Characterization of the Molecular Epidemiology of *Neisseria meningitidis* and Investigation of Two Potential Risk Factors Associated with Invasive Meningococcal Disease

by

Mark Andrew Schmidt

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Epidemiological Science)
in The University of Michigan
2009

Doctoral Committee:

Professor Janet R. Gilsdorf, Co-Chair
Professor Carl P. Simon, Co-Chair
Associate Professor Matthew L. Boulton
Assistant Professor Brenda W. Gillespie
James T. Rudrik, Michigan Department of Community Health

To Daniel

Acknowledgements

I would like to give thanks to my five committee members for their guidance through this process and, specifically, Matthew Boulton for providing me with the support to start this doctoral program and Janet Gilsdorf for leading me through its conclusion; to David Stephens, Rhonda Hobb, and Shanta Zimmer at Emory University for providing the opportunity and guidance that allowed me complete the work presented in Chapter III; to Jim Collins and Melinda Wilkins at the Michigan Department of Community Health and Ann Thomas and Paul Cieslak at the Oregon Public Health Division for their embracing of my professional development; to Rick Riolo at the University of Michigan for his technical assistance, programming expertise, and ever-willingness to help; to the Center for the Study of Complex Systems for computational resources necessary to conduct modeling experiments; to Janie Tierheimer at the Oregon State Public Health Laboratory for her PFGE work; to Mark and Ellen Sullivan for providing endless inspiration; to my parents for their unconditional love, financial support, and encouragement to follow my dreams; to Daniel Sapp, whose love, support, and sacrifice has meant the world to me; and to my family, friends, and coworkers, who've helped push me along – I could not have done this without you.

Table of Contents

List of Figures

Figure

List of Tables

Table

Abstract

The occurrence of invasive meningococcal disease (IMD) is the result of a complex interaction of host, agent, and environmental risk factors within both individuals and populations. The goal of this dissertation research was to better understand the epidemiology of its underlying organism, *Neisseria meningitidis*, and two potential risk factors associated with invasive disease.

This dissertation is comprised of three projects. The first project better characterized invasive isolates of *N. meningitidis* recovered during peak and later periods of an epidemic of serogroup B IMD in Oregon using pulsed-field gel electrophoresis. The epidemic, characterized by increased occurrence of sporadic disease, rather than localized outbreaks, appears to have been due to the introduction and spread of a new clone within the population. Through amplification and detection of an integrated bacteriophage by polymerase chain reaction, the second project investigated whether this recently-proposed virulence factor was associated with IMD among a diverse collection of isolates from the United States. As the bacteriophage was associated with serogroup, but not isolate collection source, the results suggest it represents a genetic element acquired by certain clonal strains, rather than a virulence factor required for invasive disease. In the third project, an agent-based, simulation model was developed to represent the extent to which increasing population use of broad-spectrum antibiotics among children younger than five years of age may be having an impact on the epidemiology of IMD. By reducing the prevalence of colonizing organisms, the model demonstrated that increased population

broad-spectrum antibiotic use among children younger than five years led to a lower proportion of children with acquired immunity and a higher population susceptibility to invasive disease.

The results of this dissertation research argue for increased molecular characterization of circulating strains within IMD public health laboratory surveillance programs; underscore the continued need to identify new meningococcal virulence factors; and encourage additional research into the role of increasing population broad-spectrum antibiotic use as an environmental risk factor of IMD.

Chapter I

Introduction

Impact of Invasive Meningococcal Disease

Invasive meningococcal disease (IMD) is a serious, life-threatening disease caused by the gram-negative, diplococcal bacteria, *Neisseria meningitidis*. The most common manifestations of IMD include bacterial meningitis, meningococcemia, and bacteremic pneumonia; with septic arthritis and pericarditis occurring rarely [64, 75, 94].

Globally, the most significant burden of IMD occurs in the "Meningitis Belt" of sub-Saharan Africa, where incidence rates average 10-25/100,000 and periodic epidemics have had attack rates up to 1,000/100,000 [87, 99]. In comparison, the occurrence of IMD in the United States is much lower. For the 50 years prior to 2000, the incidence of IMD in this country had been stable at 0.9 - 1.5 cases per 100,000; since then, it has decreased to a historic low of 0.3 cases per 100,000 in 2006 [18, 74, 75].

Despite its low incidence in this country, IMD is significant with respect to fatality, morbidity, disease severity and rapid progression, and population affected. The case fatality rate for IMD remains between 10-15%, despite appropriate antibiotic therapy, and death can occur within 12 hours of initial symptom onset [49]. Long-term, debilitating sequelae, such as limb amputation, hearing loss, and/or mental retardation, may afflict an additional 10-15% of survivors [27, 49, 74, 75]. The highest rates of disease occur in children less than 2 years of age, with incidence rates peaking as high as 15.9 per 100,000 in infants aged 4-5 months [74]. A second, lower incidence peak of 1.4

per 100,000 is seen among young adults 18-24 years of age and college students living in congregate settings (i.e. dormitories) are at higher risk of invasive disease [19]. For these reasons, the financial burden of IMD is also great, estimated at $1.2-$4.8 million per life lost and $10,924 to $24,030 per hospitalization [19, 81].

Molecular Epidemiology of *N. meningitidis*

Several laboratory techniques have been applied to the study of *N. meningitidis*, allowing for a better understanding of its molecular epidemiology. Determination of serogroup – the antigenic expression of polysaccharide capsule – has been the historical laboratory standard for public health surveillance programs worldwide [55]. There are 13 recognized serogroups of *N. meningitidis*, although only four – B, C, W-135, and Y – have been commonly seen among isolates recovered in the United States [19, 43, 95]. Further discrimination of strains within serogroups is achieved by describing the complete serologic phenotype, which also includes serotype and serosubtype and is commonly noted as serogroup:serotype:serosubtype. Serotype and subtype refer to the antigenic character of the class 2 or 3 – PorB – and class 1 – PorA – outer membrane proteins, respectively [32]. Multilocus enzyme electrophoresis (MLEE) and multilocus sequence typing (MLST), which classify strains by electrophoretic type (ET) and sequence type (ST), respectively, distinguish between closely-related strains (clones) and are useful in describing global and long-term epidemiological patterns, whereas pulsed-field gel electrophoresis (PFGE) and multiple-locus variable-number tandem repeat analysis (MLVA) are useful in differentiating sporadic cases from case clusters within a

limited spatiotemporal frame [28, 54, 60, 68, 82, 100, 102]. The use of multiple molecular methods can often provide complementary information [92].

Whereas invasive meningococcal isolates – those recovered from a normally sterile site, such as blood or cerebrospinal fluid – are invariably encapsulated, between one third and one half of colonization isolates – those recovered from the nasopharynx of asymptomatic carriers – may not express capsule [15, 16, 101]. Beyond these differences, colonization strains and invasive strains differ in the diversity of the bacterial populations and their ability to cause disease. Among isolates for which the serogroup can be determined, colonization strains are more diverse than invasive strains in terms of number of serogroups, serotypes, ET, and ST identified [5, 10, 16, 101]. In one study, three phenotypes – B:15:P1.7, C:2a:P1.2,5, and C:2b:P1.2,5 – found among almost 60% of IMD strains were only found among 3.2% of colonizing strains; in another, two ET complexes (ET-5 and ET-37) responsible for 91% of IMD cases, were found among only 14% of colonizing strains [5, 16]. Whereas the carrier to case ratio for serogroup B, Y, and non-ET-37 serogroup C strains is estimated to be 9,100, 12,400 and 2,300:1, respectively, that for ET-37 serogroup C strains is less than 400:1 [70].

Meningococcal Disease Epidemics

The predominant strains associated with invasive disease do not remain static over either time or geography. Periodic waves of epidemic or hyperendemic IMD have been known to occur approximately every 10 years, with associated attack rates several times higher than during interepidemic periods [33, 74, 75]. IMD epidemiology during these waves differs from that during interepidemic periods, in that incidence increases

disproportionately among adolescents and young adults and cases are due to more homogenous, clonal strains [10].

For instance, the ET-5 complex, consisting mainly of B:15 strains, emerged in Norway in 1974 and spread – causing epidemics – throughout Europe, South Africa, Cuba, and South America through the late 1970s and 1980s [14, 80]. It was only associated with sporadic cases in the United States, however, until the mid-1990's when an epidemic of IMD due to an ET-5 clone occurred in Oregon [24].

Strains from the ET-37/ST-11 complex were responsible for meningococcal outbreaks in the 1960s and 1970s and continue to represent almost all serogroup C IMD isolates, worldwide [8, 74, 89, 97]. A single, "hypervirulent" ET-37 clone, ET-15, was identified in Canada in 1986 and was responsible for an overall increase in IMD cases, an increase in serogroup C cases, and localized outbreaks across United States, Canada, and Europe through the 1990s [8, 51, 70, 97, 98]. While the majority of strains within the ET-37/ST-11 complex express the serologic phenotype C:2a:P1.2,5, B:2a:P1.2,5 and W135:2a:P1.2,5 ET-15/ST-11 strains have also been identified, attributed to the ability of genetically-identical *N. meningitidis* organisms to switch capsular expression [47, 67, 83, 90].

A dramatic increase in serogroup Y IMD occurred in several areas of the United States through the 1990s. While serogroup Y was found among less than 2% of invasive isolates in 1989-1991, this had risen to 50-80% of all isolates by 1998 [20, 46, 60, 74, 93]. As with the other serogroups, these serogroup Y isolates tend to be highly clonal, predominantly belonging to the ET-508/ST-23 complex.

The occurrence of the periodic epidemic or hyperendemic waves is believed due to the emergence and spread of a new clone through a population, against which immunity is low, although the pathogenic and transmission potentials of the emergent clones may vary on an individual basis [46, 60, 80]. C:ET-37 and Y:ET-508 strains, both responsible for a significant proportion of IMD cases, were estimated to be carried by <0.055% and 3% of the population, respectively [46]. Based on this, it was suggested that occurrence of IMD in a population could occur either through the maintenance of a low prevalence of a highly virulent organism (C:ET-37) or a high prevalence of a strain with low pathogenicity (Y:ET-508).

Risk Factors for Invasive Disease

Exposure to *N. meningitidis* is necessary, but not sufficient, to cause IMD. *N. meningitidis* is transmitted from person-to-person through respiratory droplet spread, after which the organism usually establishes asymptomatic colonization in the nasopharynx. Humans are the only reservoir for *N. meningitidis.* The overall colonization rate of *N. meningitidis* in the population has been estimated at roughly 10%, although this is highly dependent on the population structure [12, 15, 52]. For instance, in semi-closed communities, such as among military recruits, colonization rates can exceed 60% [91]. Meningococcal colonization is transient or infrequent for roughly 40% of individuals, brief (days/weeks) for about 35%, and of long duration (months) for 25% [93]. Within the population, a dynamic process of acquisition and clearance maintains constant overall colonization rates, which do not to fluctuate seasonally [5].

The mechanisms by which *N. meningitidis* invades the bloodstream and causes disease have not been well elucidated, although host, agent, and environmental risk factors have been described. A lack of protective immunity against *N. meningitidis*, as measured by serum bactericidal activity (SBA), is the significant host factor related to IMD [36, 93]. SBA has been associated with resistance to *N. meningitidis* infection in an animal model [57]. In humans, over 50% of newborns display protective levels of SBA, due to the transplacental passage of maternal anti-meningococcal antibodies to the infant [36, 37]. The level of immunity in the population wanes to a low among infants six to 12 months of age, after which point population SBA increases by roughly 5% per year of life though childhood, reaching a plateau of 65 to 85% among adults [35, 37]. This pattern is inversely correlated to IMD incidence in the population [36, 37, 53, 66, 93]. Additionally related to immune status, an increased risk of IMD has also been associated with late complement deficiency and congenital and acquired immunodeficiencies [29, 93].

Environmental factors associated with IMD include crowded living conditions, smoking, and co-circulating infectious agents. New military recruits, college students living in dormitories, and individuals of low socioeconomic status, all variables associated with crowded living conditions, demonstrate higher incidence rates of IMD [19, 36, 74, 75]. More than four people in a home has been associated with a significant adjusted odds ratio of 1.69 and a 400 to 800 fold increased risk of IMD is seen among family members to a sporadic case [4, 65]. Active and passive smoke exposures are independently associated with IMD in a dose-response relationship. For children, smoking exposure within the home demonstrates an adjusted odds ratio of 3.6-3.8 [31, 65]. Respiratory pathogens, such as influenza, which may disrupt the nasopharyngeal

epithelium, have been associated with IMD [13, 62]. Additionally, certain enteric organisms, such as *Escherichia coli* K92, express epitopes analogous to those of *N. meningitidis* and can induce circulating IgA antibodies that block anti-meningococcal bactericidal IgG antibodies [38]. Such an immunoepidemiologic model has been proposed to explain the seasonality of IMD.

The expression of polysaccharide capsule is the primary and most important meningococcal agent virulence factor due to its antibactericidal properties [85, 93]. The roles of bacterial pili, outer membrane proteins, and iron-acquisition systems have also been investigated in respect to organism virulence, as has a recently described, chromosomally integrated genetic element, termed Meningococcal Disease Associated (MDA)-island [7, 45, 78, 93, 96].

Highlighting the interaction of host, agent, and environmental factors, the incidence of IMD was highest (38.5%) among military recruits who were both deficient in protective immunity and exposed to potentially pathogenic meningococcal strains [36].

Development of Natural Anti-Meningococcal Immunity

After the waning of passive immunity after birth, natural bactericidal immunity against IMD is acquired as a natural response to colonization by *N. meningitidis* or related species, particularly *N. lactamica* [37].

N. lactamica is a purely commensal *Neisseria* species, which lacks a polysaccharide capsule but which shares other cross-reactive antigenic structures with *N. meningitidis* that induce anti-meningococcal immunity [9, 48, 61, 88]. Supporting its role in the development of broad, anti-meningococcal immunity, 40-66% of children

acquiring *N. lactamica* developed a four-fold rise in anti-meningococcal antibodies against serogroups A, B, and C and *N. lactamica* has been found to be protective against meningococcal infection in a mouse model [35, 63].

Colonization with *N. lactamica* occurs within the first few months of life and peaks between 18 and 24 months of age, when 20-25% of children may be colonized [12, 35, 42]. By age four, 59% of children have acquired *N. lactamica* at least once [35]. Consequently, as the prevalence of *N. meningitidis* among those younger than five years of age is low (0.4-2.1%), acquisition of natural immunity against IMD in this age group seems to be driven to a greater extent by colonization with *N. lactamica* than *N. meningitidis* [12, 35, 37]. After two years of age, while the prevalence of *N. lactamica* colonization decreases, the prevalence of *N. meningitidis* colonization increases with age to a peak of 24-37% in those 15-24 years of age [12, 15, 101]. Colonization with *N. meningitidis* has been shown to result in a significant increase in serum bactericidal activity, leading to protective anti-meningococcal immunity within 14 days after acquisition of the strain. While this response is strongest against the homologous meningococcal strain, cross-reactivity with heterologous strains has also been reported [37, 44, 71, 73, 101].

The main targets of bactericidal activity include polysaccharide capsule (for non-serogroup B capsular types), outer membrane protein, and lipooligosaccharide epitopes [66]. Colonization with *N. meningitidis* has also been shown to lead to mucosal immunity within the nasopharynx [73]. However, while mucosal immunity may prevent invasion of the organism into the bloodstream, it has not been shown to prevent further colonization [2, 39].

