-54.

Hopkins, A. and J. McMenamin (2005). "Respiratory Infections: Meningococcal disease in Scotland in 2004." HPS Weekly Report **39**(48): 262.

Hotopp, J. C., R. Grifantini, N. Kumar, Y. L. Tzeng, D. Fouts, E. Frigimelica, M. Draghi, M. M. Giuliani, R. Rappuoli, D. S. Stephens, G. Grandi and H. Tettelin (2006). "Comparative genomics of *Neisseria meningitidis*: core genome, islands of horizontal transfer and pathogen-specific genes." Microbiology **152**(Pt 12): 3733-49.

Hunter, P. R. and M. A. Gaston (1988). "Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity." J Clin Microbiol **26**(11): 2465-6.

Ibarz-Pavon, A. B., C. Brehony, K. Jolley and M. C. Maiden (2004). Identification of a new clonal complex (ST-213 Complex) of hyperinvasive meningococci among disease and carriage isolates. 14th International Pathogenic Neisseria Conference, Milwaukee, USA.

Jack, D. L., J. Cole, S. C. Naylor, R. Borrow, E. B. Kaczmarski, N. J. Klein and R. C. Read (2006). "Genetic polymorphism of the binding domain of surfactant protein-A2 increases susceptibility to meningococcal disease." Clin Infect Dis **43**(11): 1426-33.

Jackson, L. A., R. Baxter, K. Reisinger, A. Karsten, J. Shah, L. Bedell and P. M. Dull (2009). "Phase III comparison of an investigational quadrivalent meningococcal conjugate vaccine with the licensed meningococcal ACWY conjugate vaccine in adolescents." Clin Infect Dis **49**(1): e1-10.

Jackson, L. A. and J. D. Wenger (1993). "Laboratory-based surveillance for meningococcal disease in selected areas, United States, 1989-1991." MMWR CDC Surveill Summ **42**(2): 21-30.

Jacobsson, S., S. T. Hedberg, P. Molling, M. Unemo, M. Comanducci, R. Rappuoli and P. Olcen (2009). "Prevalence and sequence variations of the genes encoding the five antigens included in the novel 5CVMB vaccine covering group B meningococcal disease." Vaccine **27**(10): 1579-84.

Jacobsson, S., S. Thulin, P. Molling, M. Unemo, M. Comanducci, R. Rappuoli and P. Olcen (2006). "Sequence constancies and variations in genes encoding three new meningococcal vaccine candidate antigens." Vaccine **24**(12): 2161-8.

Jansen, K. U., L. K. McNeil, V. Dragalin, A. S. Anderson, S. K. Hoiseth, A. Arora, E. E. Emini, G. W. Zlotnick and T. Jones (2008). Bivalent recombinant LP2086 vaccine to provide broad protection against *Neisseria meningitidis* B disease: Immunological correlates of protection and how to assess coverage against invasive MnB strains. 16th International Pathogenic Neisseria Conference (IPNC). Beurs World Trade Center, Rotterdam, The Netherlands.

Jelfs, J., R. Munro, E. Wedege and D. A. Caugant (2000). "Sequence variation in the *porA* gene of a clone of *Neisseria meningitidis* during epidemic spread." Clin Diagn Lab Immunol **7**(3): 390-5.

Jennings, H. J., C. W. Lugowski, F. E. Ashton and J. A. Ryan (1983). "The structure of the capsular polysaccharide obtained from a new serogroup (L) of *Neisseria meningitidis*." Carbohydr Res **112**(1): 105-11.

Jodar, L., F. M. LaForce, C. Ceccarini, T. Aguado and D. M. Granoff (2003). "Meningococcal conjugate vaccine for Africa: a model for development of new vaccines for the poorest countries." Lancet **361**(9372): 1902-4.

Jolley, K. A., C. Brehony and M. C. Maiden (2007). "Molecular typing of meningococci: recommendations for target choice and nomenclature." FEMS Microbiol Rev **31**(1): 89-96.

Jolley, K. A., E. J. Feil, M. S. Chan and M. C. Maiden (2001). "Sequence type analysis and recombinational tests (START)." Bioinformatics **17**(12): 1230-1.

Jolley, K. A., J. Kalmusova, E. J. Feil, S. Gupta, M. Musilek, P. Kriz and M. C. Maiden (2000). "Carried meningococci in the Czech Republic: a diverse recombining population." J Clin Microbiol **38**(12): 4492-8.

Jolley, K. A., J. Kalmusova, E. J. Feil, S. Gupta, M. Musilek, P. Kriz and M. C. J. Maiden (2002). "Author's Correction - carried meningococci in the Czech Republic: a diverse recombining population." J Clin Microbiol **40**(9): 3549-3550.

Jones, D. (1995). Chapter 6 - Epidemiology of Meningococcal Disease in Europe and the USA. In, Meningococcal Disease Edited by Keith Cartwright.

Jones, D. M. and E. M. Sutcliffe (1990). "Group A meningococcal disease in England associated with the Haj." J Infect **21**(1): 21-5.

Jones, G. R., M. Christodoulides, J. L. Brooks, A. R. Miller, K. A. Cartwright and J. E. Heckels (1998). "Dynamics of carriage of *Neisseria meningitidis* in a group of military recruits: subtype stability and specificity of the immune response following colonization." J Infect Dis **178**(2): 451-9.

K.S.A. (2010). "Kingdom of Saudi Arabia, Ministry of Hajj - Saudi Ministry of Health requirements." from http://www.hajinformation.com/main/p3001.htm.

Kadlubowski, M., A. Skoczynska, I. Wasko, A. Klarowicz and W. Hryniewicz (2008). Poster P153 - Invasive meningococcal disease in Poland - eleven years of the activity of the National Reference Centre for Bacterial Meningitis. 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Kahler, C. M., L. E. Martin, G. C. Shih, M. M. Rahman, R. W. Carlson and D. S. Stephens (1998). "The (alpha2-->8)-linked polysialic acid capsule and lipooligosaccharide structure both contribute to the ability of serogroup B *Neisseria meningitidis* to resist the bactericidal activity of normal human serum." Infect Immun **66**(12): 5939-47.

Kallstrom, H., M. S. Islam, P. O. Berggren and A. B. Jonsson (1998). "Cell signaling by the type IV pili of pathogenic *Neisseria*." J Biol Chem **273**(34): 21777-82.

Kallstrom, H., M. K. Liszewski, J. P. Atkinson and A. B. Jonsson (1997). "Membrane cofactor protein (MCP or CD46) is a cellular pilus receptor for pathogenic *Neisseria*." Mol Microbiol **25**(4): 639-47.

Kaltoft, M. S., U. B. Skov Sorensen, H. C. Slotved and H. B. Konradsen (2008). "An easy method for detection of nasopharyngeal carriage of multiple *Streptococcus pneumoniae* serotypes." J Microbiol Methods **75**(3): 540-4.

Kellerman, S. E., K. McCombs, M. Ray, W. Baughman, M. W. Reeves, T. Popovic, N. E. Rosenstein, M. M. Farley, P. Blake and D. S. Stephens (2002). "Genotype-specific carriage of *Neisseria meningitidis* in Georgia counties with hyper- and hyposporadic rates of meningococcal disease." J Infect Dis **186**(1): 40-8.

Kelly, D. F. and R. Rappuoli (2005). "Reverse vaccinology and vaccines for serogroup B *Neisseria meningitidis*." Adv Exp Med Biol **568**: 217-23.

Kesanopoulos, K., G. Tzanakaki, A. Velegraki, N. Tegos, D. A. Caugant, P. Menounos, J. Kourea-Kremastinou and S. Levidiotou-Stefanou (2005). "Rapid molecular identification of *Neisseria meningitidis* isolates using the polymerase chain reaction followed by single-stranded conformation polymorphism analysis." FEMS Immunol Med Microbiol **45**(2): 143-9.

Klein, N. J., C. A. Ison, M. Peakman, M. Levin, S. Hammerschmidt, M. Frosch and R. S. Heyderman (1996). "The influence of capsulation and lipooligosaccharide structure on neutrophil adhesion molecule expression and endothelial injury by *Neisseria meningitidis*." J Infect Dis **173**(1): 172-9.

Koroleva, I., G. Beloshitskij, L. Spirihina, A. Gracheva and I. Zacroeva (2009). Poster P056 - Epidemiology of meningococcal disease in Russia. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Kremastinou, J., G. Tzanakaki, E. Velonakis, A. Voyiatzi, A. Nickolaou, R. A. Elton, D. Weir and C. Blackwell (1999). "Carriage of *Neisseria meningitidis* and *Neisseria lactamica* among ethnic Greek school children from Russian immigrant families in Athens." FEMS Immunol Med Microbiol **23**(1): 13-20.

Kriz, P., M. Bobak and B. Kriz (2000). "Parental smoking, socioeconomic factors, and risk of invasive meningococcal disease in children: a population based case-control study." Arch Dis Child **83**(2): 117-21.

Kriz, P., J. Klamusova and M. Musilek (2009). Poster P037 - Epidemiological situation of invasive meningococcal disease and vaccination strategy in the Czech Republic. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Kshirsagar, N., N. Mur, U. Thatte, N. Gogtay, S. Viviani, M. P. Preziosi, C. Elie, H. Findlow, G. Carlone, R. Borrow, V. Parulekar, B. Plikaytis, P. Kulkarni, N. Imbault and F. M. LaForce (2007). "Safety, immunogenicity, and antibody persistence of a new meningococcal group A conjugate vaccine in healthy Indian adults." Vaccine **25 Suppl 1**: A101-7.

Kyaw, M. H., P. Christie, I. G. Jones and H. Campbell (2002). "The changing epidemiology of bacterial meningitis and invasive non-meningitic bacterial disease in scotland during the period 1983-99." Scand J Infect Dis **34**(4): 289-98.

Kyaw, M. H., S. C. Clarke, P. Christie, I. G. Jones and H. Campbell (2002). "Invasive meningococcal disease in Scotland, 1994 to 1999, with emphasis on group B meningococcal disease." J Clin Microbiol **40**(5): 1834-7.

Lancellotti, M., A. Guiyoule, C. Ruckly, E. Hong, J. M. Alonso and M. K. Taha (2006). "Conserved virulence of C to B capsule switched *Neisseria meningitidis* clinical isolates belonging to ET-37/ST-11 clonal complex." Microbes Infect **8**(1): 191-6.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson and D. G. Higgins (2007). "Clustal W and Clustal X version 2.0." Bioinformatics **23**(21): 2947-8.

Larrauri, A., R. Cano, M. Garcia and S. Mateo (2005). "Impact and effectiveness of meningococcal C conjugate vaccine following its introduction in Spain." Vaccine **23**(32): 4097-100.

Law, D. K., M. Lorange, L. Ringuette, R. Dion, M. Giguere, A. M. Henderson, J. Stoltz, W. D. Zollinger, P. De Wals and R. S. Tsang (2006). "Invasive meningococcal disease in Quebec, Canada, due to an emerging clone of ST-269 serogroup B meningococci with serotype antigen 17 and serosubtype antigen P1.19 (B:17:P1.19)." J Clin Microbiol **44**(8): 2743-9.

Lee, C. H., W. C. Kuo, S. Beri, S. Kapre, J. S. Joshi, N. Bouveret, F. M. LaForce and C. E. Frasch (2009). "Preparation and characterization of an immunogenic meningococcal group A conjugate vaccine for use in Africa." Vaccine **27**(5): 726-32.

Liu, T. Y., E. C. Gotschlich, F. T. Dunne and E. K. Jonssen (1971a). "Studies on the meningococcal polysaccharides. II. Composition and chemical properties of the group B and group C polysaccharide." J Biol Chem **246**(15): 4703-12.

Liu, T. Y., E. C. Gotschlich, E. K. Jonssen and J. R. Wysocki (1971b). "Studies on the meningococcal polysaccharides. I. Composition and chemical properties of the group A polysaccharide." J Biol Chem **246**(9): 2849-58.

Lowe, C. A., M. A. Diggle and S. C. Clarke (2004). "A single nucleotide polymorphism identification assay for the genotypic characterisation of *Neisseria meningitidis* using MALDI-TOF mass spectrometry." Br J Biomed Sci **61**(1): 8-10.

Lucidarme, J., M. Comanducci, J. Findlow, S. J. Gray, E. B. Kaczmarski, M. Guiver, E. Kugelberg, P. J. Vallely, P. Oster, M. Pizza, S. Bambini, A. Muzzi, C. M. Tang and R. Borrow (2009). "Characterization of fHbp, nhba (gna2132), nadA, porA, sequence type (ST), and genomic presence of IS1301 in group B meningococcal ST269 clonal complex isolates from England and Wales." J Clin Microbiol **47**(11): 3577-85.

MacLennan, J., G. Kafatos, K. Neal, N. Andrews, J. C. Cameron, R. Roberts, M. R. Evans, K. Cann, D. N. Baxter, M. C. Maiden and J. M. Stuart (2006). "Social behavior and meningococcal carriage in British teenagers." Emerg Infect Dis **12**(6): 950-7.

Madico, G., J. A. Welsch, L. A. Lewis, A. McNaughton, D. H. Perlman, C. E. Costello, J. Ngampasutadol, U. Vogel, D. M. Granoff and S. Ram (2006). "The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance." J Immunol **177**(1): 501-10.

Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman and B. G. Spratt (1998). "Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms." Proc Natl Acad Sci U S A **95**(6): 3140-5.

Maiden, M. C., A. B. Ibarz-Pavon, R. Urwin, S. J. Gray, N. J. Andrews, S. C. Clarke, A. M. Walker, M. R. Evans, J. S. Kroll, K. R. Neal, D. A. Ala'aldeen, D. W. Crook, K. Cann, S. Harrison, R. Cunningham, D. Baxter, E. Kaczmarski, J. Maclennan, J. C. Cameron and J. M. Stuart (2008). "Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity." J Infect Dis **197**(5): 737-43.

Maiden, M. C. and J. M. Stuart (2002). "Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination." Lancet **359**(9320): 1829-31.

Maiden, M. C., J. Suker, A. J. McKenna, J. A. Bygraves and I. M. Feavers (1991). "Comparison of the class 1 outer membrane proteins of eight serological reference strains of *Neisseria meningitidis*." Mol Microbiol **5**(3): 727-36.

Marks, M. I., C. E. Frasch and R. M. Shapera (1979). "Meningococcal colonization and infection in children and their household contacts." Am J Epidemiol **109**(5): 563-71.

Marshall, H., M. D. Nissen, P. Richmond, S. B. Lambert, D. M. Roberton, T. Jones, S. Lockhart, W. Gruber and A. Arona (2008). Poster 213 - A randomized, placebo-controlled, double-blinded, phase 1 trial of ascending doses of meningococcal group B rLP2086 vaccine in healthy adults. 16th International Pathogenic Neisseria Conference (IPNC)
Beurs World Trade Center, Rotterdam, The Netherlands.

Martin, D. and L. Lopez (2008). Has MeNZB™ controlled New Zealand's epidemic? . 16th International Pathogenic Neisseria Conference (IPNC). Beurs World Trade Center, Rotterdam, The Netherlands.

Martin, D. R., N. Ruijne, L. McCallum, J. O'Hallahan and P. Oster (2006). "The VR2 epitope on the PorA P1.7-2,4 protein is the major target for the immune response elicited by the strain-specific group B meningococcal vaccine MeNZB." Clin Vaccine Immunol **13**(4): 486-91.

Martin, D. R., S. J. Walker, M. G. Baker and D. R. Lennon (1998). "New Zealand epidemic of meningococcal disease identified by a strain with phenotype B:4:P1.4." J Infect Dis **177**(2): 497-500.

Martin, S. L., R. Borrow, P. van der Ley, M. Dawson, A. J. Fox and K. A. Cartwright (2000). "Effect of sequence variation in meningococcal PorA outer membrane protein on the effectiveness of a hexavalent PorA outer membrane vesicle vaccine." Vaccine **18**(23): 2476-81.

Masignani, V., M. Comanducci, M. M. Giuliani, S. Bambini, J. Adu-Bobie, B. Arico, B. Brunelli, A. Pieri, L. Santini, S. Savino, D. Serruto, D. Litt, S. Kroll, J. A. Welsch, D. M. Granoff, R. Rappuoli and M. Pizza (2003). "Vaccination against *Neisseria meningitidis* using three variants of the lipoprotein GNA1870." J Exp Med **197**(6): 789-99.

Mathew, B. M. and A. K. Chaudhuri (1986). "Meningococcal infection in Lanarkshire." Lancet **2**(8512): 923-4.

McDonald, E., Denham, B., and J. McMenamin (2010a). "Respiratory and immunisation quarterly report. Quarter four: 1 October to 31 December 2009." HPS Weekly Report **44**(2010/07): 4.

McDonald, E., Reynolds, A., Denham, B., and J. McMenamin (2010b). "Respiratory and immunisation quarterly report. Quarter two: 1 April to 30 June 2010." HPS Weekly Report **44**(2010/32): 314 - 317.

McGee, L., L. McDougal, J. Zhou, B. G. Spratt, F. C. Tenover, R. George, R. Hakenbeck, W. Hryniewicz, J. C. Lefevre, A. Tomasz and K. P. Klugman (2001). "Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network." J Clin Microbiol **39**(7): 2565-71.

Meats, E., E. J. Feil, S. Stringer, A. J. Cody, R. Goldstein, J. S. Kroll, T. Popovic and B. G. Spratt (2003). "Characterization of encapsulated and noncapsulated *Haemophilus influenzae* and determination of phylogenetic relationships by multilocus sequence typing." J Clin Microbiol **41**(4): 1623-36.

Merz, A. J. and M. So (1997). "Attachment of piliated, Opa- and Opc- gonococci and meningococci to epithelial cells elicits cortical actin rearrangements and clustering of tyrosine-phosphorylated proteins." Infect Immun **65**(10): 4341-9.

Meyer, T. F., J. Pohlner and J. P. van Putten (1994). "Biology of the pathogenic Neisseriae." Curr Top Microbiol Immunol **192**: 283-317.

Miller, E., D. Salisbury and M. Ramsay (2001). "Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story." Vaccine **20 Suppl 1**: S58-67.

Molling, P., A. Backman, P. Olcen and H. Fredlund (2001). "Comparison of serogroup W-135 meningococci isolated in Sweden during a 23-year period and those associated with a recent hajj pilgrimage." J Clin Microbiol **39**(7): 2695-9.

Molling, P., S. Jacobsson, A. Backman and P. Olcen (2002). "Direct and rapid identification and genogrouping of meningococci and porA amplification by LightCycler PCR." J Clin Microbiol **40**(12): 4531-5.

Molling, P., M. Unemo, A. Backman and P. Olcen (2000). "Genosubtyping by sequencing group A, B and C meningococci; a tool for epidemiological studies of epidemics, clusters and sporadic cases." Apmis **108**(7-8): 509-16.

Mooney, J. D., P. Christie, C. Robertson and S. C. Clarke (2004). "The impact of meningococcal serogroup C conjugate vaccine in Scotland." Clin Infect Dis **39**(3): 349-56.

Mooney, J. D. and J. McMenamin (2004). "Current epidemiology of meningococcal disease in Scotland: Recent trends and a historical perspective." SCIEH Weekly Report **38**(48): 288-9.

Morgan, C. A., N. Herman, P. A. White and G. Vesey (2006). "Preservation of micro-organisms by drying; a review." J Microbiol Methods **66**(2): 183-93.

Murphy, E., L. Andrew, K. L. Lee, D. A. Dilts, L. Nunez, P. S. Fink, K. Ambrose, R. Borrow, J. Findlow, M. K. Taha, A. E. Deghmane, P. Kriz, M. Musilek, J. Kalmusova, D. A. Caugant, T. Alvestad, L. W. Mayer, C. T. Sacchi, X. Wang, D. Martin, A. von Gottberg, M. du Plessis, K. P. Klugman, A. S. Anderson, K. U. Jansen, G. W. Zlotnick and S. K. Hoiseth (2009). "Sequence diversity of the factor H binding protein vaccine candidate in epidemiologically relevant strains of serogroup B *Neisseria meningitidis*." J Infect Dis **200**(3): 379-89.

Nadel, S., M. Levin and P. Habibi (1995). Chapter 9 - Treatment of Meningococcal Disease in Childhood. In, Meningococcal Disease Edited by Keith Cartwright, John Wiley & Sons.

Neal, K. R., J. Nguyen-Van-Tam, P. Monk, S. J. O'Brien, J. Stuart and M. Ramsay (1999). "Invasive meningococcal disease among university undergraduates: association with universities providing relatively large amounts of catered hall accommodation." Epidemiol Infect **122**(3): 351-7.

Neal, K. R., J. S. Nguyen-Van-Tam, N. Jeffrey, R. C. Slack, R. J. Madeley, K. Ait-Tahar, K. Job, M. C. Wale and D. A. Ala'Aldeen (2000). "Changing carriage rate of *Neisseria meningitidis* among university students during the first week of term: cross sectional study." Bmj **320**(7238): 846-9.

Nelson, S. J., A. Charlett, H. J. Orr, R. M. Barker, K. R. Neal, C. Taylor, P. N. Monk, M. R. Evans and J. M. Stuart (2001). "Risk factors for meningococcal disease in university halls of residence." Epidemiol Infect **126**(2): 211-7.

Newcombe, J., K. Cartwright, S. Dyer and J. McFadden (1998). "Naturally occurring insertional inactivation of the *porA* gene of *Neisseria meningitidis* by integration of IS1301." Mol Microbiol **30**(2): 453-4.

Newcombe, J., S. Dyer, L. Blackman, K. Cartwright, W. H. Palmer and J. McFadden (1997). "PCR-single-stranded confirmational polymorphism analysis for non-culture-based subtyping of meningococcal strains in clinical specimens." J Clin Microbiol **35**(7): 1809-12.