Effect of Antibiotic Use on *Neisseria* spp. Colonization

For over 30 years, a course of rifampin has been the accepted standard for antibiotic prophylaxis for close contacts to a primary IMD case [3]. Rifampin has been found to be 72-95% effective at eradicating nasopharyngeal carriage of *N. meningitidis* after the standard regimen of four doses, with colonization rates among treated individuals remaining below 20% for four weeks [3, 6, 22, 23, 34, 40, 77]. Single dose regimens of ceftriaxone, ciprofloxacin, and azithromycin are also approved for prophylactic use among close contacts and are comparable to rifampin, with 93-97% efficacy and at least two to three weeks duration of nasopharyngeal eradication [26, 34, 72, 79]. Cefotaxime and sulfonamides may also provide similar protection [21, 93].

The role of penicillin antibiotics in eradicating meningococcal carriage is less clear. Parentally-administered penicillin is the recommended treatment for IMD, although it has not been found to be effective at reducing nasopharyngeal colonization of *N. meningitidis* [11]. Ampicillin was found to reduce colonization rates among a military company to less than ten percent by the third day of treatment, a level not significantly different from that in a rifampin comparison company [40]. By the third week after treatment, however, colonization rates in the ampicillin group had rebounded and were statistically higher than those in the rifampin group. It is thought that penicillins may transiently suppress, rather than eradicate, meningococcal colonization, making its presence less likely to be detected initially after treatment [1]. Regardless, penicillin is not considered adequate for eradication of colonization and individuals receiving penicillin treatment for IMD are also recommended to receive a prophylactic course of

medication to eradicate carriage [3, 40, 69, 76]. In addition to penicillin and ampicillin, erythromycin, tetracycline, chloramphenicol, and cephalexin are also considered to be ineffective at eliminating *N. meningitidis* colonization [76]. Studies of the effect of antibiotic use explicitly on colonization with *Neisseria* species other than *N. meningitidis* have not been documented in the literature.

Antibiotic Use and Trends Among Children

Through the 1990's, the overall rate of antibiotic prescriptions in children younger than 18 years of age showed a significant decline of 8% in emergency departments, 23% in ambulatory care settings, 24-33% among children in health plans, and 40-41% in outpatient physician offices, largely due to concern over increasing antibiotic resistance in organisms and an effort to promote the judicious use of antibiotics [25, 30, 41, 50, 56, 58, 59, 86]. From 1996-2000, children from three through 35 months of age received an average of 2.2 antibiotic prescriptions per year and children from three through five years of age received an average of 1.3 [30]. Among the younger age group, roughly 25% received zero prescriptions per year, 20% received one, 15% received two, 10% received three and the remainder, roughly 30%, received four or more. This is similar to another study, in which 29.3%, 22.3%, 16.7%, and 31.7% received zero, one, two, or three or more antibiotic prescriptions in the first year of life [17]. Among children three through five years of age, roughly 35% received zero prescriptions per year, 25% received one, 15% received two, less than 10% received three, and the remainder, four or more [30].

While overall antibiotic prescriptions were decreasing in the late 1990's, changes in antibiotic prescription patterns occurred, with implications for meningococcal carriage.

Penicillins continued to constitute a majority (53-59%) of antibiotic prescriptions in 2000, although their rate of prescription had decreased by 43% since 1992 [30, 58]. Cephalosporins (i.e. cefazolin and cefapririn) and erythromycin decreased by 28% and 76%, respectively. In contrast, the use of second-generation macrolides, 75% of which is azithromycin, increased between 241% and 388% from 1992-2000 [58, 84]. Among children younger than six years of age, the second-generation macrolides comprised 8.0% of all antibiotics dispensed in 2000, 60% of which were used as an initial treatment regimen. Otitis media and upper respiratory tract infections were the most common clinical manifestations for which antibiotics were prescribed in this age group [84].

Purpose of Research

The occurrence of IMD is the result of a complex interaction of host, agent, and environmental risk factors within both individuals and populations. That over 98% of all IMD cases in the U.S. are sporadic is a testament to the success of the rapid public health response to each case – namely, the provision of antibiotic prophylaxis to close contacts [3, 74]. While this approach has been largely effective at preventing additional cases, it has done so without consideration of the epidemiology of the underlying pathogen, *N. meningitidis*. The licensure of the quadrivalent meningococcal conjugate vaccine against IMD due to serogroups A, C, Y, and W-135 and the prospect of non-polysaccharide vaccines against serogroup B disease represent first steps in developing primary public health prevention to reduce IMD burden [19]. To be most effective, however, the targets of such measures – and recommendations for their use – require a greater understanding of the dynamics of *N. meningitidis* within the population, the ability of the organism to

cause invasive disease, and factors with the potential to alter the epidemiology of the meningococcus or influence host susceptibility to invasive disease.

Through an integrated approach, the goal of this dissertation research was to achieve a greater understanding of meningococcal epidemiology and factors related to invasive disease. Exemplified by PFGE characterization of isolates from the Oregon serogroup B meningococcal disease epidemic, Chapter II aims to better describe the molecular epidemiology of *N. meningitidis*. An investigation into the association between the recently-described integrated bacteriophage genetic element and meningococcal disease isolates collected in the United States, presented in Chapter III, aims to characterize a potential, novel meningococcal virulence factor [7]. Through the development of an agent-based simulation model, Chapter IV considers if, and to what extent, increases in the population use of broad spectrum antibiotics among children younger than five years of age would impact the prevalence of colonization, development of protective immunity, and occurrence of invasive disease in this age group and, as such, whether it may be considered an increasingly important environmental risk factor for IMD.

Results of the analyses presented herein shall add to the breadth of knowledge regarding the epidemiology of invasive meningococcal disease, with relevance to public health policies and recommendations for its control.

References:

1. Abramson JS, Spika JS. Persistence of Neisseria meningitidis in the upper respiratory tract after intravenous antibiotic therapy for systemic meningococcal disease. *J Infect Dis*. 1985;151:370-1.

2. Ala'Aldeen DAA, Neal KR, Ait-Tahar K, Nguyen-Van-Tam JS, English A, Falla TF, Hawkey PM, Slack RCB. Dynamics of Meningococcal Long-Term Carriage among University Students and Their Implications for Mass Vaccination. *J Clin Microbiol*. 2000;38:2311-6.

3. American Academy of Pediatrics. Meningococcal Infections. In: Pickering LK, ed. *Red Bood: 2006 Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2006; 433-4.

4. Analysis of endemic meningococcal disease by serogroup and evaluation of chemoprophylaxis. *J Infect Dis*. 1976;134:201-4.

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

6. Beam WE, Newberg NR, Devine LF, Pierce WE, Davies JA. The Effect of Rifampin on the Nasopharyngeal Carriage of *Neisseria meningitidis* in a Military Population. *J Infect Dis*. 1971;124:39-46.

7. Bille E. Zahar JR. Perrin A. Morelle S. Kriz P. Jolley KA. Maiden MC. Dervin C. Nassif X. Tinsley CR. A chromosomally integrated bacteriophage in invasive meningococci. *J Exp Microbiol*. 2005;201:1905-13.

8. Boras A, Jeren T, Sacchi CT, Schmink S, Boxinovic D, Barsic B, Rosenstein NE, Popovic T. Establishment of an Active Laboratory-Based Surveillance for Bacterial Meningitidis in Croatia and Molecular Characterization of *Neisseria meningitidis* Isolates Causing Meningococcal Disease That Were Collected in the Year 2000, the First Year of Activity. *J Clin Microbiol*. 2004;42:1803-6.

9. Braun JM, Beuth J, Blackwell CC, Giersen S, Higgins PG, Tzanakaki G, Unverhau H, Weir DM. *Neisseria meningitidis*, *Neisseria lactamica* and *Moraxella catarrhalis* share cross-reactive carbohydrate antigens. *Vaccine*. 2004;22:898-908.

10. Broud DD, Griffiss JM, Baker CJ. Heterogeneity of Serotypes of *Neisseria meningitidis* that Cause Endemic Disease. *J Infect Dis*. 1979;140:465-70.

11. Cartwright K, Reilly S, White D, Stuart J. Early treatment with parenteral penicillin in meningococcal disease. *BMJ*. 1992;305:143-7.

12. Cartwright KA, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and Neisseria lactamica. *Epidemiol Infect*. 1987;99:591-601.

13. Cartwright KAV, Jones DM, Smith AJ, Stuart JM, Kaczmarski EB, Palmer SR. Influenza A and meningococcal disease. *Lancet*. 1991;338:554-7.

14. Caugant DA, Frøholm LO, Bøvre K, Holten E, Frasch CE, Mocca LF, Zollinger WD, Selander RK. Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease. *Proc Natl Acad Sci USA*. 1986;83:4927-31.

15. Caugant DA, Hoiby EA, Magnus P, Scheel O, Hoel T, Bjune G, Wedege E, Eng J, Froholm LO. Asymptomatic Carriage of *Neisseria meningitidis* in a Randomly Sampled Population. *Journal of Clinical Microbiology*. 1994;32:323-30.

16. Caugant DA, Kristiansen BE, Froholm LO, Bovre K, Selander RK. Clonal Diversity of *Neisseria meningitidis* from a Population of Asymptomatic Carriers. *Infect Immun*. 1988;56:2060-8.

17. Celedón JC, Litonjua AA, Ryan L, Weiss ST, Gold DR. Lack of Association between Antibiotic Use in the First Year of Life and Asthma, Allergic Rhinitis, or Eczema at Age 5 Years. *Am J Respir Crit Care Med*. 2002;166:72-5.

18. Centers for Disease Control and Prevention. 2007. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Neisseria meningitidis*, 2006. Available at: http//www.cdc.gov/ncidod/dbmd/abcs/survreports/mening00.pdf. Accessed: September 27, 2008.

19. Centers for Disease Control and Prevention. Prevention and Control of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2005;54(No. RR-7).

20. Centers for Disease Control and Prevention. Serogroup Y Meningococcal Disease – Illinois, Connecticut, and Selected Areas, United States, 1989-1996. MMWR 1996;45:1010-3.

21. Clark J, Lakshman R, Galloway A, Cant A. Does cefotaxime eradicate nasopharyngeal carriage of *N. meningitidis*. *Arch Dis Child*. 2002;87:449.

22. Deal WB, Sanders E. Efficacy of rifampin in treatment of meningococcal carriers. *New Eng J Med*. 1969;281:641-5.

23. Devine LF, Johnson DP, Hagerman CR, Pierce WE, Rhode SL, Peckinpaugh RO. Rifampin: Levels in Serum and Saliva and Effect on The Meningococcal Carrier State. *JAMA*. 1970;214:1055-9.

24. Diermayer M, Hedberg K, Hoesly F, Fischer M, Perkins B, Reeves M, Fleming D. Epidemic Serogroup B Meningococcal Disease in Oregon: The Evolving Epidemiology of the ET-5 Strain. *JAMA*. 1999;281:1493-7.

25. Dowell SF. Principles of judicious use of antimicrobial agents for pediatric upper respiratory infections. *Pediatrics*. 1998;101(Suppl 1):163-84.

26. Dworzack DL, Sanders CC, Horowitz EA, Allais JM, Sookpranee M, Sanders WE, Ferraro FM. Evaluation of Single-Dose Ciprofloxacin in the Eradication of *Neisseria meningitidis* from Nasopharyngeal Carriers. *Antimicrob Agents Chemother*. 1988;32:1740-1.

27. Edwards MS, Baker CJ. Complications and sequelae of meningococcal infections in children. *J Pediatr*. 1981;99:540-5.

28. Enright MC, Spratt BG. Multilocus sequence typing. *Trends Microbiol*. 1999;7:482-7.

29. Figueroa J, Andreoni J, Densen P. Complement deficiency states and meningococcal disease. *Immunol Res*. 1993;12:295-311.

30. Finkelstein JA, Stille C, Nordin J, Davis R, Raebel MA, Roblin D, Go AS, Smith D, Johnson CC, Kleinman K, Chan KA, Platt R

31. Fischer M, Hedberg K, Cardosi P,et al. Tobacco smoke as a risk factor for meningococcal disease. *Pediatr Infect Dis J*. 1997;16:979-83.

32. Frasch CE, Zollinger WD, Poolman JT. Serotype Antigens of *Neisseria meningitidis* and a Proposed Scheme for Designation of Serotypes. *Rev Infect Dis*. 1985;7:504-510.

33. Fraser DW, Thornsberry C, Feldman RA. Trends in meningococcal disease. *J Infect Dis*. 1972;125:443-6.

34. Girgis N, Sultan Y, Frenck R, El-Gendy A, Farid, Z, Mateczun A. Azithromycin compared with rifampin for eradication of nasopharyngeal colonization by *Neisseria meningitidis*. *Pediatr Infect Dis J*. 1998;17:816-9.

35. Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in Infants and Children. *J Infect Dis*. 1978;137:112-21.

36. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med*. 1969;129:1307-26.

37. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. *J Exp Med*. 1969;129:1327-48.

38. Griffiss JM. Epidemic Meningococcal Disease: Synthesis of a Hypothetical Immunoepidemiologic Model. *Rev Infect Dis*. 1982;4:159-72.

39. Griffiss JM. Mechanism of host immunity. In *Meningococcal Disease*, pp. 36-70. Edited by K. Cartwright. Chichester, UK: Wiley & Sons. 1995.

40. Guttler RB, Counts GW, Avent CK, Beaty HN. Effect of Rifampin and Minocycline on Meningococcal Carrier Rates. *J Infect Dis*. 1971;124:199-205.

41. Halasa NB, Griffin MR, Zhu Y, Edwards KM. Decreased number of antibiotic prescriptions in office-based settings from 1993-1999 in children less than five years of age. *Pediatr Infect Dis J*. 2002;21:1023-8.

42. Harrison LM, Morris JA, Telford DR, Brown SM, Jones K. The nasopharyngeal bacterial flora in infancy: effects of age, gender, season, viral upper respiratory tract infection and sleeping position. *FEMS Immunol Med Microbiol*. 1999;25:19-28.

43. Jackson LA, Wenger JD. Laboratory-based surveillance for meningococcal disease in selected areas, United States, 1989-1991. In: CDC surveillance summaries (June). MMWR 1993;42(no. SS-2):21-30.

44. Jones GR, Christodoulides M, Brooks JL, Miller AR, Cartwright KA, Heckels JE. 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*. 1998;178:451-9.

45. Kallstrom H, Liszewski MK, Atkinson JP, Jonsson AB. Membrane cofactor protein (MCP or CD46) is a cellular pilus receptor for pathogenic *Neisseria*. *Mol Microbiol*. 1997;25:639-47.

46. Kellerman SE, McCombs K, Ray M, Baughman W, Reeves MW, Popovic T, Rosenstein NE, Farley MM, Blake P, Stephens DS, and the Georgia Emerging Infections Program. Genotype-Specific Carriage of *Neisseria meningitidis* in Georgia Counties with Hyper- and Hyposporadic Rates of Meningococcal Disease. *J Infect Dis*. 2002;186:40-8.

47. Kertesz DA, Coulthart MB, Ryan JA, Johnson WM, Ashton FE. Serogroup B, Electrophoretic Type 15 *Neisseria meningitidis* in Canada. *J Infect Dis*. 1998;177:1754-7.

48. Kim JJ, Mandrell RE, Griffiss JM. *Neisseria lactamica* and *Neisseria meningitidis* Share Lipooligosaccharide Epitopes but Lack Common Capsular and Class 1, 2, and 3 Protein Epitopes. *Infect Immun*. 1989;57:602-8.

49. Kirsch EA, Barton RP, Kitchen L, Giroir B. Pathophysiology, Treatment and Outcome of Meningococcemia: A Review and Recent Experience. *Pediatr Infect Dis J*. 1996;15:967-79.

50. Kozyrskyj AL, Carrie AG, Mazowita GB, Lix LM, Klassen TP, Law BJ. Decrease in antibiotic use among children in the 1990s: not all antibiotics, not all children *CMAJ*. 2004;171:133-8.

51. Kremastinou J, Tzanakaki G, Kansouzidou A, Pagalis A, Danielides V, Kouppari G, Lada E, Kriz P, Musilek M, Weir DM, Blackwell CC. Recent emergence of serogroup C meningococcal disease in Greece. *FEMS Immunol Med Microbiol*. 1999;23:49-55.

52. Kremastinou J, Tzanakaki G, Levidiotou S, Markou F, Themeli E, Voyiatzi A, Psoma E, Theodoridou M, Blackwell CC. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in northern Greece. *FEMS Immunol Med Microbiol*. 2003;39:23-9.

53. Kristiansen BE, Lind KW, Mevold K, Sorensen B, Froholm LO, Bryn K, Tjade T, Bovre K. Meningococcal Phenotypic and Genotypic Characteristics and Human Antibody Levels. *J Clin Microbiol*. 1988;26:1988-92.

54. Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zerth K, Caugant DA, Feavers IM, Achtman M, Spratt BG. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA*. 1998;95:3140-5.

55. Maiden MCJ, Feavers IM. Meningococcal typing. *J Med Microbiol*. 1994;40:157-8.

56. Mainous AG 3rd, Hueston WJ, Davis MP, Pearson WS. Trends in antimicrobial prescribing for bronchitis and upper respiratory infections among adults and children. *Am J Pub Health*. 2003;93:1910-4.

57. Matsunami T, Kolmer JA. The relation of the meningococcidal activity of the blood to resistance to virulent meningococci. *J Immunol*. 1918;III:201-12.

58. McCaig LF, Besser RE, Hughes JM. Antimicrobial Drug Prescriptions in Ambulatory Care Settings, United States, 1992-2000. *Emerg Infect Dis*. 2003;9:432-7.

59. McCaig LF, Besser RE, Hughes JM. Trends in Antimicrobial Prescribing Rates for Children and Adolescents. *JAMA*. 2002;287:3096-3102.

60. McEllistrem MC, Kolano JA, Pass MA, Caugant DA, Mendelsohn AB, Pacheco AGF, Shutt KA, Razeq J, Harrison LH, and Maryland Emerging Infections Program. Correlating Epidemiologic Trends with the Genotypes Causing Meningococcal Disease, Maryland. *Emerg Infect Dis*. 2004;10:451-6.

61. Mitchell MS, Rhoden DL, King EO. Lactose-Fermenting Organisms Resembling *Neisseria meningitidis*. *J Bacteriol*. 1965;90:560.

62. Moore PS, Hierholzer J, Dewitt W, Gouan K, Djore D, Lippeveld T, Plikaytis B, Broome CV. Respiratory viruses and mycoplasma as cofactors for epidemic group A meningococcal meningitis. *JAMA*. 1990;264:1271-5.

63. Oliver KJ, Reddin KM, Bracegirdle P, Hudson MJ, Borrow R, Feavers IM, Robinson A, Cartwright K, Gorringe AR. *Neisseria lactamica* Protects against Experimental Meningococcal Infection. *Infect Immun*. 2002;70:3621-6.

64. Paret G, Keller N, Barzilai A, Zemach M, Guttman D, Vardi A, Shatzberg G, Cohen H, Barzilay Z. Invasive Meningococcal Disease: Patient and Strain Characteristics Set New Challenge for Prevention and Control. *Infection*. 1999;27:261-4.

65. Pereiró I, Díez-domingo J, Segarra L, Ballester A, Albert A, Morant A. Risk factors for invasive disease among children in Spain. *J Infect*. 2004;48:320-9.

66. Pollard AJ, Frasch C. Development of natural immunity to *Neisseria meningitidis*. *Vaccine*. 2001;19:1327-46.

67. Pollard AJ, Ochnio J, Ho M, Callaghan M, Bigham M, Dobson S. Disease Susceptibility to ST11 Complex Meningococci Bearing Serogroup C or W135 Polysaccharide Capsules, North America. *Emerg Infect Dis*. 2004;10:1812-5.

68. Popovic T, Schmink S, Rosenstein NA, Ajello GW, Reeves MW, Plikaytis B, Hunter SB, Ribot EM, Boxrud D, Tondella ML, Kim C, Noble C, Mothershed E, Besser J, Perkins BA. Evaluation of Pulsed-Field Gel Electrophoresis in Epidemiological Investigations of Meningococcal Disease Outbreaks Caused by *Neisseria meningitidis* Serogroup C. *J Clin Microbiol*. 2001;39:75-85.

69. Purcell B, Samuelsson S, Hahné SJM, Ehrhard I, Heuberger S, Camaroni I, Charlett A, Stuart JM. Effectiveness of antibiotics in preventing meningococcal disease after a case: systematic review. *BMJ*. 2004;328:1339-43.

70. Raymond NJ, Reeves M, Ajello G, Baughman W, Gheesling LL, Carlone GM, Wenger JD, Stephens DS. Molecular Epidemiology of Sporadic (Endemic) Serogroup C Meningococcal Disease. *J Infect Dis*. 1997;176:1277-84.

71. Reller LB, MacGregor RR, Beaty HN. Bactericidal antibody after colonization with *Neisseria meningitidis*. *J Infect Dis*. 1973;127:56-62.

72. Renkonen OV, Sivonen A, Visakorpi R. Effect of Ciprofloxacin on Carrier Rate of *Neisseria meningitidis* in Army Recruits in Finland. *Antimicrob Agents Chemother*. 1987;31:962-3.

73. Robinson K, Neal KR, Howard C, Stockton J, Atkinson K, Scarth E, Moran J, Robins A, Todd I, Kaczmarski E, Gray S, Muscat I, Slack R, Ala'Aldeen DAA. Characterization of humoral and cellular immune responses elicited by meningococcal carriage. *Infect Immun*. 2002;70:1301-9.

74. Rosenstein NE, Perkins BA, Stephens DS, Lefkowitz L, Cartter ML, Danila R, Cieslak P, Shutt KA, Popovic T, Schuchat A, Harrison LH, Reingold AL. The Changing Epidemiology of Meningococcal Disease in the United States, 1992-1996. *J Infect Dis*. 1999;180:1894-901.

75. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal Disease. *NEJM*. 2001;344:1378-88.

76. Sanders E, Deal WB. Prevention of meningococcal infections. *J Infect Dis*. 1970;121:449-51.

77. Schaad UB. Chemoprophylaxis for the prevention of bacterial meningitis. *Infection*. 1984; 12 Suppl 1:S65-71.

78. Schryvers AB, Stojiljkovic I. Iron acquisition systems in the pathogenic *Neisseria*. *Mol Microbiol*. 1999;32:1117-23.

79. Schwartz B, Al-Tobaiqi A, Al-Ruwais A, Fontaine RE, A'Ashi J, Hightower AW, Broome CV, Music SI. Comparative Efficacy of Ceftriaxone and Rifampicin in Eradicating Pharyngeal Carriage of Group A *Neisseria meningitidis*. *Lancet*. 1988;331:1239-42.

80. Schwartz B, Moore PS, Broome CV. Global Epidemiology of Meningococcal Disease. *Clin Microbiol Rev*. 1989;2(Supp);S118-24.

81. Scott RD 2nd, Meltzer MI, Erickson LJ, De Wals P, Rosenstein NE. Vaccinating first-year college students living in dormitories for Meningococcal disease: an economic analysis. *Am J Prev Med*. 2002;23:98-105.

82. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of Multilocus Enzyme Electrophoresis for Bacterial Population Genetics and Systematics. Appl Environ Microbiol. 1986;51:873-84.

83. Stefanelli P, Fazio C, Neri A, Sofia T, Mastrantonio P. First Report of Capsule Replacement among Electrophoretic Type 37 *Neisseria meningitidis* Strains in Italy. *J Clin Microbiol*. 2003;41:5783-6.

84. Stille CJ, Andrade SE, Huang SS, Nordin J, Raebel MA, Go AS, Chan KA, Finkelstein JA. Increased Use of Second-Generation Macrolide Antibiotics in Children in Nine Health Plans in the United States. *Pediatrics*. 2004;114:1206-11.

85. Taha MK, Deghmane AE, Antignac A, Zarantonelli ML, Larribe M, Alonso JM. The duality of virulence and transmissibility in *Neisseria meningitidis*. *Trends Microbiol*. 2002;10:376-82.

86. Thorpe JM, Smith SR, Trygstad TK. Trends in Emergency Department Antibiotic Prescribing for Acute Respiratory Tract Infections. *Ann Pharmacother*. 2004;38:928-35.

87. Tikhomirov E, Santamaria M, Esteves K. Meningococcal disease: public health burden and control. *Wld Hlth Statist. Quart*. 1997;50.

88. Troncoso G, Sánchez S, Criado MT, Ferreirós C. Analysis of *Moraxella catarrhalis* outer membrane antigens cross-reactive with *Neisseria meningitidis* and *Neisseria lactamica*. *FEMS Imm Med Microbiol*. 2004;40:89-94.

89. Tsang RSW, Squires SG, Tam TWS. Characterization of *Neisseria meningitidis* strains isolated from invasive meningococcal disease cases in Canada in 2001. *Can J Microbiol*. 2003;49:633-8.

90. Tyler S, Tsang R. Genetic analysis of Canadian isolates of C:2a:P1.2,5 and B:2a:P1.2,5 *Neisseria meningitidis* strains belonging to the hypervirulent clone of ET-15. *Can J Microbioli*. 2004;50:433-43.

91. Tyski S, Grzybowska W, Dulny G, Berthelsen L, Lind I. Phenotypical and genotypical characterization of *Neisseria meningitidis* carrier strains isolated from Polish recruits in 1998. *Eur J Clin Microbiol Infect Dis*. 2001;20:350-3.

92. Tzanakaki G, Urwin R, Musilek M, Kriz P, Kremastinou J, Pangalis A, Blackwell CC, Maiden MC. Phenotypic and genotypic approaches to characterization of isolates of *Neisseria meningitidis* from patients and their close family contacts. *J Clin Microbiol*. 2001;39:1235-40.

93. Tzeng YL, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microb Infect*. 2000;2:687-700.

94. van Duren M, Brandtzaeg P, van der Meer, JWM. Update on Meningococcal Disease with Emphasis on Pathogenesis and Clinical Management. *Clin Micro Rev*. 2000;13:144-66.

95. Vedros NA. Development of meningococcal serogroups. In Evolution of meningococcal disease. *Edited by*: NA Vedros. CRC Press, Boca Raton, FL. Pp. 33-37. 1987002E.

96. Vogel U, Frosch M. Mechanisms of neisserial serum resistance. *Mol Microbiol*. 1999;32:1133-9.

97. Wang JF, Caugant DA, Morelli G, Koumaré B, Achtman M. Antigenic and Epidemiologic Properties of the ET-37 Complex of *Neisseria meningitidis*. *J Infect Dis*. 1993;167:1320-9.

98. Whalen CM, Hockin JC, Ryan A, Ashton F. The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992. Emergence of a virulent clone of *Neisseria meningitidis*. *JAMA*. 1995;273:390-4.

99. World Health Organization Working Group. Control of epidemic meningococcal diseases: WHO practical guidelines. Lyon, France: Edition Foundation Marcel Merieux, 1995.

100. Yakubu DE, Abadi FJR, Pennington TH. Molecular typing methods for *Neisseria meningitidis*. *J Med Microbiol*. 1999;48:1055-64.

101. Yazdankhah SP, Kriz P, Tzanakaki G, Kremastinou J, Kalmusova J, Musilek M, Alvestad T, Jolley KA, Wilson DJ, McCarthy ND, Caugant DA, Maiden MCJ. Distribution of Serogroups and Genotypes among Disease-Associated and Carried Isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *J Clin Microbiol*. 2004;42:5146-53.

102. Zerouali K, Castelli P, Van Looveren M, El Mdaghri N, Boudouma M, Benbachir M, Nicolas P. Use of multilocus sequence typing and pulsed-field gel electrophoresis for the study of serogroup B *Neisseria meningitidis* isolates from Casablanca (Morocco). *Path Biol*. 2006;54:166-70.

Chapter II

Pulsed-Field Gel Electrophoresis Evaluation of the Oregon Serogroup B Meningococcal Epidemic

Introduction

Invasive meningococcal disease (IMD), the most common clinical manifestations of which include meningitis, meningococcemia, and bacteremic pneumonia, is a serious, life-threatening disease caused by the gram-negative, diplococcal bacteria, *Neisseria meningitidis* [27, 33, 45]. Despite appropriate antibiotic therapy, 10 to 15% of IMD cases are fatal and long-term, debilitating sequelae, such as limb amputation, hearing loss, and/or mental retardation, afflict an additional 10-15% of survivors [10, 21, 32, 33]. Until 2000, the overall U.S. annual incidence rate of sporadic IMD – comprising 98% of all cases – had remained stable for over 50 years at 0.9-1.5 cases per 100,000 persons, with periods of hyperendemic or epidemic IMD occurring in roughly 10-year cycles, characterized by attack rates several times higher [14, 32, 33].

Isolates of *N. meningitidis* are typically defined by serogroup, the antigenic expression of polysaccharide capsule by the organism. Although 13 serogroups have been identified globally, only four have been commonly seen among meningococcal isolates in the U.S.: B, C, W-135, and Y [8, 19, 46]. Serogroup determination, as the historical standard for laboratory-based meningococcal surveillance programs, has been useful in describing broad patterns of disease, supporting the epidemiologic identification of potential clusters of IMD, and guiding appropriate public health interventions in response

to IMD outbreaks [8]. However, as serogroup does not fully capture the genotypic and phenotypic diversity of the organism, other laboratory techniques have been developed to better elucidate its molecular epidemiology [24].

The full serologic profile (serogroup:serotype:serosubtype), the multilocus enzyme electrophoresis (MLEE)-derived electrophoretic type (ET), and the multilocus sequence typing (MLST)-derived sequence type (ST) have been used to describe global epidemiological patterns of clonal strains over periods of years and decades [11, 15, 23, 36, 48, 50]. For example, serogroup B, ET-5/ST-32 complex strains emerged in Norway in 1974 and spread – causing epidemics – throughout Europe, South Africa, Cuba, and South America through the late 1970s and 1980s; the ET-37/ST-11 complex of serogroup C strains were responsible for meningococcal outbreaks in the 1960s, 1970s, and 1990s, and still constitute the majority of serogroup C IMD cases; and a dramatic increase in serogroup Y IMD, due to the highly clonal ET-508/ST-23 complex, occurred in several areas of the United States through the 1990s [4, 6, 20, 22, 26, 31, 32, 35, 42, 44, 47, 49].

The ability of these techniques to discern between strains on a global, long-term scale is based on their respective characterization of highly-conserved, and therefore relatively stable, phenotypic traits (serologic profile and ET) or genotypic regions (ST) [23, 36, 48]. The three techniques produce highly concordant results, although MLST has become the preferred typing method due to its low cost and high degree of comparability between laboratories [23, 37]. Pulsed-field gel electrophoresis (PFGE), on the other hand, relies on the presence of certain genetic sequences present throughout the bacterial chromosome, including within regions more susceptible to variation through mutation or transformation and recombination, common events for the meningococcus [13, 40].

Consequently, changes in PFGE patterns can occur much more rapidly (i.e. weeks and months). Although some concordance between PFGE and the other strain typing techniques has been shown in the context of meningococcal epidemics, this technique is better adept at – and therefore more commonly use for – discriminating between sporadic IMD cases and epidemiologically linked cases clustering within defined communities over shorter time frames [26, 28, 51, 52]. As they reveal different aspects of the molecular epidemiology of *N. meningitidis*, the use of both global strain typing techniques and PFGE can provide complementary information on meningococcal strains circulating in an area [43].