Nicolas, P., N. Ait M'barek, S. Al-Awaidy, S. Al Busaidy, N. Sulaiman, M. Issa, J. Mahjour, P. Molling, D. A. Caugant, P. Olcen and M. Santamaria (2005). "Pharyngeal carriage of serogroup W135 *Neisseria meningitidis* in Hajjees and their family contacts in Morocco, Oman and Sudan." Apmis **113**(3): 182-6.

Nicolas, P., L. Decousset, V. Riglet, P. Castelli, R. Stor and G. Blanchet (2001). "Clonal expansion of sequence type (ST-)5 and emergence of ST-7 in serogroup A meningococci, Africa." Emerg Infect Dis **7**(5): 849-54.

Nicolas, P., S. Djibo, A. Moussa, B. Tenebray, P. Boisier and S. Chanteau (2005). "Molecular epidemiology of meningococci isolated in Niger in 2003 shows serogroup A sequence type (ST)-7 and serogroup W135 ST-11 or ST-2881 strains." J Clin Microbiol **43**(3): 1437-8.

Nicolas, P., G. Norheim, E. Garnotel, S. Djibo and D. A. Caugant (2005). "Molecular epidemiology of neisseria meningitidis isolated in the African Meningitis Belt between 1988 and 2003 shows dominance of sequence type 5 (ST-5) and ST-11 complexes." J Clin Microbiol **43**(10): 5129-35.

Okoko, B. J., O. T. Idoko and R. A. Adegbola (2009). "Prospects and challenges with introduction of a mono-valent meningococcal conjugate vaccine in Africa." Vaccine **27**(14): 2023-9.

Olcen, P., J. Kjellander, D. Danielsson and B. L. Lindquist (1981). "Epidemiology of *Neisseria meningitidis*; prevalence and symptoms from the upper respiratory tract in family members to patients with meningococcal disease." Scand J Infect Dis **13**(2): 105-9.

Oster, P., D. Lennon, J. O'Hallahan, K. Mulholland, S. Reid and D. Martin (2005). "MeNZB: a safe and highly immunogenic tailor-made vaccine against the New Zealand *Neisseria meningitidis* serogroup B disease epidemic strain." Vaccine **23**(17-18): 2191-6.

Parent du Chatelet, I., M.-K. Taha, A.-E. Deghmane, A. Lepoutre and D. Levy-Bruhl (2009). Poster P066 - Invasive meningococcal disease in France, 2007-2008. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Parkhill, J., M. Achtman, K. D. James, S. D. Bentley, C. Churcher, S. R. Klee, G. Morelli, D. Basham, D. Brown, T. Chillingworth, R. M. Davies, P. Davis, K. Devlin, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, S. Leather, S. Moule, K. Mungall, M. A. Quail, M. A. Rajandream, K. M. Rutherford, M. Simmonds, J. Skelton, S. Whitehead, B. G. Spratt and B. G. Barrell (2000). "Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491." Nature **404**(6777): 502-6.

Patel, M. S. (2007). "Australia's century of meningococcal disease: development and the changing ecology of an accidental pathogen." Med J Aust **186**(3): 136-41.

Patrick, D. M., S. Champagne, S. H. Goh, G. Arsenault, E. Thomas, C. Shaw, T. Rahim, F. Taha, M. Bigham, V. Dubenko, D. Skowronski and R. C. Brunham (2003). "*Neisseria meningitidis* carriage during an outbreak of serogroup C disease." Clin Infect Dis **37**(9): 1183-8.

Pavlopoulou, I. D., G. L. Daikos, H. Alexandrou, E. Petridou, A. Pangalis, M. Theodoridou and V. P. Syriopoulou (2004). "Carriage of *Neisseria meningitidis* by Greek children: risk factors and strain characteristics." Clin Microbiol Infect **10**(2): 137-42.

Peeters, C. C., H. C. Rumke, L. C. Sundermann, E. M. Rouppe van der Voort, J. Meulenbelt, M. Schuller, A. J. Kuipers, P. van der Ley and J. T. Poolman (1996). "Phase I clinical trial with a hexavalent PorA containing meningococcal outer membrane vesicle vaccine." Vaccine **14**(10): 1009-15.

Peng, J., L. Yang, F. Yang, J. Yang, Y. Yan, H. Nie, X. Zhang, Z. Xiong, Y. Jiang, F. Cheng, X. Xu, S. Chen, L. Sun, W. Li, Y. Shen, Z. Shao, X. Liang, J. Xu and Q. Jin (2008). "Characterization of ST-4821 complex, a unique *Neisseria meningitidis* clone." Genomics **91**(1): 78-87.

Petering, H., S. Hammerschmidt, M. Frosch, J. P. van Putten, C. A. Ison and B. D. Robertson (1996). "Genes associated with meningococcal capsule complex are also found in *Neisseria gonorrhoeae*." J Bacteriol **178**(11): 3342-5.

Pizza, M., J. Donnelly and R. Rappuoli (2008). "Factor H-binding protein, a unique meningococcal vaccine antigen." Vaccine **26 Suppl 8**: I46-8.

Pizza, M., V. Scarlato, V. Masignani, M. M. Giuliani, B. Arico, M. Comanducci, G. T. Jennings, L. Baldi, E. Bartolini, B. Capecchi, C. L. Galeotti, E. Luzzi, R. Manetti, E. Marchetti, M. Mora, S. Nuti, G. Ratti, L. Santini, S. Savino, M. Scarselli, E. Storni, P. Zuo, M. Broeker, E. Hundt, B. Knapp, E. Blair, T. Mason, H. Tettelin, D. W. Hood, A. C. Jeffries, N. J. Saunders, D. M. Granoff, J. C. Venter, E. R. Moxon, G. Grandi and R. Rappuoli (2000). "Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing." Science **287**(5459): 1816-20.

Plant, L. and A. B. Jonsson (2003). "Contacting the host: insights and implications of pathogenic *Neisseria* cell interactions." Scand J Infect Dis **35**(9): 608-13.

Poland, G. A. (2010). "Prevention of meningococcal disease: current use of polysaccharide and conjugate vaccines." Clin Infect Dis **50 Suppl 2**: S45-53.

Pollard, A. J. (2004). "Global epidemiology of meningococcal disease and vaccine efficacy." Pediatr Infect Dis J **23**(12 Suppl): S274-9.

Pollard, A. J. and C. Frasch (2001). "Development of natural immunity to *Neisseria meningitidis*." Vaccine **19**(11-12): 1327-46.

Popovic, T., G. Ajello and R. R. Facklam (1999). Laboratory Methods for the diagnosis of meningitis by *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* W. H. Organization, World Health Organization.

Ramsay, M. E., N. J. Andrews, C. L. Trotter, E. B. Kaczmarski and E. Miller (2003). "Herd immunity from meningococcal serogroup C conjugate vaccination in England: database analysis." Bmj **326**(7385): 365-6.

Rappuoli, R. (2001). "Reverse vaccinology, a genome-based approach to vaccine development." Vaccine **19**(17-19): 2688-91.

Rappuoli, R. (2008). The application of reverse vaccinology, Novartis MenB vaccine developed by design. 16th International Pathogenic Neisseria Conference (IPNC). Beurs World Trade Center, Rotterdam, The Netherlands.

Read, R. C., N. J. Camp, F. S. di Giovine, R. Borrow, E. B. Kaczmarski, A. G. Chaudhary, A. J. Fox and G. W. Duff (2000). "An interleukin-1 genotype is associated with fatal outcome of meningococcal disease." J Infect Dis **182**(5): 1557-60.

Reisinger, K. S., R. Baxter, S. L. Block, J. Shah, L. Bedell and P. M. Dull (2009). "Quadrivalent meningococcal vaccination of adults: phase III comparison of an investigational conjugate vaccine, MenACWY-CRM, with the licensed vaccine, Menactra." Clin Vaccine Immunol **16**(12): 1810-5.

Richmond, P., H. Marshall, M. D. Nissen, S. Lambert, T. Jones, W. Gruber and A. Arora (2008). Poster 212 - A randomized, observer-blinded, active control, phase 1 trial of meningococcal serogroup B rLP2086 vaccine in healthy children and adolescents aged 8 to 14 years. 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Riordan, T., K. Cartwright, N. Andrews, J. Stuart, A. Burris, A. Fox, R. Borrow, T. Douglas-Riley, J. Gabb and A. Miller (1998). "Acquisition and carriage of meningococci in marine commando recruits." Epidemiol Infect **121**(3): 495-505.

Roberts, J., B. Greenwood and J. Stuart (2009). "Sampling methods to detect carriage of *Neisseria meningitidis*; literature review." J Infect **58**(2): 103-7.

Rodriguez, A. P., F. Dickinson, A. Baly and R. Martinez (1999). "The epidemiological impact of antimeningococcal B vaccination in Cuba." Mem Inst Oswaldo Cruz **94**(4): 433-40.

Rosenstein, N. E., B. A. Perkins, D. S. Stephens, L. Lefkowitz, M. L. Cartter, R. Danila, P. Cieslak, K. A. Shutt, T. Popovic, A. Schuchat, L. H. Harrison and A. L. Reingold (1999). "The changing epidemiology of meningococcal disease in the United States, 1992-1996." J Infect Dis **180**(6): 1894-901.

Rosenstein, N. E., B. A. Perkins, D. S. Stephens, T. Popovic and J. M. Hughes (2001). "Meningococcal disease." N Engl J Med **344**(18): 1378-88.

Rots, N. Y. and D. E. Kleijne (2008). Safety of a Nonavalent Meningococcal serogroup B vaccine in healthy adult volunteers in a randomised, controlled,

single blind study. 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Russell, J. E., K. A. Jolley, I. M. Feavers, M. C. Maiden and J. Suker (2004). "PorA variable regions of *Neisseria meningitidis*." Emerg Infect Dis **10**(4): 674-8.

Russell, J. E., R. Urwin, S. J. Gray, A. J. Fox, I. M. Feavers and M. C. Maiden (2008). "Molecular epidemiology of meningococcal disease in England and Wales 1975-1995, before the introduction of serogroup C conjugate vaccines." Microbiology **154**(Pt 4): 1170-7.

Sadler, F., A. Fox, K. Neal, M. Dawson, K. Cartwright and R. Borrow (2003). "Genetic analysis of capsular status of meningococcal carrier isolates." Epidemiol Infect **130**(1): 59-70.

Saitou, N. and M. Nei (1987). "The neighbor-joining method: a new method for reconstructing phylogenetic trees." Mol Biol Evol **4**(4): 406-25.

Salleras, L., A. Dominguez and N. Cardenosa (2003). "Impact of mass vaccination with polysaccharide conjugate vaccine against serogroup C meningococcal disease in Spain." Vaccine **21**(7-8): 725-8.

Sardinas, G., Y. Climent, Y. Rodriguez, S. Gonzalez, D. Garcia, K. Cobas, E. Caballero, Y. Perez, C. Brookes, S. Taylor, A. Gorringe, M. Delgado, R. Pajon and D. Yero (2009). "Assessment of vaccine potential of the Neisseria-specific protein NMB0938." Vaccine **27**(49): 6910-7.