In addition to overall higher occurrence, the epidemiology of IMD during periodic waves of epidemic or hyperendemic disease differs from sporadic cases occurring during interepidemic periods, in that incidence increases disproportionately among adolescents and young adults and cases are due to more homogenous, clonal strains [5]. Serogroup B IMD occurrence in Oregon in the mid-1990s fit this epidemic profile: the overall rate of serogroup B IMD doubled from the 1989-1992 baseline rate; that among individuals 15-19 years of age was 13 times higher than baseline; and 89% of all isolates tested by MLEE were ET-5 complex strains [9]. While this high proportion of isolates sharing one ET designation is consistent with the hypothesis that IMD epidemics are due to the emergence and spread of a clonal strain through a non-immune population, it is insufficient evidence to conclude that the Oregon serogroup B epidemic was the direct result of the same phenomenon, with regard to ET-301 [26, 35].

Through the use of PFGE, the goal of this research was to better characterize the Oregon serogroup B meningococcal disease epidemic. Our aims were to demonstrate the

concordance of PFGE with previous strain typing techniques in identifying the circulating epidemic clonal strain; to assess whether the PFGE profile of isolates recovered from cases occurring during a later epidemic period was more diverse than that of isolates recovered from cases occurring during the peak epidemic, thus supporting the hypothesis that the epidemic was due to the spread of a clonal strain in the population; and to describe the relatedness of PFGE patterns from cases with known epidemiological links and those occurring within a narrow spatiotemporal frame.

Materials and Methods

Meningococcal Strains and Study Population

All clinical laboratories in the state are required to submit specimens of *Neisseria meningitidis* isolated from a normally sterile site to the Oregon State Public Health Laboratory (OSPHL) (Oregon Administrative Rules [OARS] 333-018-0018). Upon receipt of isolates, OSPHL determines serogroup through slide agglutination, using standard methods [48]. While typing of meningococcal isolates beyond serogroup is not routinely performed by OSPHL, it had been done on all viable isolates to describe the nature of the circulating strains within specific time frames, as special project funding became available. Specifically, during the peak of the epidemic (January 1, 1994 through December 31, 1996) strain typing was performed using a combination of MLEE and serologic typing and subtyping; during a later epidemic period (January 1, 2001 through December 31, 2003), typing was performed by MLST [11, 17, 23, 36]. Demonstrating the concordance between MLEE and MLST, 100% of a sample of isolates tested from the peak of the Oregon serogroup B epidemic were ET-301, ST-32, and B:15:P1.7,16 [16].

Overall, 207 peak period and 111 later epidemic period serogroup B meningococcal isolates had been previously collected and archived at OSPHL. Of these, further strain type characterization had been completed on 151 (73%) peak and 96 (86%) later epidemic period isolates, for a total of 247 isolates eligible for inclusion in this study.

For these analyses, the "epidemic clone" was defined as serogroup B isolates, known to be ET-5/ST-32 complex, by MLEE or MLST, or B:15:P1.7,16, by serologic typing; all other MLEE, MLST, and phenotypic designations were considered as "non-epidemic clone."

PFGE

PFGE was conducted on eligible isolates using a standardized protocol. Briefly, plugs were made by suspending bacterial subculture (incubated overnight at 37^{o}C on a TSA-5% sheep blood agar plate) into 100 mM Tris: 100mM EDTA buffer and mixing with 1% SeaKem Gold / 1% sodium dodecyl sulfate agarose solution (10 mM Tris: 1 mM EDTA) and Proteinase K (2%). Cell lysis was done within the plugs using 50 mM Tris: 50 mM EDTA + 1% sodium lauroyl sarconsine buffer and 2% Proteinase K in a 54^{o}C water bath for 1.5-2 hours. Plugs were then washed in 10 mM: 1 mM EDTA buffer in a 56^{o}C water bath. Enzyme restriction digestion of bacterial DNA was done by mixing plug slices with 170 uL sterile water, 20 uL 10x restriction buffer, and 50 U *Nhe* I (New England Biolabs, Beverly, MA) and incubating at 37^{o}C for 1.5-2 hours. The resultant restriction cleavage products were resolved on a 1% gel with switch times of 2.2 – 35 seconds for 18 hours at 14^{o}C, at a 120 degree angle, and at 6.0 volts. Subsequently, the

gel was stained using ethidium bromide and digitized using a Bio-Rad Gel Doc 2000 system (Bio-Rad, Hercules, CA). Dendograms were created using the unweighted pair group method using arithmetic averages and a position tolerance of 1.5% by the BioNumerics software (Bio-Rad, Hercules, CA). The percent similarity was calculated for each dendogram.

Isolates with PFGE patterns demonstrating no band differences (100% similarity) were defined as indistinguishable and shared the same, arbitrary PFGE pattern number; isolates with PFGE patterns with one to three band differences from the predominant pattern were considered "probably-related strains"; isolates with PFGE patterns with four to six band differences from the predominant pattern were considered "possibly-related strains," and isolates with PFGE patterns with greater than six band differences were considered unrelated strains [41]. The predominant PFGE group, "Group I," was defined by isolates with PFGE patterns demonstrating ≥ 80% similarity, correlating to fewer than seven band differences (unpublished data).

Demographic and Clinical Information

In addition to the submission of meningococcal isolates from clinical laboratories to OSPHL, physicians are required to report IMD cases to the local health department through communicable disease reporting rules (OARS 333-018-0015). Oregon has also participated in active IMD surveillance through the Active Bacterial Core surveillance (ABCs) component of the Centers for Disease Control and Prevention Emerging Infections Program since 1995. For all isolates included in this study, demographic information (age, sex, street address, and city and county of residence) was obtained from

either the communicable disease reporting database (1994 isolates) or the ABCs database (1995-1996; 2001-2003 isolates). An additional data element noting the presence of an epidemiological link to another confirmed or presumptive case was obtained from the communicable disease reporting database for all isolates studied, as this information was not available in the ABCs database.

Epidemiologically-linked Cases and Spatiotemporal Clusters

We identified multiple cases with matching street address and city of residence; multiple cases with matching last name; and cases for whom the value of the epidemiological link variable in the communicable disease reporting database was 'Yes' from among serogroup B cases reported during the peak and later time periods. We manually reviewed the individual records of all identified cases to determine whether they were epidemiologically linked. Cases in this study were defined as having an epidemiological link if they shared a matching street address and city of residence; or if they were noted to have had an epidemiological link with another IMD case or had matching last names AND information in the communicable disease records unambiguously identified a link with another case included in this study (i.e. case identification number or linked-case name).

Spatiotemporal clusters were defined as the occurrence of two or more serogroup B IMD cases in a county, with isolate culture dates between successive cases less than or equal to 21 days apart. Although this is more sensitive to the detection of clusters than that described by the Centers for Disease Control and Prevention, we chose this time interval – twice the length of the maximum and five times the length of the mean

incubation period – to capture cases that may be associated within several intermediate contacts [8, 18]. While a formal evaluation of the Oregon IMD surveillance system has not been done, investigative efforts during the peak of the serogroup B epidemic and participation in ABCs, which requires active surveillance for disease through clinical laboratories, has resulted in a high sensitivity for IMD surveillance in Oregon: isolates were submitted to OSPHL for 207/211 (98%) and 111/111 (100%) of serogroup B IMD cases reported during the peak and later epidemic periods, respectively.

All epidemiologically-linked case isolates and all isolates occurring within spatiotemporal clusters were analyzed by PFGE, regardless of whether or not previous strain typing had been performed.

Data Analysis

The overall similarity of the PFGE dendogram, cophrenetic coefficient, and pairwise similarities of isolates were calculated by BioNumerics software (Version 3.2; Bio-Rad, Hercules, CA). Statistical analyses were performed using SAS (Version 9.1; SAS Institute; Cary, NC).

Differences in the proportions of the predominant indistinguishable PFGE pattern; probably-, possibly-, and unrelated strains; and PFGE groups, by epidemic period (peak versus later), and PFGE groups by age group (<15 years, 15-24 years, and ≥25 years) and strain type (epidemic versus unrelated) were assessed through the chi-squared test. Pairwise similarity of isolates was calculated for each comparison group, with the predominant PFGE pattern, NEME-003, used as the referent strain. The median and 25th and 75th percentiles for groups were presented; differences in the distributions of these

pairwise similarities between groups were assessed through the Kruskal-Wallis rank sum test. Difference in the distribution of ages and number of band differences from the predominant PFGE pattern, by epidemic period, were assessed through the Wilcoxon signed-rank test.

Results

PFGE was completed on 221/247 (89%) eligible isolates for which strain typing information was available, including 129/151 peak period isolates (85%) and 92/96 later period isolates (96%) (p=0.0094). The remaining 26 isolates were no longer available or were non-viable at the time of this study. The age distribution of isolates for which PFGE was completed did not differ from that of cases not included in this study (p=0.84).

Results and select demographic characteristics of the 221 completed isolates from the peak and later epidemic periods are shown in Table 2-1 and Figure 2-1. This population included 61 distinguishable PFGE patterns, with an overall dendogram similarity of 50.3% and a cophrenetic coefficient of 95% (Figure 2-1). The predominant pattern, NEME-003, was seen among 82 isolates (37%), followed by NEME-005 (23 isolates, 10%); NEME-006 (18 isolates, 8%); NEME-039 (10 isolates, 5%) and NEME-025 (7 isolates, 3%). Sixteen patterns were shared by five or fewer isolates each and 40 patterns were unique. One hundred eighty seven isolates (85%) comprised the predominant PFGE group, Group I. As no more than five of the remaining 34 isolates grouped together, analysis of other PFGE groups was not performed. PFGE Group I isolates had a higher pairwise similarity (median: 95.7%, [25th%-ile: 90.9%, 75th%-ile: 100%]) than did non-Group I isolates (61.2% [57.1%, 72.7%]) (p<0.0001). One hundred

thirty one isolates (59%) were cultured from individuals less than 15 years of age, compared with 45 (20%) each among those 15-24 and 25 and older. The proportion of isolates identified as Group I and the pairwise similarity of Group I isolates were not significantly different between the age groups (p=0.14 and p=0.29).

Of the 221 total isolates analyzed, 185 (83.7%) identified as PFGE Group I/epidemic clone and 24 (10.9%) identified as non-Group I/non-epidemic clone for an observed agreement between PFGE and previous strain typing methods of 94.6% (Kappa=0.77; p<0.0001) (Table 2-2). For the 12 remaining isolates, ten identified as the epidemic clone but did not segregate into PFGE Group I and two identified as PFGE Group I, but did not carry the markers used to identify the epidemic clone.

During the peak of the epidemic, 31 distinguishable patterns were identified among the 129 isolates, compared to 40 distinguishable patterns identified among the 92 later epidemic period isolates. Only the predominant PFGE pattern, NEME-003, significantly differed between epidemic periods: 48% of isolates during the peak period and 22% during the late period (p<0.0001) shared this pattern. The proportion of probably-related strains did not change between the peak (41%) and later (47%) periods (p=0.4); the proportion of possibly-related strains increased from 2% to 9% (p=0.03); and the proportion of unrelated strains increased from 9% to 23%, p=0.0029. The proportion of those isolates classified as Group I decreased from 89% to 78% (p=0.027), the pairwise similarity of Group I isolates decreased from 100.0% (91.7%, 100.0%) to 91.7% (87.0%, 95.7%) (p=<0.0001), and the median number of band differences from the predominant PFGE pattern increased from one to three (p<0.0001) from the peak to the later epidemic period (Table 2-3). In contrast, the pairwise similarity of non-Group I

isolates did not significantly change p=0.44. This overall trend was seen within each of the three age groups, as well, with a decreasing proportion of Group I isolates (albeit non-significant) and decreasing similarity of Group I isolates between the peak and later epidemic periods.

We identified three pairs of cases with an epidemiological link (EL) and 25 spatiotemporal clusters (ST) with no epidemiological link (Table 2-4). Two of the three EL pairs were household contacts and one was identified through matching last names, with case notes indicating 'secondary case' due to a non-household contact. Isolates for the two household pairs, EL-1 and EL-2, were collected one and two days apart, respectively, and shared indistinguishable PFGE patterns. Isolates for the third pair, EL-3, were collected 114 days apart and were distinguishable by PFGE. Of the 25 unlinked ST clusters, four (16%) consisted of isolates sharing identical ET/ST and PFGE patterns; four (16%) consisted of isolates sharing an identical PFGE pattern with no ET/ST information available; and 17 (68%) consisted of isolates distinguishable by ET (1), PFGE (12), or both (4). Of the eight ST clusters consisting of isolates with indistinguishable PFGE patterns (17 total isolates), four were comprised of isolates identified as NEME-003 (nine isolates, 53%), three were comprised of isolates identified as NEME-005 (six isolates, 33%), and one was comprised of isolates identified as NEME-016 (two isolates, 11%).

Discussion

These results clearly demonstrate the concordance of PFGE with other strain typing methods in identifying the circulating serogroup B epidemic strain in Oregon;

provide further evidence that IMD epidemic in the mid-1990s was the result of the spread of a clonal strain within the population; and suggest that localized clusters of IMD were due to hyperendemic, sporadic cases, rather than community outbreaks.

In this study, PFGE and strain typing by MLEE, MLST, or phenotypic profile, differentiated between the circulating epidemic clonal strain and non-epidemic strains with an observed agreement of 95%. While a previous study noted a correlation between PFGE and MLST when analyzing hyperendemic meningococcal disease due to serogroups C and Y, this is the first such study to find similar results among serogroup B isolates [26]. Discordant results for the remaining five percent of isolates, including the greater number of epidemic clone isolates failing to identify as PFGE Group I than vice-versa, were likely due to the differences in the molecular targets used for classification among the techniques and their respective sensitivity to genetic variation [13, 23, 36, 48, 40].

McEllistrem et al. also found a significant difference in the proportion of clonal strains and degree of similarity among isolates by age group [26]. While the same pattern was seen in our results, that this association was not statistically significant was likely due to a higher proportion of our isolates identified as the predominant PFGE clonal group and a greater degree of similarity of our clonal isolates than among the 74 serogroup C strains analyzed in that study.

Meningococcal epidemics due to the introduction of a new clone into the population manifest as the identification of a large proportion of clonal isolates early in the epidemic [5, 26]. The exchange of genetic material between the epidemic strain and other circulating *Neisseria* species, mainly through transformation and recombination,

subsequently lead to an increased diversity of the bacterial population over the course of the epidemic [7, 13, 25]. Our results, demonstrating the high proportion (89%) and similarity (98%) of Group I isolates during the peak epidemic period, followed by an increase in the number of distinguishable PFGE patterns, decrease in the proportions of NEME-003 and PFGE Group I isolates, increase in the proportions of possibly-related and unrelated strains, and decrease in the similarity of Group I isolates between the peak and later epidemic periods are consistent with this profile and support the hypothesis that the serogroup B meningococcal epidemic was due to the emergence of this epidemic clone in Oregon.

The highly clonal nature of serogroup B strains found in our study is atypical of *N. meningitidis* epidemiology. Transformation and recombination affects serogroup B isolates to a greater extent than isolates from other serogroups [12, 13, 30, 39]. Consequently, serogroup B isolates are generally panmictic and dominant serogroup B clones seen during hyperendemic periods are more unstable than are those of other serogroups [3, 7, 25, 52]. This manifests as a greater number of less similar strain types and/or PFGE patterns among serogroup B isolates than comparable analyses of serogroup C or Y isolates during both epidemic and interepidemic periods [1, 28, 51, 52]. The diversity of serogroup B isolates extends to strains sharing the same ET designation and has been shown specifically for ET-5 complex strains [6, 25, 38]. In this study, the panmictic nature of the non-Group I isolates is demonstrated by the near parity in the number of isolates to number of distinguishable PFGE patterns and by the low degree of similarity between isolates that does not change over time.