Saunders, N. J. and S. Butcher (2003). "The use of complete genome sequences in vaccine design." Methods Mol Med **87**: 301-12.

Schoen, C., J. Blom, H. Claus, A. Schramm-Gluck, P. Brandt, T. Muller, A. Goesmann, B. Joseph, S. Konietzny, O. Kurzai, C. Schmitt, T. Friedrich, B. Linke, U. Vogel and M. Frosch (2008). "Whole-genome comparison of disease and carriage strains provides insights into virulence evolution in *Neisseria meningitidis*." Proc Natl Acad Sci U S A **105**(9): 3473-8.

Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour and T. S. Whittam (1986). "Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics." Appl Environ Microbiol **51**(5): 873-84.

Shakir, E., C. Cameron, B. Denham and J. McMenamin (2008). "Respiratory and immunisation quarterly report: 1 April to 30 June 2008 (Meningococcal disease, Invasive pneumococcal disease, *Haemophilus influenzae*) " HPS Weekly Report **42**(2008/32): 282-284.

Shao, Z., W. Li, J. Ren, X. Liang, L. Xu, B. Diao, M. Li, M. Lu, H. Ren, Z. Cui, B. Zhu, Z. Dai, L. Zhang, X. Chen, B. Kan and J. Xu (2006). "Identification of a

new *Neisseria meningitidis* serogroup C clone from Anhui province, China." Lancet **367**(9508): 419-23.

Shigematsu, M., K. L. Davison, A. Charlett and N. S. Crowcroft (2002). "National enhanced surveillance of meningococcal disease in England, Wales and Northern Ireland, January 1999-June 2001." Epidemiol Infect **129**(3): 459-70.

Sierra, G. V., H. C. Campa, N. M. Varcacel, I. L. Garcia, P. L. Izquierdo, P. F. Sotolongo, G. V. Casanueva, C. O. Rico, C. R. Rodriguez and M. H. Terry (1991). "Vaccine against group B *Neisseria meningitidis*: protection trial and mass vaccination results in Cuba." NIPH Ann **14**(2): 195-207; discussion 208-10.

Simoes, M. J., M. Cunha, F. Almeida, C. Furtado and L. Brum (2009). "Molecular surveillance of *Neisseria meningitidis* capsular switching in Portugal, 2002-2006." Epidemiol Infect **137**(2): 161-5.

Smart, L. E., Christie, P., Clarke, S. C., Reid, J. A., Thom, L., Denham, B. C. and G. F. S. Edwards (1999). "Meningococcal Disease in Scotland 1997." SCIEH Weekly Report **33**(01): 2-6.

Smart, L. E., O'Brien, S. J., Thom, L., Reid, J. A. and R. W. A. Girdwood (1997). "Meningococcal Disease in Scotland 1996." SCIEH Weekly Report **31**(47): 248-53.

Smith, A. J., J. Jefferies, S. C. Clarke, C. Dowson, G. F. Edwards and T. J. Mitchell (2006). "Distribution of epidemic antibiotic-resistant pneumococcal clones in Scottish pneumococcal isolates analysed by multilocus sequence typing." Microbiology **152**(Pt 2): 361-5.

Snape, M. D. (2008). Immunogenicity and reactogenicity of a novel serogroup B *Neisseria meningitidis* vaccine administered from 6 months of age. 16th International Pathogenic Neisseria Conference (IPNC). Beurs World Trade Center, Rotterdam, The Netherlands.

Sorhouet, C., M. Regueira, M.-K. Taha and M. Mollerach (2008). Poster P232 - Genotypic characterisation of invasive *Neisseria meningitidis* serogroup B (MenB) in Argentina 2006. 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Speers, D. J. and J. Jelfs (1997). "Typing of *Neisseria meningitidis* by restriction analysis of the amplified *porA* gene." Pathology **29**(2): 201-5.

Spinosa, M. R., C. Progida, A. Tala, L. Cogli, P. Alifano and C. Bucci (2007). "The *Neisseria meningitidis* capsule is important for intracellular survival in human cells." Infect Immun **75**(7): 3594-603.

Spratt, B. G., W. P. Hanage, B. Li, D. M. Aanensen and E. J. Feil (2004). "Displaying the relatedness among isolates of bacterial species -- the eBURST approach." FEMS Microbiol Lett **241**(2): 129-34.

St-Martin, G., J. Christensen, K. Molbak and L. Lambertsen (2009). Poster P036 - Epidemiological and laboratory surveillance of invasive disease in Denmark 2007 and 2008. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Stefanelli, P., C. Fazio and P. Mastrantonio (2001). "Typing of *Neisseria meningitidis* isolates from patients with invasive disease by molecular analysis of porin genes." New Microbiol **24**(2): 149-55.

Stefanelli, P., C. Fazio, A. Neri, T. Sofia and P. Mastrantonio (2003). "First report of capsule replacement among electrophoretic type 37 Neisseria meningitidis strains in Italy." J Clin Microbiol **41**(12): 5783-6.

Stefanelli, P., C. Fazio, T. Sofia, A. Neri and P. Mastrantonio (2009). Poster P071 - Laboratory surveillance of invasive meningococcal diseases in Italy: 2007 - 2008. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Stephens, D. S. (2007). "Conquering the meningococcus." FEMS Microbiol Rev **31**(1): 3-14.

Steven, N. and M. Wood (1995). Chapter 8 - The Clinical Spectrum of Meningococcal Disease. In, Meningococcal Disease Edited by Keith Cartwright, John Wiley & Sons.

Stuart, J. M., K. A. Cartwright, D. M. Jones, N. D. Noah, R. J. Wall, C. C. Blackwell, A. E. Jephcott and I. R. Ferguson (1987). "An outbreak of meningococcal disease in Stonehouse: planning and execution of a large-scale survey." Epidemiol Infect **99**(3): 579-89.

Suker, J., I. M. Feavers, M. Achtman, G. Morelli, J. F. Wang and M. C. Maiden (1994). "The *porA* gene in serogroup A meningococci: evolutionary stability and mechanism of genetic variation." Mol Microbiol **12**(2): 253-65.

Sullivan, C. B. (2008). PhD thesis: A molecular epidemiological analysis of meningococcal isolates within Scotland 1972 - 1998. Division of Infection and Immunity, Institute of Biomedical and Life Sciences. Glasgow, University of Glasgow. **Doctor of Philosophy:** 240.

Sullivan, C. B., J. M. Jefferies, M. A. Diggle and S. C. Clarke (2006). "Automation of MLST using third-generation liquid-handling technology." Mol Biotechnol **32**(3): 219-26.

Swaminathan, B., G. M. Matar, M. W. Reeves, L. M. Graves, G. Ajello, W. F. Bibb, L. O. Helsel, M. Morales, H. Dronavalli, M. el-Swify, W. DeWitt and S. B. Hunter (1996). "Molecular subtyping of *Neisseria meningitidis* serogroup B: comparison of five methods." J Clin Microbiol **34**(6): 1468-73.

Swartley, J. S., J. H. Ahn, L. J. Liu, C. M. Kahler and D. S. Stephens (1996). "Expression of sialic acid and polysialic acid in serogroup B *Neisseria meningitidis*: divergent transcription of biosynthesis and transport operons through a common promoter region." J Bacteriol **178**(14): 4052-9.

Swartley, J. S., L. J. Liu, Y. K. Miller, L. E. Martin, S. Edupuganti and D. S. Stephens (1998). "Characterization of the gene cassette required for biosynthesis of the (alpha1-->6)-linked N-acetyl-D-mannosamine-1-phosphate capsule of serogroup A *Neisseria meningitidis*." J Bacteriol **180**(6): 1533-9.

Swartley, J. S., A. A. Marfin, S. Edupuganti, L. J. Liu, P. Cieslak, B. Perkins, J. D. Wenger and D. S. Stephens (1997). "Capsule switching of *Neisseria meningitidis*." Proc Natl Acad Sci U S A **94**(1): 271-6.

Taha, M. K., M. Achtman, J. M. Alonso, B. Greenwood, M. Ramsay, A. Fox, S. Gray and E. Kaczmarski (2000). "Serogroup W135 meningococcal disease in Hajj pilgrims." Lancet **356**(9248): 2159.

Taha, M. K., J. M. Alonso, M. Cafferkey, D. A. Caugant, S. C. Clarke, M. A. Diggle, A. Fox, M. Frosch, S. J. Gray, M. Guiver, S. Heuberger, J. Kalmusova, K. Kesanopoulos, A. M. Klem, P. Kriz, J. Marsh, P. Molling, K. Murphy, P. Olcen, O. Sanou, G. Tzanakaki and U. Vogel (2005). "Interlaboratory comparison of PCR-based identification and genogrouping of *Neisseria meningitidis*." J Clin Microbiol **43**(1): 144-9.

Tayal, S. C., S. Rashid, K. M. Muttu and A. J. Hildreth (1997). "Meningococcal carriage: prevalence and sex-related risk factors." J Infect **34**(2): 101-5.

Tettelin, H., N. J. Saunders, J. Heidelberg, A. C. Jeffries, K. E. Nelson, J. A. Eisen, K. A. Ketchum, D. W. Hood, J. F. Peden, R. J. Dodson, W. C. Nelson, M. L. Gwinn, R. DeBoy, J. D. Peterson, E. K. Hickey, D. H. Haft, S. L. Salzberg, O. White, R. D. Fleischmann, B. A. Dougherty, T. Mason, A. Ciecko, D. S. Parksey, E. Blair, H. Cittone, E. B. Clark, M. D. Cotton, T. R. Utterback, H. Khouri, H. Qin, J. Vamathevan, J. Gill, V. Scarlato, V. Masignani, M. Pizza, G. Grandi, L. Sun, H. O. Smith, C. M. Fraser, E. R. Moxon, R. Rappuoli and J. C. Venter (2000). "Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58." Science **287**(5459): 1809-15.

Thompson, E. A., I. M. Feavers and M. C. Maiden (2003). "Antigenic diversity of meningococcal enterobactin receptor FetA, a vaccine component." Microbiology **149**(Pt 7): 1849-58.

Thomson, W. and T. F. Jackson (1987). "Deaths from meningococcal infection in Lanarkshire, 1984 -1986." Public Health **101**(6): 443-5.

Thulin, S., P. Olcen, H. Fredlund and M. Unemo (2008). "Combined real-time PCR and pyrosequencing strategy for objective, sensitive, specific, and high-throughput identification of reduced susceptibility to penicillins in *Neisseria meningitidis*." Antimicrob Agents Chemother **52**(2): 753-6.

Tondella, M. L., T. Popovic, N. E. Rosenstein, D. B. Lake, G. M. Carlone, L. W. Mayer and B. A. Perkins (2000). "Distribution of Neisseria meningitidis serogroup B serosubtypes and serotypes circulating in the United States. The Active Bacterial Core Surveillance Team." J Clin Microbiol **38**(9): 3323-8.