Within its broad context, the profile of IMD epidemics and hyperendemic periods is affected by its localized transmission dynamics within the context of the immune status of the population. For instance, epidemics of serogroup C IMD, largely due to the emergence of a hypervirulent ST-11/ET-37 strain, have been marked by community clusters of disease with isolates demonstrating indistinguishable PFGE patterns [2, 31, 34, 42, 49]. Conversely, hyperendemic occurrence of serogroup Y disease seems to be driven to a greater extent by the higher community prevalence of strains, manifesting in more diverse isolate patterns and without associated clustering [8, 20, 29]. Our results show that the Oregon serogroup B epidemic is more similar to that due to serogroup Y than that due to serogroup C. The majority of clustered isolates analyzed were distinguishable by one or more molecular methods, demonstrating that disease was largely due to an increased number of sporadic cases rather than an increase in localized community outbreaks. While previously unascertained community associations cannot be ruled out, in only one cluster – that in which isolates shared the rare PFGE pattern, NEME-016 – is such a link highly suggested. The nature of the remaining clusters, associated with the most common patterns identified, cannot be fully determined. These results lend further support to our hypothesis of the spread of an epidemic clone through an immunologically naïve population. Localized, community outbreaks of IMD from which isolates with indistinguishable PFGE patterns were recovered would be expected with the introduction of a strain into small pockets of susceptible individuals within the context of an otherwise immune population; hyperendemic, sporadic disease due to distinguishable isolates, as seen in our results, would be expected with general, widespread circulation of strains in a largely susceptible population.

The main limitation of this study was that proportion of eligible isolates analyzed by PFGE from the later epidemic period (96%) was higher than that from the peak epidemic period (85%, p=0.0094), potentially introducing bias into our results. For instance, if all unanalyzed isolates had been identified as PFGE non-Group I, the decrease in the proportion of Group I isolates from the peak to the later epidemic would no longer have been significant (p=0.84). Additionally, that no isolates from prior to the peak of the epidemic were available for analysis prevents us from describing the distribution of strains during that time or definitively concluding if and when the epidemic clone was introduced into the population. As our results for the pairwise similarity of Group I isolates would not have been affected by these limitations, however, the high degree of similarity of Group I isolates, compared to non-Group I isolates, and the increasing diversity of Group I isolates over time are most consistent with this theory. Other hypotheses, such as an increased virulence of strains – even if Group I strains had been circulating earlier – would likely not have resulted in this same similarity profile and are, therefore, less probable.

Two additional limitations may have had an impact on our results, particularly with regard to the description of spatiotemporal clusters. While the use of one rare-cutting restriction enzyme is common among PFGE analysis of meningococcal isolates, the use of two enzymes would provide greater resolution in distinguishing isolates. Second, epidemiological links between cases may not have been fully ascertained during the original public health investigation into each IMD case – especially those separated by more than one generation – and as a result would have been missed in our study. The effect of both limitations, however, would likely be to overestimate the number of

spatiotemporal clusters due to indistinguishable isolates and potential community associations in these analyses, further supporting our conclusions.

Through the use of MLST, a previous report concluded that, although serogroup B IMD rates had decreased in Oregon since the peak of the epidemic, the associated clonal strain had persisted in the population [16]. The use of PFGE in this study, however, indicates that this has not been a static process. Rather, the epidemic strain has evolved over time, becoming increasingly diverse. This process is likely to continue until the clonal strain is no longer identifiable from among a panmictic bacterial population.

In addition to providing greater insight into the nature of meningococcal epidemics, the use of molecular techniques beyond serogroup can assist public health professionals in implementing appropriate meningococcal prevention and control activities, such as the provision of antibiotics or use of the meningococcal conjugate vaccine for non-serogroup B meningococcal outbreaks [8]. For example, a potential organizational serogroup B cluster of two confirmed and one presumptive IMD cases was identified among children in a preschool setting in 2007. After the second confirmed case, institution-wide provision of antibiotic prophylaxis was undertaken. While not done at the time, later analysis of these two isolates revealed distinguishable PFGE patterns, suggesting that such an aggressive public health intervention may not have been indicated. Real-time molecular typing methods for *N. meningitidis*, including PFGE, should therefore be considered as a component of laboratory-based meningococcal surveillance activities.

Table 2-1: PFGE Results and Characteristics of 221 Oregon Serogroup B Meningococcal Isolates Cultured During the Peak (1994-1996) and Later (2001-2003) Epidemic Periods.

Lab ID Number	*Age / Sex*	*County of Residence*	*Culture Month/Year*	*Clone†*	*PFGE Group I*
940033	*13/M*	*Clackamas*	*1/1994*	*Epidemic*	*Yes*
940040	*54/F*	*Washington*	*1/1994*	*Epidemic*	*No*
940045	*6/M*	*Lincoln*	*1/1994*	*Epidemic*	*Yes*
940066	*1/M*	*Marion*	*1/1994*	*Epidemic*	*Yes*
940113	*6/M*	*Washington*	*2/1994*	*Epidemic*	*Yes*
940117	*37/M*	*Coos*	*2/1994*	*Epidemic*	*Yes*
940118	*22/M*	*Washington*	*2/1994*	*Epidemic*	*Yes*
940119	*14/M*	*Columbia*	*2/1994*	*Epidemic*	*Yes*
940127	*18/F*	*Yamhill*	*2/1994*	*Epidemic*	*Yes*
940128	*7/M*	*Linn*	*2/1994*	*Epidemic*	*Yes*
940138	*5/F*	*Multnomah*	*3/1994*	*Epidemic*	*Yes*
940160	*44/M*	*Washington*	*3/1994*	*Epidemic*	*Yes*
940167	*19/F*	*Jackson*	*3/1994*	*Epidemic*	*Yes*
940168	*88/F*	*Deschutes*	*3/1994*	*Epidemic*	*Yes*
940174	*15/M*	*Marion*	*3/1994*	*Epidemic*	*Yes*
940185	*69/F*	*Columbia*	*4/1994*	*Epidemic*	*Yes*
940191	*7/M*	*Jackson*	*4/1994*	*Epidemic*	*Yes*
940192	*42/F*	*Clackamas*	*4/1994*	*Epidemic*	*Yes*
940198	*18/M*	*Linn*	*4/1994*	*Epidemic*	*Yes*
940219	*16/M*	*Marion*	*4/1994*	*Epidemic*	*Yes*
940220	*38/M*	*Multnomah*	*4/1994*	*Epidemic*	*Yes*
940233	*2/M*	*Lane*	*5/1994*	*Epidemic*	*Yes*
940241	*77/F*	*Marion*	*5/1994*	*Epidemic*	*Yes*
940242	*2/M*	*Marion*	*5/1994*	*Epidemic*	*Yes*
940245	*39/F*	*Clackamas*	*5/1994*	*Epidemic*	*Yes*
940247	*8/M*	*Linn*	*5/1994*	*Epidemic*	*Yes*
940248	*16/M*	*Multnomah*	*5/1994*	*Epidemic*	*Yes*
940260	*16/M*	*Clackamas*	*5/1994*	*Epidemic*	*Yes*
940276	*20/M*	*Lane*	*6/1994*	*Epidemic*	*Yes*
940286	*2/F*	*Wallowa*	*6/1994*	*Epidemic*	*No*
940287	*13/F*	*Clackamas*	*6/1994*	*Epidemic*	*No*
940303	*16/F*	*Multnomah*	*6/1994*	*Epidemic*	*Yes*
940312	*15/M*	*Washington*	*6/1994*	*Epidemic*	*Yes*
940334	*46/F*	*Hood River*	*7/1994*	*Epidemic*	*Yes*
940378	*2/M*	*Columbia*	*8/1994*	*Epidemic*	*Yes*
940380	*21/F*	*Multnomah*	*8/1994*	*Epidemic*	*Yes*
940408	*2/M*	*Coos*	*8/1994*	*Epidemic*	*Yes*
940419	*2/F*	*Washington*	*9/1994*	*Epidemic*	*Yes*
940425	*64/F*	*Linn*	*9/1994*	*Epidemic*	*Yes*
940430	*1/M*	*Marion*	*9/1994*	*Epidemic*	*Yes*

Lab ID Number	*Age / Sex*	*County of Residence*	*Culture Month/Year*	*Clone†*	*PFGE Group I*
940461	*20/F*	*Marion*	*10/1994*	*Epidemic*	*Yes*
940463	*13/F*	*Washington*	*10/1994*	*Epidemic*	*Yes*
940490	*18/F*	*Marion*	*10/1994*	*Epidemic*	*Yes*
940508	*4/F*	*Marion*	*10/1994*	*Epidemic*	*No*
940516	*21/M*	*Washington*	*11/1994*	*Epidemic*	*Yes*
940522	*6/F*	*Marion*	*11/1994*	*Epidemic*	*Yes*
940535	*65/M*	*Multnomah*	*11/1994*	*Epidemic*	*Yes*
940539	*6/M*	*Marion*	*11/1994*	*Epidemic*	*Yes*
940543	*0/M*	*Washington*	*11/1994*	*Epidemic*	*Yes*
940547	*20/F*	*Multnomah*	*11/1994*	*Epidemic*	*Yes*
940555	*9/F*	*Tillamook*	*11/1994*	*Epidemic*	*Yes*
940560	*0/F*	*Multnomah*	*12/1994*	*Epidemic*	*Yes*
940573	*1/F*	*Marion*	*12/1994*	*Epidemic*	*Yes*
940603	*59/F*	*Jackson*	*12/1994*	*Epidemic*	*Yes*
940607	*1/M*	*Marion*	*12/1994*	*Epidemic*	*Yes*
950002	*13/M*	*Polk*	*12/1994*	*Epidemic*	*Yes*
950350	*12/F*	*Douglas*	*7/1995*	*Non-Epidemic*	*Yes*
950355	*62/M*	*Lane*	*7/1995*	*Epidemic*	*Yes*
950392	*2/F*	*Washington*	*8/1995*	*Non-Epidemic*	*No*
950416	*28/F*	*Washington*	*8/1995*	*Epidemic*	*Yes*
950424	*16/F*	*Multnomah*	*8/1995*	*Epidemic*	*Yes*
950433	*4/M*	*Multnomah*	*8/1995*	*Epidemic*	*Yes*
950444	*1/M*	*Linn*	*8/1995*	*Epidemic*	*Yes*
950466	*16/F*	*Clackamas*	*9/1995*	*Epidemic*	*Yes*
950542	*14/F*	*Polk*	*9/1995*	*Epidemic*	*Yes*
950510	*15/M*	*Washington*	*10/1995*	*Epidemic*	*Yes*
950520	*3/M*	*Douglas*	*10/1995*	*Non-Epidemic*	*No*
950557	*1/M*	*Clackamas*	*10/1995*	*Epidemic*	*Yes*
950604	*10/F*	*Clackamas*	*11/1995*	*Epidemic*	*Yes*
950628	*3/F*	*Multnomah*	*11/1995*	*Epidemic*	*Yes*
950636	*1/M*	*Marion*	*11/1995*	*Epidemic*	*Yes*
950681	*16/F*	*Clackamas*	*12/1995*	*Non-Epidemic*	*No*
950722	*8/F*	*Multnomah*	*12/1995*	*Epidemic*	*Yes*
950737	*2/M*	*Marion*	*12/1995*	*Epidemic*	*Yes*
950740	*6/M*	*Clackamas*	*12/1995*	*Epidemic*	*Yes*
950742	*5/M*	*Multnomah*	*12/1995*	*Epidemic*	*Yes*
950753	*17/F*	*Josephine*	*12/1995*	*Epidemic*	*No*
950768	*4/M*	*Josephine*	*12/1995*	*Epidemic*	*No*
960026	*11/F*	*Multnomah*	*1/1996*	*Epidemic*	*Yes*
960041	*68/F*	*Linn*	*1/1996*	*Epidemic*	*Yes*
960078	*3/M*	*Marion*	*1/1996*	*Epidemic*	*Yes*
960103	*61/F*	*Clackamas*	*1/1996*	*Non-Epidemic*	*No*
960126	*6/M*	*Marion*	*1/1996*	*Epidemic*	*Yes*
960141	*0/F*	*Marion*	*1/1996*	*Epidemic*	*Yes*

Lab ID Number	*Age / Sex*	*County of Residence*	*Culture Month/Year*	*Clone†*	*PFGE Group I*
960117	*2/F*	*Clackamas*	*2/1996*	*Epidemic*	*Yes*
960156	*40/F*	*Clackamas*	*2/1996*	*Epidemic*	*Yes*
960164	*17/F*	*Multnomah*	*2/1996*	*Epidemic*	*Yes*
960196	*18/M*	*Clackamas*	*2/1996*	*Epidemic*	*Yes*
960205	*17/M*	*Deschutes*	*2/1996*	*Epidemic*	*Yes*
960207	*40/M*	*Washington*	*2/1996*	*Epidemic*	*Yes*
960242	*1/M*	*Clackamas*	*3/1996*	*Epidemic*	*Yes*
960270	*6/M*	*Multnomah*	*3/1996*	*Epidemic*	*Yes*
960272	*5/M*	*Lane*	*3/1996*	*Epidemic*	*Yes*
960285	*75/M*	*Lane*	*3/1996*	*Non-Epidemic*	*No*
960287	*6/F*	*Clackamas*	*3/1996*	*Non-Epidemic*	*Yes*
960292	*2/M*	*Linn*	*3/1996*	*Epidemic*	*Yes*
960308	*0/M*	*Clackamas*	*3/1996*	*Non-Epidemic*	*No*
960314	*16/M*	*Linn*	*4/1996*	*Epidemic*	*Yes*
960336	*0/F*	*Marion*	*4/1996*	*Epidemic*	*Yes*
960348	*17/M*	*Marion*	*4/1996*	*Epidemic*	*Yes*
960363	*41/M*	*Marion*	*4/1996*	*Epidemic*	*Yes*
960365	*0/F*	*Clackamas*	*4/1996*	*Epidemic*	*Yes*
960409	*0/F*	*Multnomah*	*5/1996*	*Epidemic*	*Yes*
960417	*16/M*	*Linn*	*5/1996*	*Epidemic*	*Yes*
960432	*2/F*	*Clackamas*	*5/1996*	*Epidemic*	*Yes*
960447	*9/F*	*Washington*	*5/1996*	*Epidemic*	*Yes*
960452	*7/M*	*Washington*	*5/1996*	*Epidemic*	*Yes*
960453	*32/M*	*Linn*	*5/1996*	*Epidemic*	*Yes*
960478	*0/F*	*Multnomah*	*5/1996*	*Epidemic*	*Yes*
960489	*3/F*	*Multnomah*	*6/1996*	*Epidemic*	*No*
960512	*3/F*	*Multnomah*	*6/1996*	*Epidemic*	*Yes*
960650	*2/F*	*Multnomah*	*6/1996*	*Epidemic*	*Yes*
960676	*2/M*	*Lincoln*	*6/1996*	*Epidemic*	*Yes*
960691	*22/M*	*Marion*	*6/1996*	*Epidemic*	*Yes*
960693	*6/M*	*Multnomah*	*7/1996*	*Epidemic*	*Yes*
960757	*4/M*	*Lincoln*	*7/1996*	*Epidemic*	*Yes*
960770	*13/M*	*Yamhill*	*7/1996*	*Epidemic*	*Yes*
960772	*6/M*	*Multnomah*	*8/1996*	*Epidemic*	*Yes*
960835	*13/F*	*Linn*	*8/1996*	*Epidemic*	*Yes*
960850	*28/M*	*Marion*	*9/1996*	*Epidemic*	*Yes*
960904	*2/F*	*Yamhill*	*9/1996*	*Epidemic*	*Yes*
960905	*3/M*	*Multnomah*	*9/1996*	*Epidemic*	*No*
960990	*16/F*	*Marion*	*10/1996*	*Epidemic*	*Yes*
961006	*5/M*	*Multnomah*	*11/1996*	*Epidemic*	*Yes*
961012	*7/M*	*Linn*	*11/1996*	*Epidemic*	*Yes*
961020	*3/M*	*Linn*	*11/1996*	*Epidemic*	*Yes*
961067	*10/F*	*Deschutes*	*11/1996*	*Epidemic*	*Yes*
961094	*1/F*	*Linn*	*12/1996*	*Epidemic*	*Yes*