Toropainen, M., H. Kayhty and P. Ruutu (2009). Poster P057 - Epidemiology of meningococcal disease in Finland in 1995 - 2008. 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Trotter, C. L., M. Chandra, R. Cano, A. Larrauri, M. E. Ramsay, C. Brehony, K. A. Jolley, M. C. Maiden, S. Heuberger and M. Frosch (2007). "A surveillance network for meningococcal disease in Europe." FEMS Microbiol Rev **31**(1): 27-36.

Trotter, C. L., A. J. Fox, M. E. Ramsay, F. Sadler, S. J. Gray, R. Mallard and E. B. Kaczmarski (2002). "Fatal outcome from meningococcal disease--an association with meningococcal phenotype but not with reduced susceptibility to benzylpenicillin." J Med Microbiol **51**(10): 855-60.

Trotter, C. L. and N. J. Gay (2003). "Analysis of longitudinal bacterial carriage studies accounting for sensitivity of swabbing: an application to *Neisseria meningitidis*." Epidemiol Infect **130**(2): 201-5.

Trotter, C. L., M. E. Ramsay, S. Gray, A. Fox and E. Kaczmarski (2006). "No evidence for capsule replacement following mass immunisation with meningococcal serogroup C conjugate vaccines in England and Wales." Lancet Infect Dis **6**(10): 616-7; author reply 617-8.

Tsang, R. S., D. K. Law, S. D. Tyler, G. S. Stephens, M. Bigham and W. D. Zollinger (2005). "Potential capsule switching from serogroup Y to B: The characterization of three such *Neisseria meningitidis* isolates causing invasive meningococcal disease in Canada." Can J Infect Dis Med Microbiol **16**(3): 171-4.

Tully, J., R. M. Viner, P. G. Coen, J. M. Stuart, M. Zambon, C. Peckham, C. Booth, N. Klein, E. Kaczmarski and R. Booy (2006). "Risk and protective factors for meningococcal disease in adolescents: matched cohort study." Bmj **332**(7539): 445-50.

Tzanakaki, G. and J. Kremastinou (2009). Poster P028 - Epidemiology and surveillance of meningococcal disease in Greece (2007 - 2008). 10th Meeting of the European Meningococcal Disease Society (EMGM), Radisson Edwardian Manchester Hotel, Manchester, United Kingdom.

Tzanakaki, G., R. Urwin, M. Musilek, P. Kriz, J. Kremastinou, A. Pangalis, C. C. Blackwell and M. C. Maiden (2001). "Phenotypic and genotypic approaches to characterization of isolates of *Neisseria meningitidis* from patients and their close family contacts." J Clin Microbiol **39**(4): 1235-40.

Tzeng, Y. L., A. K. Datta, C. A. Strole, M. A. Lobritz, R. W. Carlson and D. S. Stephens (2005). "Translocation and surface expression of lipidated serogroup B

capsular Polysaccharide in Neisseria meningitidis." Infect Immun **73**(3): 1491-505.

Tzeng, Y. L., C. Noble and D. S. Stephens (2003). "Genetic basis for biosynthesis of the (alpha 1-->4)-linked N-acetyl-D-glucosamine 1-phosphate capsule of Neisseria meningitidis serogroup X." Infect Immun **71**(12): 6712-20.

Tzeng, Y. L., J. S. Swartley, Y. K. Miller, R. E. Nisbet, L. J. Liu, J. H. Ahn and D. S. Stephens (2001). "Transcriptional regulation of divergent capsule biosynthesis and transport operon promoters in serogroup B *Neisseria meningitidis*." Infect Immun **69**(4): 2502-11.

Uli, L., L. Castellanos-Serra, L. Betancourt, F. Dominguez, R. Barbera, F. Sotolongo, G. Guillen and R. Pajon Feyt (2006). "Outer membrane vesicles of the VA-MENGOC-BC vaccine against serogroup B of *Neisseria meningitidis*: Analysis of protein components by two-dimensional gel electrophoresis and mass spectrometry." Proteomics **6**(11): 3389-99.

Ure, R., S. J. Gray and M. C. J. Maiden (2004). Genotypic Characterisation of Invasive Meningococci Isolated in England and Wales Preceding Introduction of the Meningococcal Conjugate Serogroup C Vaccine. 14th International Pathogenic Neisseria Conference (IPNC), Milwaukee, Wisconsin, USA.

Ure, R., Reid, J., Thom, L., Diggle, M., Edwards, G. (2008). Poster P159 - The epidemiology of clinically relevant *Neisseria meningitidis* isolated in Scotland between 2003 and 2008. 16th International Pathogenic Neisseria Conference (IPNC), Beurs World Trade Center, Rotterdam, The Netherlands.

Urwin, R., J. E. Russell, E. A. Thompson, E. C. Holmes, I. M. Feavers and M. C. Maiden (2004). "Distribution of surface protein variants among hyperinvasive meningococci: implications for vaccine design." Infect Immun **72**(10): 5955-62.

van den Dobbelsteen, G. P., H. H. van Dijken, S. Pillai and L. van Alphen (2007). "Immunogenicity of a combination vaccine containing pneumococcal conjugates and meningococcal PorA OMVs." Vaccine **25**(13): 2491-6.

van der Ende, A., C. T. Hopman and J. Dankert (1999). "Deletion of *porA* by recombination between clusters of repetitive extragenic palindromic sequences in *Neisseria meningitidis*." Infect Immun **67**(6): 2928-34.

van der Ende, A., C. T. Hopman and J. Dankert (2000). "Multiple mechanisms of phase variation of PorA in *Neisseria meningitidis*." Infect Immun **68**(12): 6685-90.

van der Ende, A., C. T. Hopman, W. C. Keijzers, L. Spanjaard, E. B. Lodder, P. H. van Keulen and J. Dankert (2003). "Outbreak of meningococcal disease caused by PorA-deficient meningococci." J Infect Dis **187**(5): 869-71.

van der Ende, A., C. T. Hopman, S. Zaat, B. B. Essink, B. Berkhout and J. Dankert (1995). "Variable expression of class 1 outer membrane protein in *Neisseria meningitidis* is caused by variation in the spacing between the -10 and -35 regions of the promoter." J Bacteriol **177**(9): 2475-80.

van der Kaaden, A., G. J. Gerwig, J. P. Kamerling, J. F. Vliegenthart and R. H. Tiesjema (1985). "Structure of the capsular antigen of *Neisseria meningitidis* serogroup K." Eur J Biochem **152**(3): 663-8.

van der Kaaden, A., J. I. van Doorn-van Wakeren, J. P. Kamerling, J. F. Vliegenthart and R. H. Tiesjema (1984). "Structure of the capsular antigen of *Neisseria meningitidis* serogroup H." Eur J Biochem **141**(3): 513-9.

van der Ley, P., J. E. Heckels, M. Virji, P. Hoogerhout and J. T. Poolman (1991). "Topology of outer membrane porins in pathogenic Neisseria spp." Infect Immun **59**(9): 2963-71.

van der Voort, E. R., P. van der Ley, J. van der Biezen, S. George, O. Tunnela, H. van Dijken, B. Kuipers and J. Poolman (1996). "Specificity of human bactericidal antibodies against PorA P1.7,16 induced with a hexavalent meningococcal outer membrane vesicle vaccine." Infect Immun **64**(7): 2745-51.

van der Voort, E. R., H. van Dijken, B. Kuipers, J. van der Biezen, P. van der Ley, J. Meylis, I. Claassen and J. Poolman (1997). "Human B- and T-cell responses after immunization with a hexavalent PorA meningococcal outer membrane vesicle vaccine." Infect Immun **65**(12): 5184-90.

van Deuren, M., P. Brandtzaeg and J. W. van der Meer (2000). "Update on meningococcal disease with emphasis on pathogenesis and clinical management." Clin Microbiol Rev **13**(1): 144-66, table of contents.

Vazquez-Bermudez, M. F., S. Barroso, K. Walter, A. J. Alvarez, A. Alarcon, M. Lopez-Trascasa, I. Wichmann, F. Aguilar, A. Nunez-Roldan and B. Sanchez (2003). "Complement component C7 deficiency in a Spanish family." Clin Exp Immunol **133**(2): 240-6.

Vázquez, J. A. (2007). "Resistance testing of meningococci: the recommendations of the European Monitoring Group on Meningococci." FEMS Microbiology Reviews **31**(1): 97-100.

Vázquez, J. A., R. Enriquez, R. Abad, B. Alcalá, C. Salcedo and L. Arreaza (2007). "Antibiotic resistant meningococci in Europe: Any need to act?" FEMS Microbiology Reviews **31**(1): 64-70.

Verdu, M. E., P. Coll, D. Fontanals, F. March, I. Pons, D. Van Esso and G. Prats (1999). "Comparison of conventional ribotyping and PCR-RFLP ribotyping for the analysis of endemic strains of *Neisseria meningitidis* isolated in a local community over 7 years." FEMS Microbiol Lett **179**(2): 247-53.

Vipond, C., J. Suker, C. Jones, C. Tang, I. M. Feavers and J. X. Wheeler (2006). "Proteomic analysis of a meningococcal outer membrane vesicle vaccine prepared from the group B strain NZ98/254." Proteomics **6**(11): 3400-13.

Vipond, C., J. X. Wheeler, C. Jones, I. M. Feavers and J. Suker (2005). "Characterization of the protein content of a meningococcal outer membrane vesicle vaccine by polyacrylamide gel electrophoresis and mass spectrometry." Hum Vaccin **1**(2): 80-4.

Virji, M., H. Kayhty, D. J. Ferguson, C. Alexandrescu, J. E. Heckels and E. R. Moxon (1991). "The role of pili in the interactions of pathogenic *Neisseria* with cultured human endothelial cells." Mol Microbiol **5**(8): 1831-41.

Virji, M., K. Makepeace, D. J. Ferguson, M. Achtman, J. Sarkari and E. R. Moxon (1992). "Expression of the Opc protein correlates with invasion of epithelial and endothelial cells by *Neisseria meningitidis*." Mol Microbiol **6**(19): 2785-95.

Virji, M., S. M. Watt, S. Barker, K. Makepeace and R. Doyonnas (1996). "The N-domain of the human CD66a adhesion molecule is a target for Opa proteins of *Neisseria meningitidis* and *Neisseria gonorrhoeae*." Mol Microbiol **22**(5): 929-39.

Vogel, U., H. Claus and M. Frosch (2004a). "Genetic lineages and their traits in *Neisseria meningitidis*." Int J Med Microbiol **294**(2-3): 75-82.

Vogel, U., H. Claus, L. von Muller, D. Bunjes, J. Elias and M. Frosch (2004b). "Bacteremia in an immunocompromised patient caused by a commensal *Neisseria meningitidis* strain harboring the capsule null locus (cnl)." J Clin Microbiol **42**(7): 2898-901.