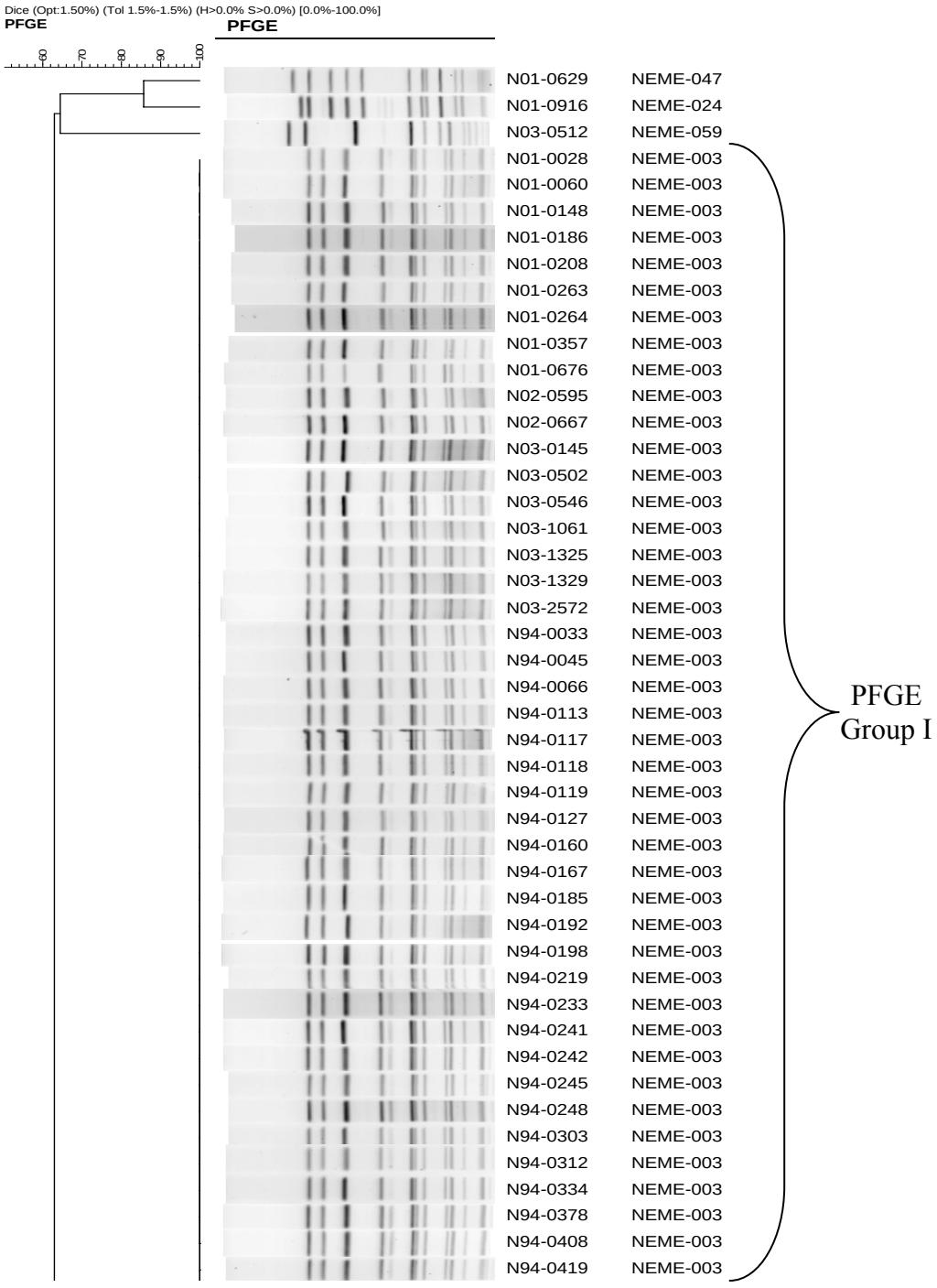
Lab ID Number	*Age / Sex*	*County of Residence*	*Culture Month/Year*	*Clone†*	*PFGE Group I*
961103	*42/M*	*Washington*	*12/1996*	*Epidemic*	*Yes*
010028	*19/M*	*Douglas*	*1/2001*	*Epidemic*	*Yes*
010033	*2/M*	*Clackamas*	*1/2001*	*Epidemic*	*Yes*
010060	*0/F*	*Yamhill*	*1/2001*	*Epidemic*	*Yes*
010133	*0/F*	*Clatsop*	*2/2001*	*Non-Epidemic*	*No*
010139	*19/F*	*Lane*	*2/2001*	*Epidemic*	*Yes*
010146	*20/M*	*Benton*	*2/2001*	*Epidemic*	*Yes*
010148	*71/M*	*Douglas*	*2/2001*	*Epidemic*	*Yes*
010150	*1/M*	*Clackamas*	*2/2001*	*Epidemic*	*Yes*
010155	*0/M*	*Gilliam*	*2/2001*	*Epidemic*	*Yes*
010186	*62/F*	*Jackson*	*2/2001*	*Epidemic*	*Yes*
010208	*8/F*	*Yamhill*	*3/2001*	*Epidemic*	*Yes*
010209	*3/F*	*Lincoln*	*3/2001*	*Epidemic*	*Yes*
010263	*3/F*	*Marion*	*3/2001*	*Epidemic*	*Yes*
010264	*8/M*	*Marion*	*3/2001*	*Epidemic*	*Yes*
010320	*17/F*	*Marion*	*4/2001*	*Epidemic*	*Yes*
010357	*1/F*	*Marion*	*4/2001*	*Epidemic*	*Yes*
010358	*0/F*	*Marion*	*4/2001*	*Epidemic*	*Yes*
010359	*0/M*	*Marion*	*4/2001*	*Epidemic*	*Yes*
010360	*2/F*	*Marion*	*4/2001*	*Epidemic*	*Yes*
010385	*1/M*	*Yamhill*	*4/2001*	*Epidemic*	*Yes*
010431	*3/M*	*Lincoln*	*5/2001*	*Epidemic*	*Yes*
010477	*11/M*	*Clackamas*	*5/2001*	*Epidemic*	*Yes*
010492	*0/M*	*Multnomah*	*5/2001*	*Non-Epidemic*	*No*
010507	*17/M*	*Washington*	*6/2001*	*Epidemic*	*Yes*
010594	*2/M*	*Clatsop*	*7/2001*	*Epidemic*	*Yes*
010629	*85/F*	*Linn*	*7/2001*	*Non-Epidemic*	*No*
010657	*15/F*	*Yamhill*	*7/2001*	*Epidemic*	*Yes*
010676	*10/F*	*Multnomah*	*8/2001*	*Epidemic*	*Yes*
010685	*36/M*	*Lane*	*8/2001*	*Epidemic*	*Yes*
010732	*82/M*	*Jackson*	*8/2001*	*Epidemic*	*No*
010827	*6/M*	*Yamhill*	*10/2001*	*Epidemic*	*Yes*
010877	*19/M*	*Lane*	*10/2001*	*Epidemic*	*Yes*
010879	*0/F*	*Lane*	*10/2001*	*Non-Epidemic*	*No*
010897	*18/M*	*Douglas*	*10/2001*	*Epidemic*	*Yes*
010916	*44/M*	*Lane*	*10/2001*	*Non-Epidemic*	*No*
011083	*24/F*	*Clackamas*	*11/2001*	*Epidemic*	*Yes*
011088	*13/M*	*Multnomah*	*11/2001*	*Non-Epidemic*	*No*
020007	*3/M*	*Washington*	*12/2001*	*Epidemic*	*Yes*
020126	*8/M*	*Linn*	*2/2002*	*Epidemic*	*Yes*
020132	*1/F*	*Crook*	*2/2002*	*Epidemic*	*Yes*
020162	*12/F*	*Polk*	*2/2002*	*Epidemic*	*Yes*
020164	*58/F*	*Lane*	*2/2002*	*Epidemic*	*Yes*
020221	*16/M*	*Josephine*	*2/2002*	*Non-Epidemic*	*No*

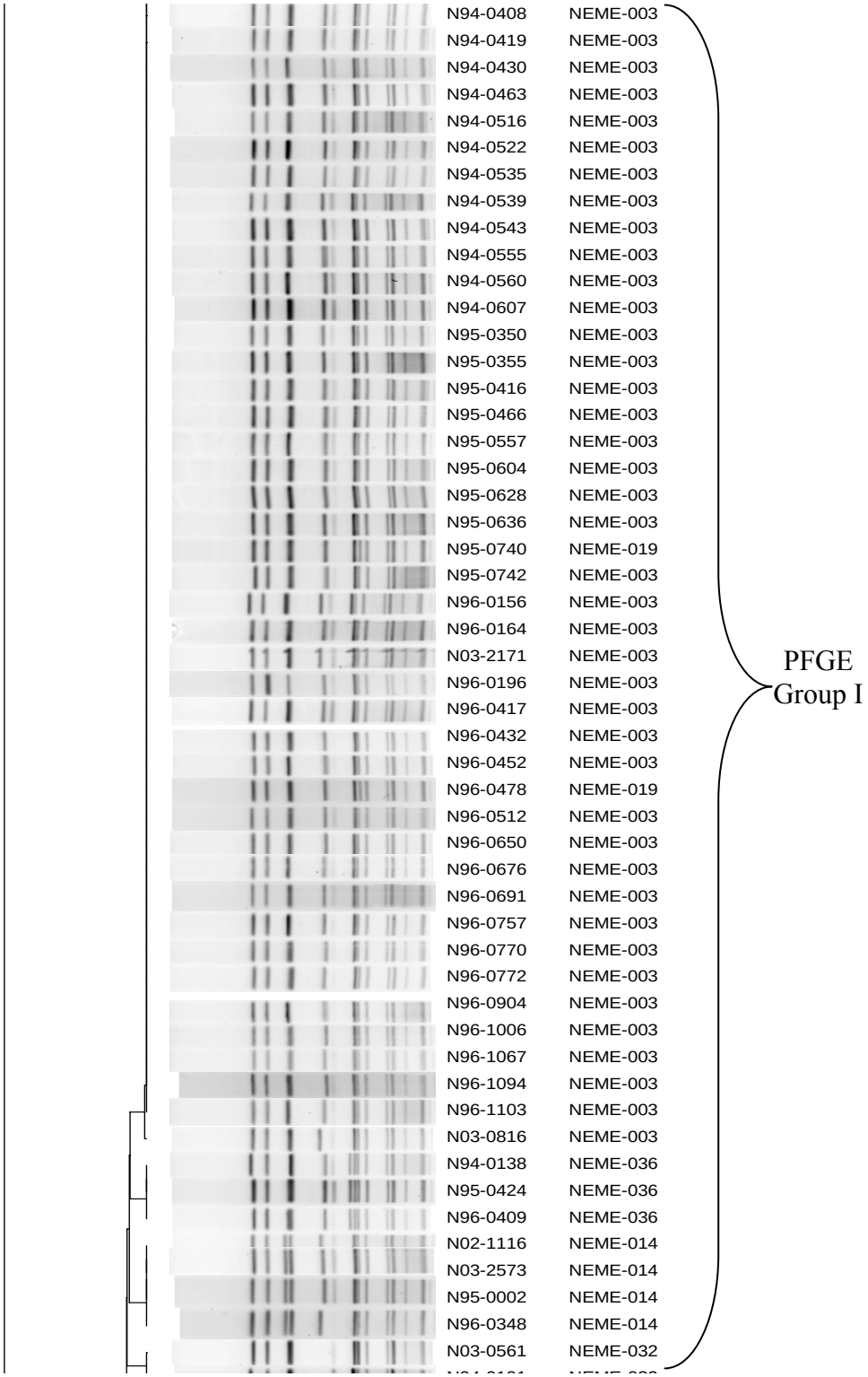
Lab ID Number	*Age / Sex*	*County of Residence*	*Culture Month/Year*	*Clone†*	*PFGE Group I*
020296	*72/F*	*Lane*	*3/2002*	*Epidemic*	*Yes*
020425	*1/F*	*Lane*	*4/2002*	*Epidemic*	*Yes*
020459	*14/M*	*Linn*	*4/2002*	*Epidemic*	*Yes*
020509	*17/M*	*Linn*	*5/2002*	*Epidemic*	*Yes*
020525	*17/M*	*Clackamas*	*5/2002*	*Epidemic*	*No*
020578	*2/F*	*Linn*	*5/2002*	*Epidemic*	*Yes*
020595	*0/M*	*Multnomah*	*5/2002*	*Epidemic*	*Yes*
020618	*4/M*	*Deschutes*	*5/2002*	*Epidemic*	*Yes*
020667	*14/M*	*Douglas*	*6/2002*	*Epidemic*	*Yes*
020711	*1/M*	*Multnomah*	*6/2002*	*Non-Epidemic*	*No*
021116	*5/F*	*Jackson*	*9/2002*	*Epidemic*	*Yes*
021262	*0/M*	*Clackamas*	*10/2002*	*Non-Epidemic*	*No*
030099	*36/F*	*Washington*	*1/2003*	*Non-Epidemic*	*No*
030102	*1/M*	*Washington*	*1/2003*	*Epidemic*	*Yes*
030137	*11/F*	*Clackamas*	*1/2003*	*Epidemic*	*Yes*
030145	*42/F*	*Washington*	*1/2003*	*Epidemic*	*Yes*
030384	*17/M*	*Jackson*	*2/2003*	*Epidemic*	*Yes*
030502	*0/M*	*Multnomah*	*2/2003*	*Epidemic*	*Yes*
030511	*0/M*	*Klamath*	*2/2003*	*Non-Epidemic*	*No*
030512	*2/M*	*Marion*	*2/2003*	*Non-Epidemic*	*No*
030518	*5/M*	*Clackamas*	*2/2003*	*Epidemic*	*Yes*
030529	*73/M*	*Lane*	*3/2003*	*Non-Epidemic*	*No*
030530	*69/F*	*Lane*	*3/2003*	*Epidemic*	*Yes*
030546	*49/M*	*Lane*	*3/2003*	*Epidemic*	*Yes*
030561	*0/M*	*Linn*	*3/2003*	*Epidemic*	*Yes*
030816	*0/F*	*Jackson*	*4/2003*	*Epidemic*	*Yes*
030841	*83/F*	*Marion*	*4/2003*	*Non-Epidemic*	*No*
030868	*2/M*	*Douglas*	*4/2003*	*Non-Epidemic*	*No*
031061	*0/M*	*Multnomah*	*5/2003*	*Epidemic*	*Yes*
031325	*51/M*	*Multnomah*	*6/2003*	*Epidemic*	*Yes*
031329	*35/F*	*Yamhill*	*6/2003*	*Epidemic*	*Yes*
031478	*44/F*	*Polk*	*7/2003*	*Epidemic*	*Yes*
031791	*1/M*	*Jackson*	*8/2003*	*Epidemic*	*Yes*
031808	*69/F*	*Marion*	*8/2003*	*Epidemic*	*Yes*
031976	*4/M*	*Washington*	*9/2003*	*Epidemic*	*Yes*
032061	*15/F*	*Lane*	*9/2003*	*Epidemic*	*Yes*
032117	*0/M*	*Umatilla*	*10/2003*	*Epidemic*	*Yes*
032130	*7/M*	*Tillamook*	*10/2003*	*Epidemic*	*Yes*
032131	*1/M*	*Washington*	*10/2003*	*Epidemic*	*Yes*
032171	*3/M*	*Jackson*	*10/2003*	*Epidemic*	*Yes*
032197	*0/M*	*Columbia*	*10/2003*	*Non-Epidemic*	*No*
032221	*13/M*	*Lane*	*10/2003*	*Epidemic*	*Yes*
032309	*58/F*	*Deschutes*	*11/2003*	*Epidemic*	*Yes*
032451	*5/M*	*Multnomah*	*11/2003*	*Epidemic*	*Yes*