Vogel, U., A. Weinberger, R. Frank, A. Muller, J. Kohl, J. P. Atkinson and M. Frosch (1997). "Complement factor C3 deposition and serum resistance in isogenic capsule and lipooligosaccharide sialic acid mutants of serogroup B *Neisseria meningitidis*." Infect Immun **65**(10): 4022-9.

von Gottberg, A., M. du Plessis, C. Cohen, E. Prentice, S. Schrag, L. de Gouveia, G. Coulson, G. de Jong and K. Klugman (2008). "Emergence of endemic serogroup W135 meningococcal disease associated with a high mortality rate in South Africa." Clin Infect Dis **46**(3): 377-86.

Von Loewenich, F. D., E. Wintermeyer, M. Dumig and M. Frosch (2001). "Analysis of transcriptional control mechanisms of capsule expression in *Neisseria meningitidis*." Int J Med Microbiol **291**(5): 361-9.

Weber, M. V., H. Claus, M. C. Maiden, M. Frosch and U. Vogel (2006). "Genetic mechanisms for loss of encapsulation in polysialyltransferase-gene-positive meningococci isolated from healthy carriers." Int J Med Microbiol **296**(7): 475-84.

Wedege, E., E. A. Hoiby, E. Rosenqvist and G. Bjune (1998). "Immune responses against major outer membrane antigens of *Neisseria meningitidis* in vaccinees and controls who contracted meningococcal disease during the Norwegian serogroup B protection trial." Infect Immun **66**(7): 3223-31.

Wedege, E., J. Kolberg, A. Delvig, E. A. Hoiby, E. Holten, E. Rosenqvist and D. A. Caugant (1995). "Emergence of a new virulent clone within the electrophoretic type 5 complex of serogroup B meningococci in Norway." Clin Diagn Lab Immunol **2**(3): 314-21.

Wells, D. B., P. J. Tighe, K. G. Wooldridge, K. Robinson and D. A. Ala' Aldeen (2001). "Differential gene expression during meningeal-meningococcal interaction: evidence for self-defense and early release of cytokines and chemokines." Infect Immun **69**(4): 2718-22.

Welte, R., G. van den Dobbelsteen, J. M. Bos, H. de Melker, L. van Alphen, L. Spanjaard, H. C. Rumke and M. J. Postma (2004). "Economic evaluation of meningococcal serogroup C conjugate vaccination programmes in The Netherlands and its impact on decision-making." Vaccine **23**(4): 470-9.

WHO, W. H. O. (2006). "Outbreak news. Meningococcal disease, African meningitis belt, epidemic season 2006." Wkly Epidemiol Rec **81**(13): 119-20.

Wiese-Posselt, M., W. Hellenbrand, A. Siedler and C. Mayer (2006). "Universal childhood immunisation with pneumococcal vaccine and meningococcal serogroup C vaccine introduced in Germany." Euro Surveill **11**(9): E060907 4.

Wilder-Smith, A. (2003). "W135 meningococcal carriage in association with the Hajj pilgrimage 2001: the Singapore experience." Int J Antimicrob Agents **21**(2): 112-5.

Wilder-Smith, A., T. M. Barkham, S. K. Chew and N. I. Paton (2003a). "Absence of *Neisseria meningitidis* W-135 electrophoretic Type 37 during the Hajj, 2002." Emerg Infect Dis **9**(6): 734-7.

Wilder-Smith, A., T. M. Barkham, S. Ravindran, A. Earnest and N. I. Paton (2003b). "Persistence of W135 *Neisseria meningitidis* carriage in returning Hajj pilgrims: risk for early and late transmission to household contacts." Emerg Infect Dis **9**(1): 123-6.

Wilder-Smith, A., N. I. Paton, T. M. Barkham and A. Earnest (2003). "Meningococcal carriage in Umra pilgrims returning from Saudi Arabia." J Travel Med **10**(3): 147-9.

Woods, T. C., L. O. Helsel, B. Swaminathan, W. F. Bibb, R. W. Pinner, B. G. Gellin, S. F. Collin, S. H. Waterman, M. W. Reeves, D. J. Brenner and et al. (1992). "Characterization of *Neisseria meningitidis* serogroup C by multilocus enzyme electrophoresis and ribosomal DNA restriction profiles (ribotyping)." J Clin Microbiol **30**(1): 132-7.

Yakubu, D. E., F. J. Abadi and T. H. Pennington (1994). "Molecular epidemiology of recent United Kingdom isolates of *Neisseria meningitidis* serogroup C." Epidemiol Infect **113**(1): 53-65.

Yakubu, D. E., F. J. Abadi and T. H. Pennington (1999). "Molecular typing methods for *Neisseria meningitidis*." J Med Microbiol **48**(12): 1055-64.

Yang, L., X. Zhang, J. Peng, Y. Zhu, J. Dong, J. Xu and Q. Jin (2009). "Distribution of surface-protein variants of hyperinvasive meningococci in China." J Infect **58**(5): 358-67.

Yazdankhah, S. P. and D. A. Caugant (2004). "*Neisseria meningitidis*: an overview of the carriage state." J Med Microbiol **53**(Pt 9): 821-32.

Yazdankhah, S. P., K. Kesanopoulos, G. Tzanakaki, J. Kremastinou and D. A. Caugant (2005). "Variable-number tandem repeat analysis of meningococcal isolates belonging to the sequence type 162 complex." J Clin Microbiol **43**(9): 4865-7.

Yazdankhah, S. P., P. Kriz, G. Tzanakaki, J. Kremastinou, J. Kalmusova, M. Musilek, T. Alvestad, K. A. Jolley, D. J. Wilson, N. D. McCarthy, D. A. Caugant and M. C. Maiden (2004). "Distribution of serogroups and genotypes among disease-associated and carried isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway." J Clin Microbiol **42**(11): 5146-53.

Yazdankhah, S. P., B. A. Lindstedt and D. A. Caugant (2005). "Use of variable-number tandem repeats to examine genetic diversity of *Neisseria meningitidis*." J Clin Microbiol **43**(4): 1699-705.

Young, H., A. B. Harris, D. H. Robertson and R. J. Fallon (1983). "Ano-genital gonorrhoea and pharyngeal colonisation with meningococci: a serogroup analysis." J Infect **6**(1): 49-54.

Yusuf, H. R., R. W. Rochat, W. S. Baughman, P. M. Gargiullo, B. A. Perkins, M. D. Brantley and D. S. Stephens (1999). "Maternal cigarette smoking and invasive meningococcal disease: a cohort study among young children in metropolitan Atlanta, 1989-1996." Am J Public Health **89**(5): 712-7.

Zhang, X., Z. Shao, Y. Zhu, L. Xu, X. Xu, L. W. Mayer, J. Xu and Q. Jin (2008). "Genetic characteristics of serogroup A meningococci circulating in China, 1956-2005." Clin Microbiol Infect **14**(6): 555-61.

Zhu, P., X. Hu and L. Xu (1995). "Typing Neisseria meningitidis by analysis of restriction fragment length polymorphisms in the gene encoding the class 1 outer membrane protein: application to assessment of epidemics throughout the last 4 decades in China." J Clin Microbiol **33**(2): 458-62.

9 Appendix

9.1 *N. meningitidis* carriage strain collection database

An electronic record of the carried *N. meningitidis* strain collection characterised during this study was created from the meningococcal isolate database at SHLMPRL. All results of the genogroup-specific and genogroup non-specific PCR tests, the MLST allelic profiles and STs, the *porA* VRs and capsule null locus allele sequencing were recorded onto the electronic record. This database is stored as a Microsoft Excel file named "KScott-PhD" located in the folder "Common" within the SHLMPRL network and is only accessible by SHLMPRL staff.

9.2 *N. meningitidis* carriage strain collection sequence electropherograms

At the SHLMPRL, sequence trace files were routinely archived in duplicate. One copy was archived to a remote server and one copy was archived locally onto compact disc. This was also performed for sequence trace files for all MLST alleles, *porA* VRs and *cnl* alleles obtained during this project. A paper record of the sequencing results was also maintained during the project. To allow retrospective identification of the individual trace files details of the individual MegaBACE 1000 sequencing run file (or files) which contained the individual DNA sequence electropherograms for each MLST allele, *porA* VR or *cnl* allele was recorded in the paper record. At the time of writing this paper record was located in room 8 of the SHLMPRL. The archived sequence data files and the paper record of the sequencing results can only be accessed by SHLMPRL staff.

9.3 Examples of agarose gel electrophoresis pictures for the genotypic serogrouping, *porA* and MLST PCRs

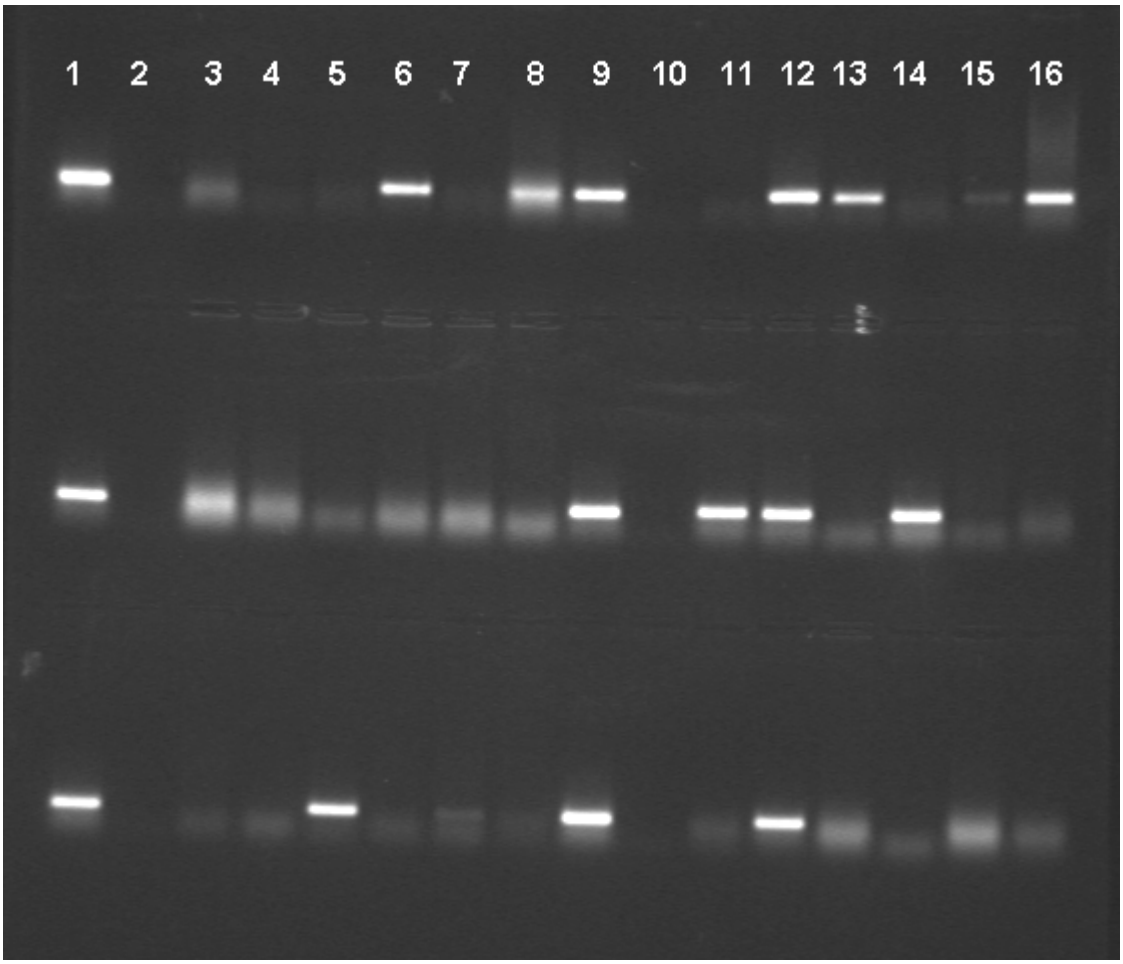

Figure 9.1 – Example of an agarose gel electrophoresis gel picture for the genotypic serogroup B (*siaD*$_B$) PCR of *N. meningitidis* strains

All rows; Lane 1: *N. meningitidis* serogroup B strain ATCC 13090 positive control, Lane 2: 18MΩ H_2O negative control, Lanes 3 – 8, *N. meningitidis* isolates; Lane 9: *N. meningitidis* serogroup B strain ATCC 13090 positive control, Lane 10: 18MΩ H_2O negative control, Lanes 11 – 16: *N. meningitidis* isolates.