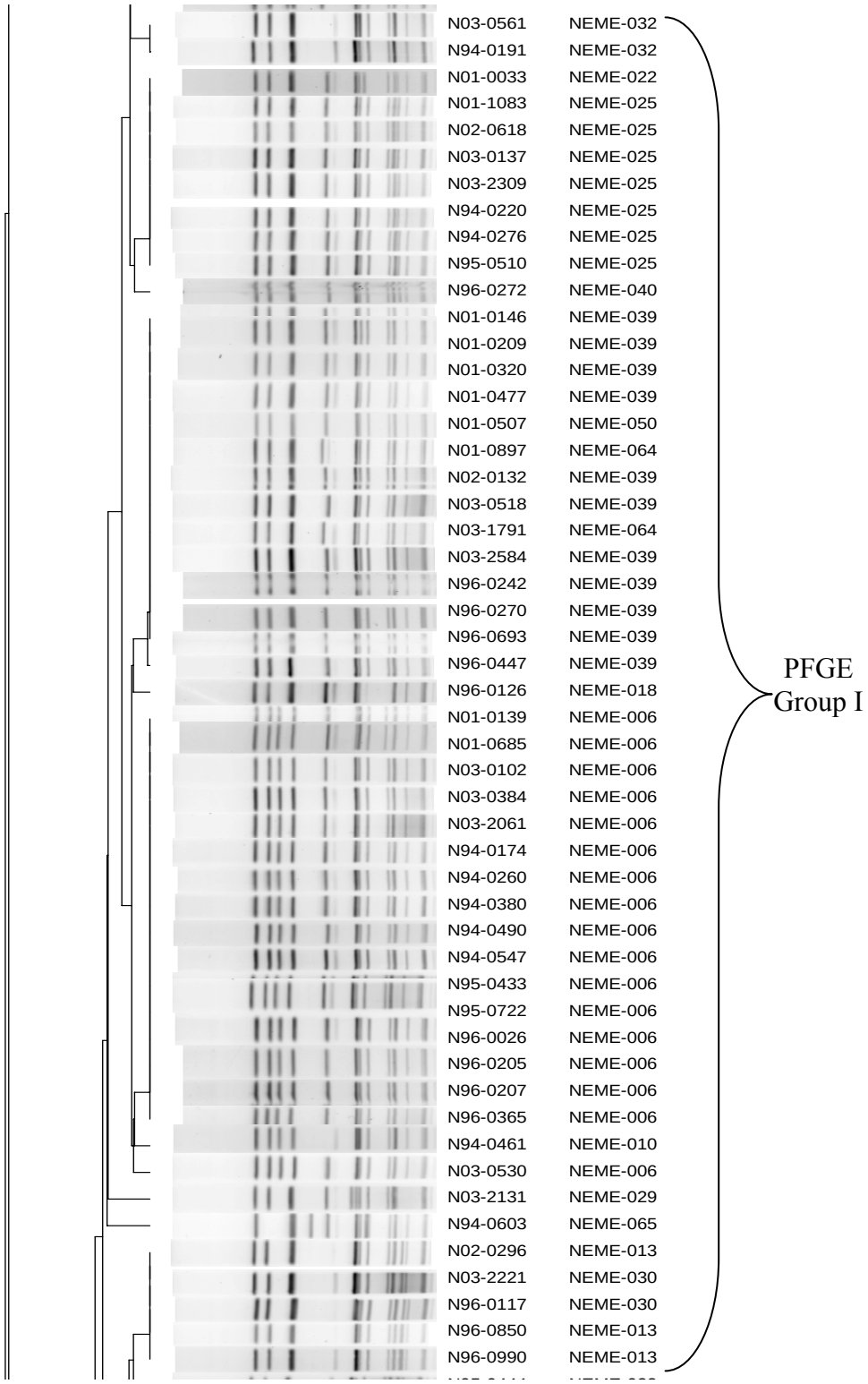
Lab ID Number	*Age / Sex*	*County of Residence*	*Culture Month/Year*	*Clone†*	*PFGE Group I*
032572	*8/F*	*Washington*	*12/2003*	*Epidemic*	*Yes*
032573	*47/M*	*Jackson*	*12/2003*	*Epidemic*	*Yes*
032584	*23/F*	*Multnomah*	*12/2003*	*Epidemic*	*Yes*
032621	*64/F*	*Umatilla*	*12/2003*	*Non-Epidemic*	*No*
032685	*15/M*	*Josephine*	*12/2003*	*Non-Epidemic*	*No*

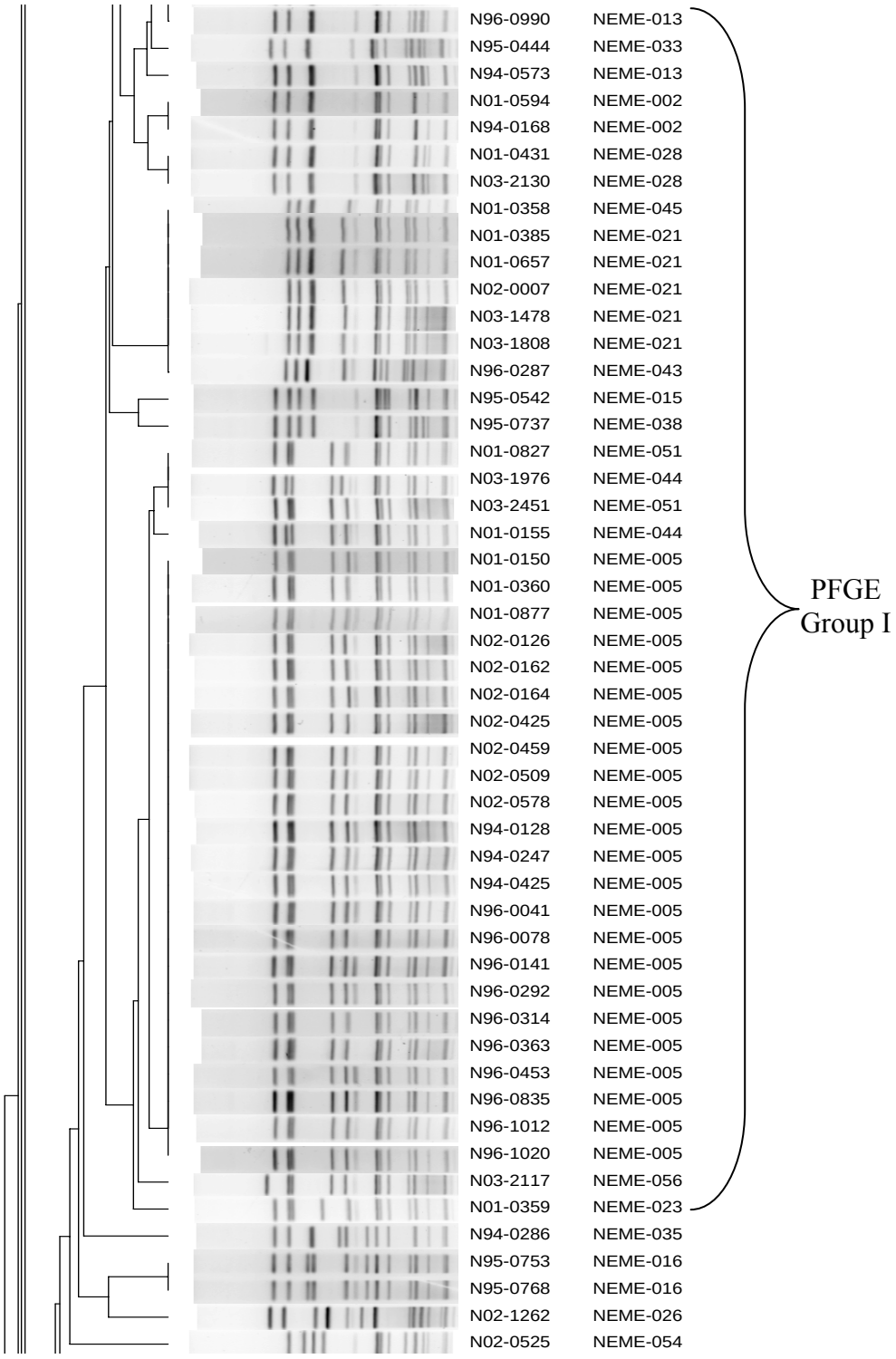
† "Epidemic clone" was defined as serogroup B isolates known to be ET-5/ST-32 complex, by multi-locus enzyme electrophoresis (MLEE) or multi-locus sequence typing (MLST), or B:15:P1.7,16, by serologic typing; all other MLEE, MLST, and serologic designations were considered a "non-epidemic clone."

Figure 2-1: Pulsed-Field Gel Electrophoresis Patterns of 221 Isolates from Peak (1994-1996) and Later (2001-2003) Periods of the Oregon Serogroup B Epidemic.




N94-0408 NEME-003
N94-0419 NEME-003
N94-0430 NEME-003
N94-0463 NEME-003
N94-0516 NEME-003
N94-0522 NEME-003
N94-0535 NEME-003
N94-0539 NEME-003
N94-0543 NEME-003
N94-0555 NEME-003
N94-0560 NEME-003
N94-0607 NEME-003
N95-0350 NEME-003
N95-0355 NEME-003
N95-0416 NEME-003
N95-0466 NEME-003
N95-0557 NEME-003
N95-0604 NEME-003
N95-0628 NEME-003
N95-0636 NEME-003
N95-0740 NEME-019
N95-0742 NEME-003
N96-0156 NEME-003
N96-0164 NEME-003
N03-2171 NEME-003
N96-0196 NEME-003
N96-0417 NEME-003
N96-0432 NEME-003
N96-0452 NEME-003
N96-0478 NEME-019
N96-0512 NEME-003
N96-0650 NEME-003
N96-0676 NEME-003
N96-0691 NEME-003
N96-0757 NEME-003
N96-0770 NEME-003
N96-0772 NEME-003
N96-0904 NEME-003
N96-1006 NEME-003
N96-1067 NEME-003
N96-1094 NEME-003
N96-1103 NEME-003
N03-0816 NEME-003
N94-0138 NEME-036
N95-0424 NEME-036
N96-0409 NEME-036
N02-1116 NEME-014
N03-2573 NEME-014
N95-0002 NEME-014
N96-0348 NEME-014
N03-0561 NEME-032
PFGE
Group I


N03-0561 NEME-032
N94-0191 NEME-032
N01-0033 NEME-022
N01-1083 NEME-025
N02-0618 NEME-025
N03-0137 NEME-025
N03-2309 NEME-025
N94-0220 NEME-025
N94-0276 NEME-025
N95-0510 NEME-025
N96-0272 NEME-040
N01-0146 NEME-039
N01-0209 NEME-039
N01-0320 NEME-039
N01-0477 NEME-039
N01-0507 NEME-050
N01-0897 NEME-064
N02-0132 NEME-039
N03-0518 NEME-039
N03-1791 NEME-064
N03-2584 NEME-039
N96-0242 NEME-039
N96-0270 NEME-039
N96-0693 NEME-039
N96-0447 NEME-039
N96-0126 NEME-018
N01-0139 NEME-006
N01-0685 NEME-006
N03-0102 NEME-006
N03-0384 NEME-006
N03-2061 NEME-006
N94-0174 NEME-006
N94-0260 NEME-006
N94-0380 NEME-006
N94-0490 NEME-006
N94-0547 NEME-006
N95-0433 NEME-006
N95-0722 NEME-006
N96-0026 NEME-006
N96-0205 NEME-006
N96-0207 NEME-006
N96-0365 NEME-006
N94-0461 NEME-010
N03-0530 NEME-006
N03-2131 NEME-029
N94-0603 NEME-065
N02-0296 NEME-013
N03-2221 NEME-030
N96-0117 NEME-030
N96-0850 NEME-013
N96-0990 NEME-013
PFGE
Group I


N96-0990 NEME-013
N95-0444 NEME-033
N94-0573 NEME-013
N01-0594 NEME-002
N94-0168 NEME-002
N01-0431 NEME-028
N03-2130 NEME-028
N01-0358 NEME-045
N01-0385 NEME-021
N01-0657 NEME-021
N02-0007 NEME-021
N03-1478 NEME-021
N03-1808 NEME-021
N96-0287 NEME-043
N95-0542 NEME-015
N95-0737 NEME-038
N01-0827 NEME-051
N03-1976 NEME-044
N03-2451 NEME-051
N01-0155 NEME-044
N01-0150 NEME-005
N01-0360 NEME-005
N01-0877 NEME-005
N02-0126 NEME-005
N02-0162 NEME-005
N02-0164 NEME-005
N02-0425 NEME-005
N02-0459 NEME-005
N02-0509 NEME-005
N02-0578 NEME-005
N94-0128 NEME-005
N94-0247 NEME-005
N94-0425 NEME-005
N96-0041 NEME-005
N96-0078 NEME-005
N96-0141 NEME-005
N96-0292 NEME-005
N96-0314 NEME-005
N96-0363 NEME-005
N96-0453 NEME-005
N96-0835 NEME-005
N96-1012 NEME-005
N96-1020 NEME-005
N03-2117 NEME-056
N01-0359 NEME-023
N94-0286 NEME-035
N95-0753 NEME-016
N95-0768 NEME-016
N02-1262 NEME-026
N02-0525 NEME-054
PFGE
Group I

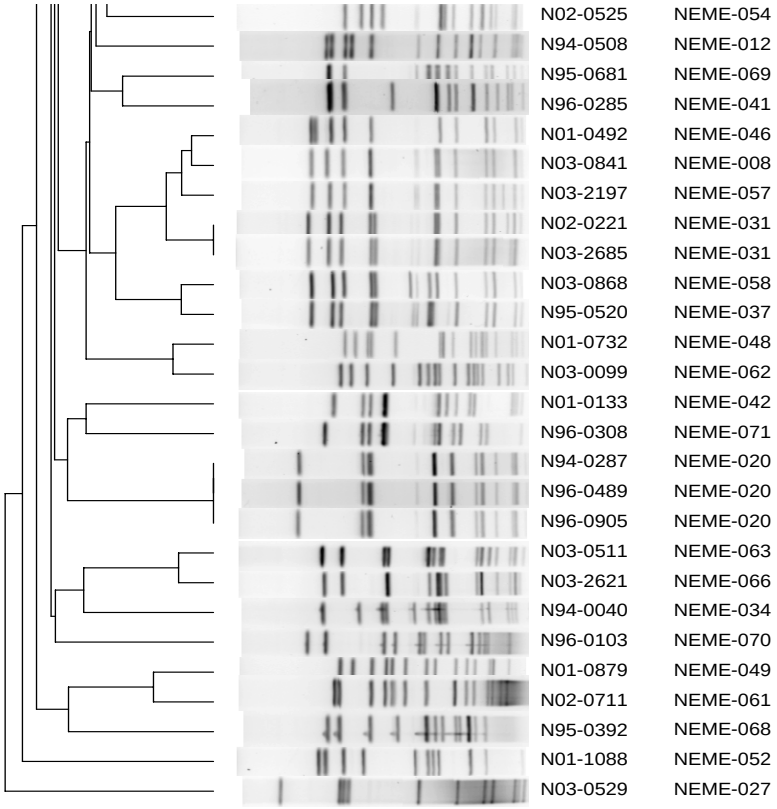

N02-0525 NEME-054
N94-0508 NEME-012
N95-0681 NEME-069
N96-0285 NEME-041
N01-0492 NEME-046
N03-0841 NEME-008
N03-2197 NEME-057
N02-0221 NEME-031
N03-2685 NEME-031
N03-0868 NEME-058
N95-0520 NEME-037
N01-0732 NEME-048
N03-0099 NEME-062
N01-0133 NEME-042
N96-0308 NEME-071
N94-0287 NEME-020
N96-0489 NEME-020
N96-0905 NEME-020
N03-0511 NEME-063
N03-2621 NEME-066
N94-0040 NEME-034
N96-0103 NEME-070
N01-0879 NEME-049
N02-0711 NEME-061
N95-0392 NEME-068
N01-1088 NEME-052
N03-0529 NEME-027

Table 2-2: Distribution of Isolates, by Pulsed-Field Gel Electrophoresis (PFGE) and Epidemic Clone†.