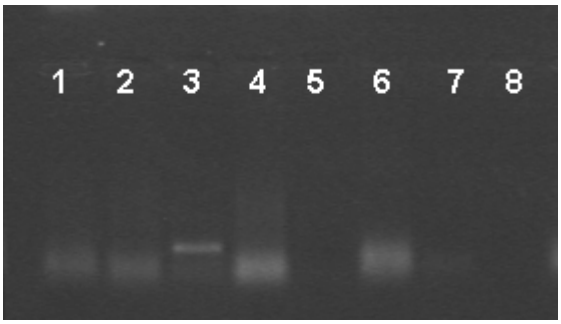

Figure 9.2 – Example of an agarose gel electrophoresis gel picture for the genotypic serogroup C (*siaD*$_C$) PCR of *N. meningitidis* strains

Lane 1: *N. meningitidis* serogroup A strain ATCC 13077, Lane 2: *N. meningitidis* serogroup B strain ATCC 13090, Lane 3: *N. meningitidis* serogroup C strain ATCC 13102, Lane 4: *N. meningitidis* serogroup W135 strain NCTC 11203, Lane 5: *N. meningitidis* serogroup Y strain ATCC 35561, Lane 6: *N. meningitidis cnl* strain GL0101366, Lane 7: 18MΩ H_2O negative control, Lane 8: *siaD*$_C$ PCR mix only.

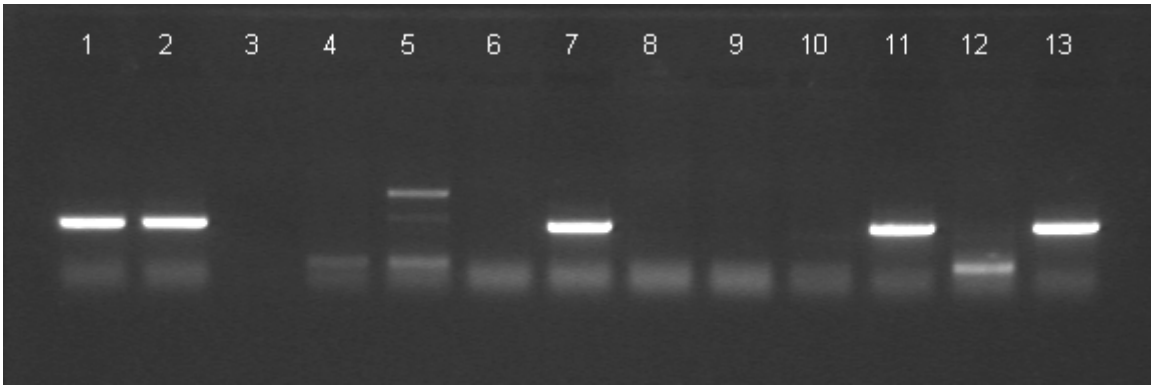

Figure 9.3 – Example of an agarose gel electrophoresis picture for the genotypic serogroup Y or W135 (*siaD*$_{Y/W135}$) PCR of *N. meningitidis* strains

Lane 1: *N. meningitidis* serogroup W135 strain NCTC 11203 positive control, Lane 2: *N. meningitidis* serogroup Y strain ATCC 35561 positive control, Lane 3: 18MΩ H_2O negative control, Lane 4: *N. meningitidis* strain 85-06321, Lane 5: *N. meningitidis* strain 85-09251, Lane 6: *N. meningitidis* strain 85-09253, Lane 7: *N. meningitidis* strain 85-09254, Lane 8: *N. meningitidis* strain 85-09260, Lane 9: *N. meningitidis* strain 85-09262, Lane 10: *N. meningitidis* strain 85-09264, Lane 11: *N. meningitidis* strain 85-09266, Lane 12: *N. meningitidis* strain 85-09274, Lane 13: *N. meningitidis* strain 85-11256.

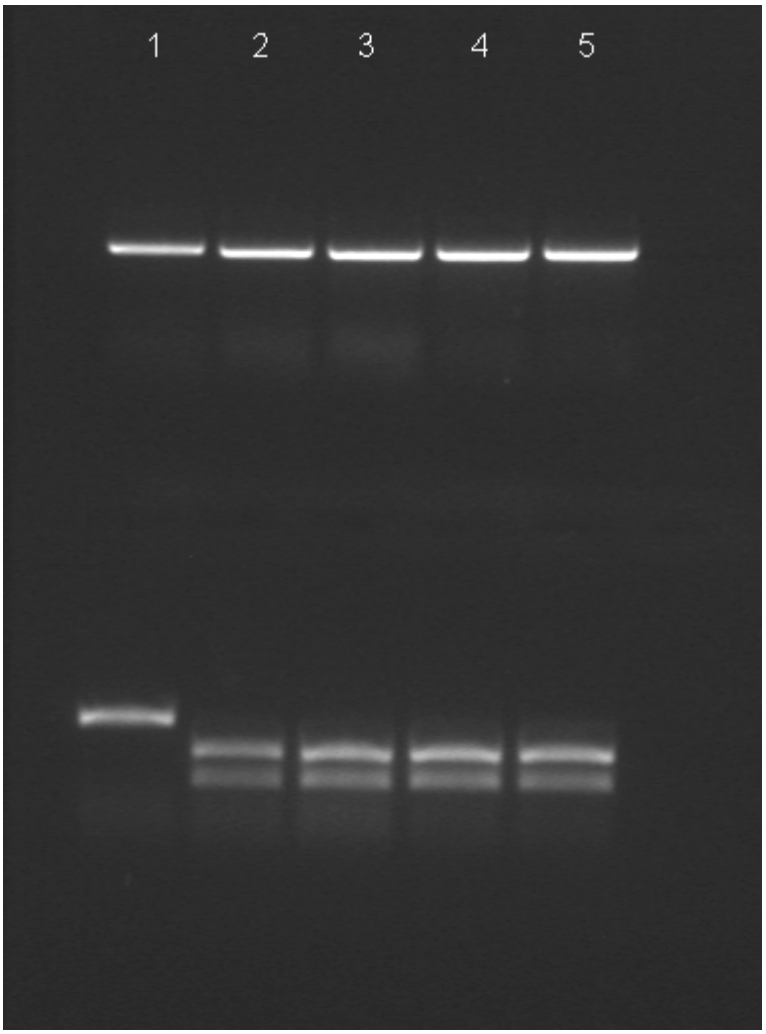

Figure 9.4 – An example of an agarose gel electrophoresis picture of *XbaI*-digested *siaD*$_{Y/W135}$ PCR-positive products

Top row: undigested *siaD* Y/W135 PCR-positive amplicons, Bottom row: *XbaI*-digested *siaD* Y/W135 PCR-positive products, Lane 1: *N. meningitidis* serogroup W135 strain NCTC 11203 (positive control), Lane 2: *N. meningitidis* serogroup Y strain ATCC 35561 (positive control), Lane 3: *N. meningitidis* strain 85-09254, Lane 4: *N. meningitidis* strain 85-09266, Lane 5: *N. meningitidis* strain 85-11256.

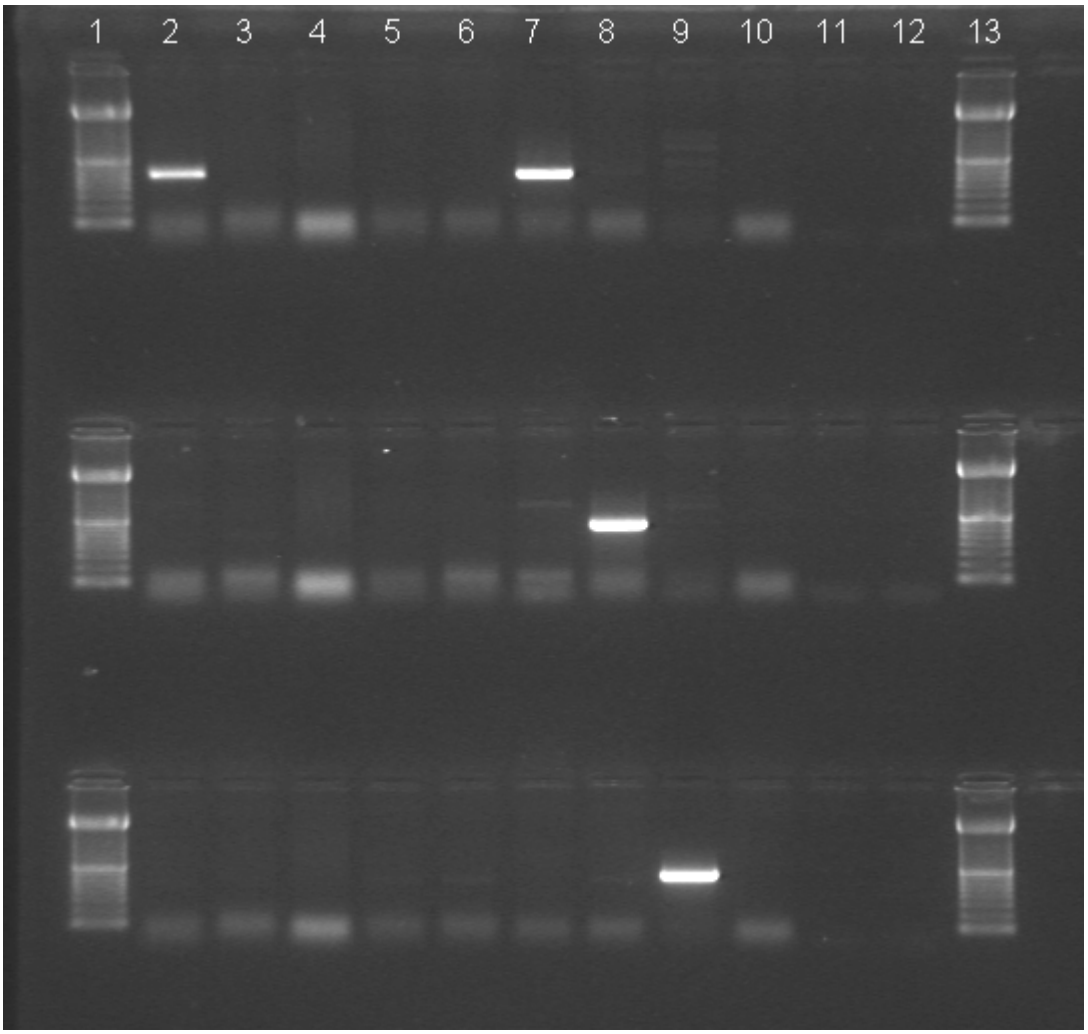

Figure 9.5 – Example of an agarose gel electrophoresis picture for the genotypic serogroup X, Z & 29E (*ctrA*$_X$, *ctrA*$_Z$ & *ctrA*$_{29E}$) PCRs of *N. meningitidis* strains

Top row: *ctrA* X PCR, Middle row: *ctrA* Z PCR, Bottom row: *ctrA* 29E PCR, Lane 1: 100bp DNA Ladder, Lane 2: *N. meningitidis* serogroup A strain ATCC 13077, Lane 3: *N. meningitidis* serogroup B strain ATCC 13090, Lane 4: *N. meningitidis* serogroup C strain ATCC 13102, Lane 5: *N. meningitidis* serogroup W135 strain NCTC 11203, Lane 6: *N. meningitidis* serogroup Y strain ATCC 35561, Lane 7: *N. meningitidis* serogroup X strain NCTC 10790, Lane 8: *N. meningitidis* serogroup Z strain NCTC 10792, Lane 9: *N. meningitidis* serogroup 29E strain NCTC 11202, Lane 10: *N. meningitidis cnl* strain GL0101366, Lane 11: *ctrA* PCR mix + 18MΩ H_2O, Lane 12: *ctrA* PCR mix only, Lane 13: 100bp DNA Ladder.

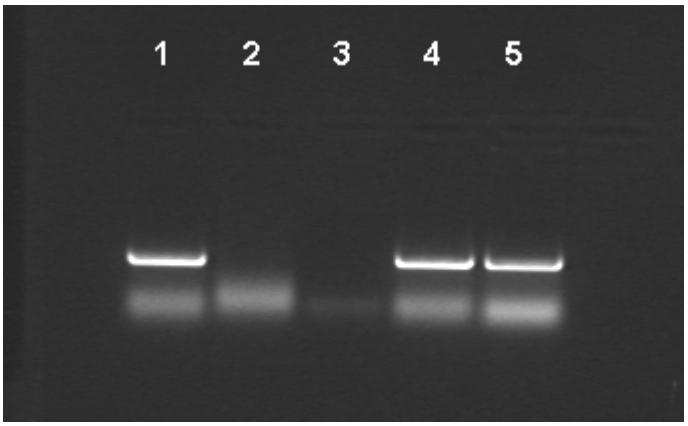

Figure 9.6 – Example of an agarose gel picture for the capsule null locus (*cnl*) PCR of *N. meningitidis* strains

Lane 1: *N. meningitidis cnl* strain GL0101366 (positive control), Lane 2: *N. meningitidis* serogroup B strain ATCC 13090 (negative control), Lane 3: 18MΩ H_2O (negative control), Lane 4: *N. meningitidis* strain 99-3792, Lane 5: *N. meningitidis* strain 02-4098.

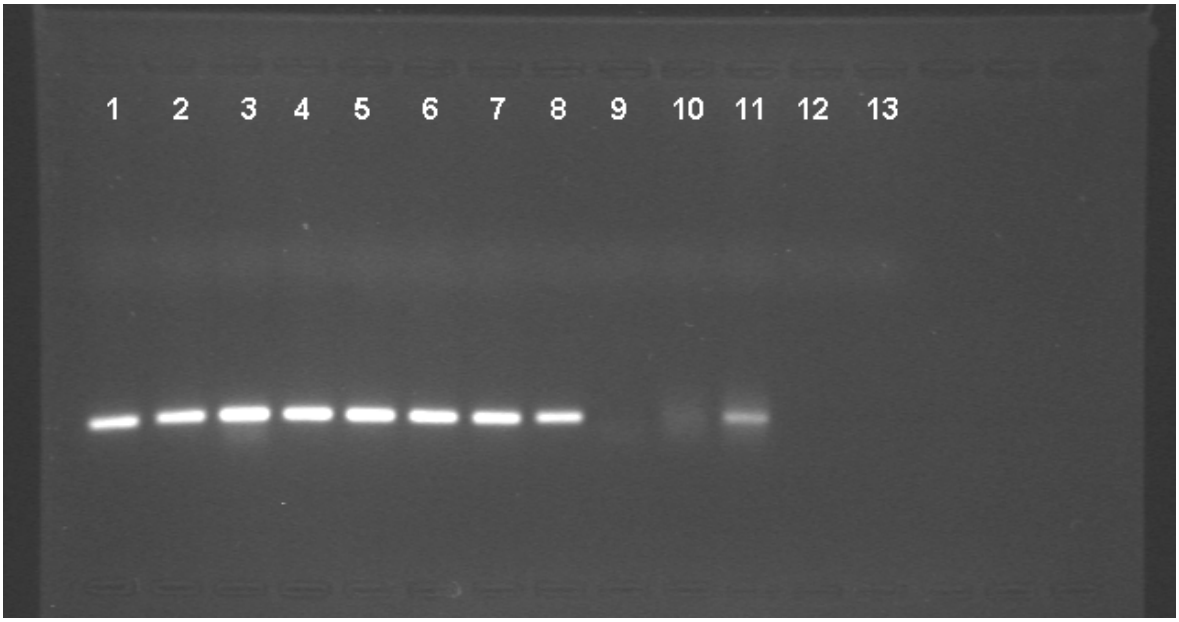

Figure 9.7 – Example of an agarose gel electrophoresis picture for the serogroup non-specific *ctrA* PCR on *N. meningitidis* strains

Lane 1: *N. meningitidis* serogroup A strain ATCC 13077, Lane 2: *N. meningitidis* serogroup B strain ATCC 13090, Lane 3: *N. meningitidis* serogroup C strain ATCC 13102, Lane 4: *N. meningitidis* serogroup W135 strain NCTC 11203, Lane 5: *N. meningitidis* serogroup Y strain ATCC 35561, Lane 6: *N. meningitidis* serogroup X strain NCTC 10790, Lane 7: *N. meningitidis* serogroup Z strain NCTC 10792, Lane 8: *N. meningitidis* serogroup 29E strain NCTC 11202, Lane 9: *N. meningitidis* cnl strain GL0101366, Lane 10: *N. meningitidis* strain 96-0628, Lane 11: *N. meningitidis* strain 97-1386, Lane 12: serogroup non-specific *ctrA* PCR mix + 18MΩ H_2O, Lane 13: serogroup non-specific *ctrA* PCR mix only.

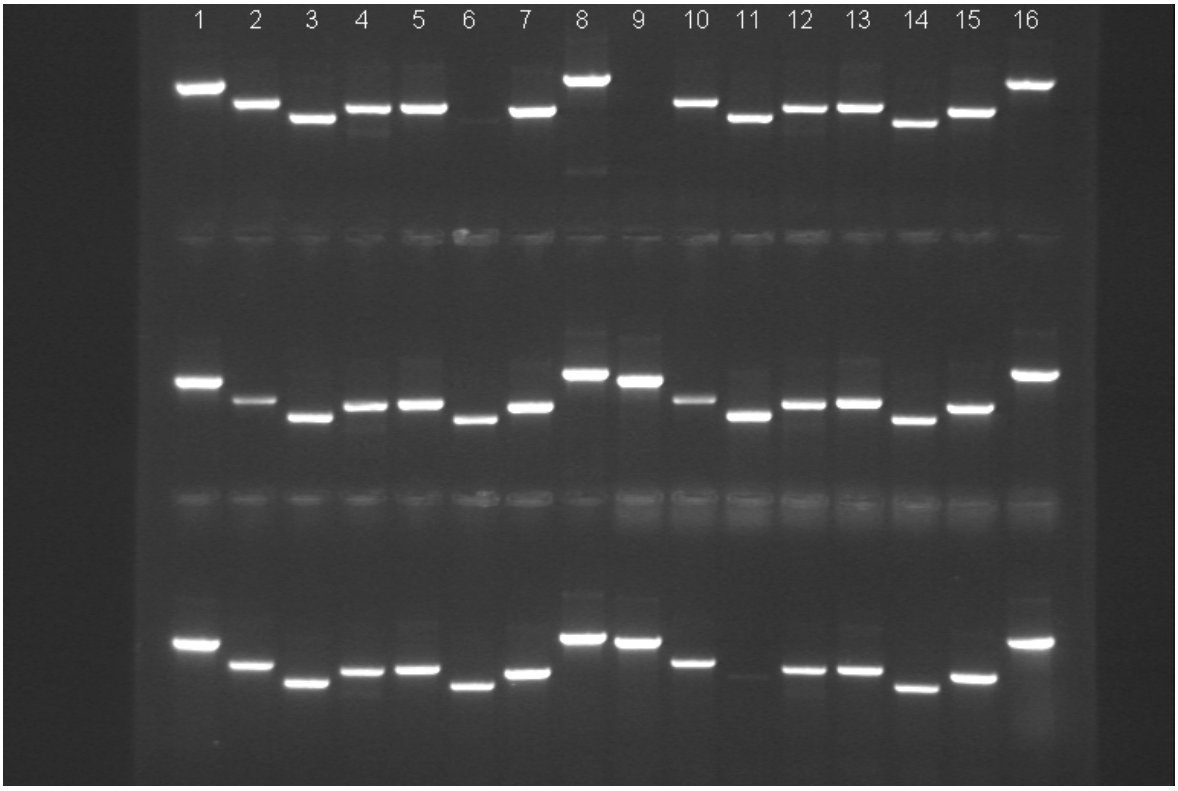

Figure 9.8 – Example of an agarose gel electrophoresis picture for the amplification of *porA* and MLST PCR products on *N. meningitidis* strains

Order of amplified gene fragments repeats every 8 lanes: *porA*, *abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC* and *pgm*, Top row Lanes 1 – 8: *N. meningitidis* serogroup A strain ATCC 13077, Top row Lanes 9 – 16: *N. meningitidis* serogroup B strain ATCC 13090, Middle row Lanes 1 – 8: *N. meningitidis* serogroup W135 strain NCTC 11203, Middle row Lanes 9 – 16: *N. meningitidis* serogroup Y strain ATCC 35561, Bottom row Lanes 1 – 8: *N. meningitidis* serogroup X strain NCTC 10790, Bottom row Lanes 9 – 16: *N. meningitidis* serogroup Z strain NCTC 10792.