	Epidemic Clone	Non-Epidemic Clone	
PFGE Group			
Group I	185 (95%)	2 (8%)	187 (85%)
Non-Group I	10 (5%)	24 (92%)	34 (15%)
Total	195 (88%)	26 (12%)	221

† "Epidemic clone" was defined as serogroup B isolates known to be ET-5/ST-32 complex, by multi-locus enzyme electrophoresis (MLEE) or multi-locus sequence typing (MLST), or B:15:P1.7,16, by serologic typing; all other MLEE, MLST, and serologic designations were considered a "non-epidemic clone."

Table 2-3: Distribution and Pairwise Similarity of Isolates, by Age and Epidemic Period.

	Peak	Later	p-value
Strain Distribution	N (%)	N (%)	
Overall			
Group I	115/129 (89%)	72/92 (78%)	0.027
Non-Group I	14/129 (11%)	20/92 (22%)	
Age-Specific†			
<15 years	67/76 (88%)	45/55 (82%)	0.31
15-24 years	27/29 (93%)	13/16 (82%)	0.23
≥25 years	21/24 (88%)	14/21 (67%)	0.094
Pairwise Similarity	Median (25-, 75-ile)	Median (25-, 75-ile)	
Overall			
Group I	100.0 (91.7, 100.0)	91.7 (87.0, 95.7)	<0.0001
Non-Group I	60.9 (57.1, 66.7)	63.8 (59.6, 68.0)	0.44
Age-Specific‡			
<15 years	100.0 (91.7, 100.0)	90.9 (87.0, 95.7)	0.0002
15-24 years	100.0 (91.7, 100.0)	95.7 (91.7, 95.7)	0.047
≥25 years	100.0 (90.9, 100.0)	95.7 (90.9, 100.0)	0.68

†Proportion of PFGE Group I isolates out of all isolates in the specified age group.
‡Pairwise similarity of PFGE Group I isolates only.

Table 2-4: Demographic and Isolate Characteristics of Epidemiologically Linked Cases (EL) and Spatiotemporal Clusters (ST) Identified During the Peak and Later Epidemic Periods, by County.

Cluster	County	Culture Date	Age	ET / ST†	PFGE Pattern (NEME-#)
EL-1	Linn	11/9/96	7	NA	005
		11/11/96	3	NA	005
EL-2	Marion	3/17/01	8	32	003
		3/18/01	3	32	003
EL-3	Jackson	8/18/03	1	32	064
		10/10/03	3	32	003
ST-1	Washington	2/15/94	22	317	003
		2/18/94	6	329	003
ST-2	Jackson	3/26/94	19	301	003
		4/10/94	7	316	032
ST-3	Marion	4/25/94	16	301	003
		5/9/94	77	301	003
		5/9/94	2	301	003
ST-4	Multnomah	4/28/94	38	301	025
		5/13/94	16	301	003
ST-5	Clackamas	5/9/94	39	317	003
		5/18/94	16	301	006
ST-6	Washington	11/3/94	21	473	003
		11/19/94	0	473	003
ST-7	Marion	11/9/94	6	301	003
		11/14/94	6	301	003
ST-8	Multnomah	11/17/94	65	475	003
		11/17/94	20	301	006
		12/6/94	0	301	003
ST-9	Marion	12/7/94	1	301	013
		12/24/94	1	301	003
ST-10	Multnomah	8/21/95	16	NA	036
		8/28/95	4	NA	006
ST-11	Clackamas	10/14/95	1	NA	003
		11/4/95	10	NA	003
ST-12	Multnomah	12/12/95	8	NA	006
		12/16/95	5	NA	003
		1/2/96	11	NA	006
ST-13	Josephine	12/16/95	17	NA	016
		12/22/95	4	NA	016
ST-14	Marion	1/12/96	3	NA	005
		1/31/96	0	NA	005

ST-15	Clackamas	2/8/96	40	1062	003
		2/18/96	18	NA	003
		2/22/96	2	NA	030
		3/9/96	1	NA	039
ST-16	Linn	3/24/96	2	NA	005
		4/1/96	16	NA	005
ST-17	Marion	4/5/96	0	NA	038
		4/9/96	41	301	005
		4/12/96	17	301	014
ST-18	Linn	5/8/96	16	301	003
		5/17/96	32	301	005
ST-19	Washington	5/17/96	9	301	039
		5/17/96	7	301	003
ST-20	Multnomah	5/31/96	0	301	019
		6/3/96	3	301	020
		6/7/96	3	301	003
		6/23/96	2	475	003
		7/2/96	6	301	006
ST-21	Marion	4/7/01	17	32	039
		4/16/01	1	32	003
		4/18/01	2	32	005
ST-22	Linn	5/2/02	17	32	005
		5/16/02	2	32	005
ST-23	Washington	1/13/03	1	32	006
		1/27/03	42	32	003
ST-24	Lane	3/2/03	69	32	006
		3/5/03	49	32	003
ST-25	Multnomah	11/19/03	5	32	051
		12/5/03	23	32	039

†The value shown represents electrophoretic type (ET) for isolates with a culture date from 1/1/1994-12/31/1996 and sequence type (ST) for isolates with a culture date from 1/1/2001-12/31/2003. NA=not available.

References:

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

2. Arreaza L, Berron S, Fernandez S, Santiago MI, Malvar A, Vazquez JA. Investigation for a more virulent variant among the C:2b:P1.2,5 Spanish meningococcal epidemic strains by molecular epidemiology. *J Med Microbiol*. 2000;49:1079-84.

3. Ashton FE, Caugant DA. The panmictic nature of *Neisseria meningitidis* serogroup B during a period of endemic disease in Canada. *Can J Microbiol*. 2001;47:283-9.

4. Boras A, Jeren T, Sacchi CT, Schmink S, Boxinovic D, Barsic B, Rosenstein NE, Popovic T. Establishment of an Active Laboratory-Based Surveillance for Bacterial Meningitidis in Croatia and Molecular Characterization of *Neisseria meningitidis* Isolates Causing Meningococcal Disease That Were Collected in the Year 2000, the First Year of Activity. *J Clin Microbiol*. 2004;42:1803-6.

5. Broud DD, Griffiss JM, Baker CJ. Heterogeneity of Serotypes of *Neisseria meningitidis* that Cause Endemic Disease. *J Infect Dis*. 1979;140:465-70.

6. Caugant DA, Frøholm LO, Bøvre K, Holten E, Frasch CE, Mocca LF, Zollinger WD, Selander RK. Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease. *Proc Natl Acad Sci USA*. 1986;83:4927-31.

7. Caugant DA, Tzanakaki G, Kriz P. Lessons from meningococcal carriage studies. *FEMS Microbiol Rev*. 2007;31:52-63.

8. Centers for Disease Control and Prevention. Prevention and Control of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2005;54(No. RR-7).

9. Diermayer M, Hedberg K, Hoesly F, Fischer M, Perkins B, Reeves M, Fleming D. Epidemic Serogroup B Meningococcal Disease in Oregon: The Evolving Epidemiology of the ET-5 Strain. *JAMA*. 1999;281:1493-7.

10. Edwards MS, Baker CJ. Complications and sequelae of meningococcal infections in children. *J Pediatr*. 1981;99:540-5.

11. Enright MC, Spratt BG. Multilocus sequence typing. *Trends Microbiol*. 1999;7:482-7.

12. Feavers IM, Heath AB, Bygraves JA, Maiden MC. Role of horizontal genetic exchange in the antigenic variation of the class 1 outer membrane protein of *Neisseria meningitidis*. *Mol Microbiol*. 1992;489-95.

13. Feil EJ, Enright MC, Spratt BG. Estimating the relative contributions of mutation and recombination to clonal diversification: a comparison between *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Res Microbiol*. 2000;151:465-9.

14. Fraser DW, Thornsberry C, Feldman RA. Trends in meningococcal disease. *J Infect Dis*. 1972;125:443-6.

15. Frasch CE, Zollinger WD, Poolman JT. Serotype Antigens of *Neisseria meningitidis* and a Proposed Scheme for Designation of Serotypes. *Rev Infect Dis*. 1985;7:504-510.

16. Haley CC, Hedberg K, Harrison LH, Cieslak PR. Evolution of the Oregon Serogroup B Meningococcal Epidemic – Persistence of the B:15:P1.7,16 Clone. *Submitted for publication*.

17. Harrison LH, Jolley KA, Shutt KA et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA*. 1998;95:140-5.

18. Heymann DL (ed.) Meningococcal Meningitis *In*: Control of Communicable Diseases Manual. American Public Health Association. 2004: 359-66.

19. Jackson LA, Wenger JD. Laboratory-based surveillance for meningococcal disease in selected areas, United States, 1989-1991. In: CDC surveillance summaries (June). MMWR 1993;42(no. SS-2):21-30.

20. Kellerman SE, McCombs K, Ray M, Baughman W, Reeves MW, Popovic T, Rosenstein NE, Farley MM, Blake P, Stephens DS, and the Georgia Emerging Infections Program. Genotype-Specific Carriage of *Neisseria meningitidis* in Georgia Counties with Hyper- and Hyposporadic Rates of Meningococcal Disease. *J Infect Dis*. 2002;186:40-8.

21. Kirsch EA, Barton RP, Kitchen L, Giroir B. Pathophysiology, Treatment and Outcome of Meningococcemia: A Review and Recent Experience. *Pediatr Infect Dis J*. 1996;15:967-79.

22. Kremastinou J, Tzanakaki G, Levidiotou S, Markou F, Themeli E, Voyiatzi A, Psoma E, Theodoridou M, Blackwell CC. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in northern Greece. *FEMS Immunol Med Microbiol*. 2003;39:23-9.

23. Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zerth K, Caugant DA, Feavers IM, Achtman M, Spratt BG. Multilocus sequence

typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA*. 1998;95:3140-5.

24. Maiden MCJ, Feavers IM. Meningococcal typing. *J Med Microbiol*. 1994;40:157-8.

25. Maiden MCJ. Population genetics of a transformable bacterium: The influence of horizontal genetic exchange on the biology of *Neisseria meningitidis*. *FEMS Microbiol Let*. 1993; 112:243-50.

26. McEllistrem MC, Kolano JA, Pass MA, Caugant DA, Mendelsohn AB, Pacheco AGF, Shutt KA, Razeq J, Harrison LH, and Maryland Emerging Infections Program. Correlating Epidemiologic Trends with the Genotypes Causing Meningococcal Disease, Maryland. *Emerg Infect Dis*. 2004;10:451-6.

27. Paret G, Keller N, Barzilai A, Zemach M, Guttman D, Vardi A, Shatzberg G, Cohen H, Barzilay Z. Invasive Meningococcal Disease: Patient and Strain Characteristics Set New Challenge for Prevention and Control. *Infection*. 1999;27:261-4.

28. Popovic T, Schmink S, Rosenstein NA, Ajello GW, Reeves MW, Plikaytis B, Hunter SB, Ribot EM, Boxrud D, Tondella ML, Kim C, Noble C, Mothershed E, Besser J, Perkins BA. Evaluation of Pulsed-Field Gel Electrophoresis in Epidemiological Investigations of Meningococcal Disease Outbreaks Caused by *Neisseria meningitidis* Serogroup C. *J Clin Microbiol*. 2001;39:75-85.

29. Racoosin JA, Whitney CG, Conover CS, Diaz PS. Serogroup Y Meningococcal Disease in Chicago, 1991-1997. *JAMA*. 1998;280:2094-8.

30. Radstrom P, Fermer C, Kristiansen BE, Jenkins A, Skold O, Swedberg G. Transformational exchanges in the dihydropterate synthase gene of *Neisseria meningitidis*: a novel mechanism for the acquisition of sulfonamide resistance. *J Bacteriol*. 1992;174:5961-8.

31. Raymond NJ, Reeves M, Ajello G, Baughman W, Gheesling LL, Carlone GM, Wenger JD, Stephens DS. Molecular Epidemiology of Sporadic (Endemic) Serogroup C Meningococcal Disease. *J Infect Dis*. 1997;176:1277-84.

32. Rosenstein NE, Perkins BA, Stephens DS, Lefkowitz L, Cartter ML, Danila R, Cieslak P, Shutt KA, Popovic T, Schuchat A, Harrison LH, Reingold AL. The Changing Epidemiology of Meningococcal Disease in the United States, 1992-1996. *J Infect Dis*. 1999;180:1894-901.

33. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal Disease. *NEJM*. 2001;344:1378-88.

34. Schmink S, Watson JT, Coulson GB, Jones RC, Diaz PS, Mayer LW, Wilkins PP, Messonnier N, Gerber SI, Fischer M. Molecular Epidemiology of *Neisseria*

meningitidis Isolates from an Outbreak of Meningococcal Disease among Men Who Have Sex with Men, Chicago, Illinois, 2003. *J Clin Microbiol*. 2007; 45:3768-70.

35. Schwartz B, Moore PS, Broome CV. Global Epidemiology of Meningococcal Disease. *Clin Microbiol Rev*. 1989;2(Supp);S118-24.

36. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of Multilocus Enzyme Electrophoresis for Bacterial Population Genetics and Systematics. Appl Environ Microbiol. 1986;51:873-84.

37. Smith I, Caugant DA, Hoiby EA, Wentzel-Larsen T, Halstensen A. High case-fatality rates of meningococcal disease in Western Norway caused by serogroup C strains belonging to both sequence type (ST)-32 and ST-11 complexes, 1985-2002. *Epidemiol Infect*. 2006;134:1195-202.

38. Smith JM, Smith NH, O'Rourke M, Spratt BG. How clonal are bacteria? *Proc Natl Acad Sci USA*. 1993;90:4384-8.

39. Spratt BG, Bowler LD, Zhang QY, Zhou J, Smith JM. Role of interspecies transfer of chromosomal genes in the evolution of penicillin resistance in pathogenic and commensal *Neisseria* species. *J Mol Evol*. 1992;34:115-25.

40. Swaminathan B, Matar GM. Molecular typing methods *In* Persing DH, Smith TF, Tenover FC, White TJ (ed.) Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C. 1993:26-50.

41. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis. *J Gen Microbiol*. 1995;33:2233-9.

42. Tsang RSW, Squires SG, Tam TWS. Characterization of *Neisseria meningitidis* strains isolated from invasive meningococcal disease cases in Canada in 2001. *Can J Microbiol*. 2003;49:633-8.

43. Tzanakaki G, Urwin R, Musilek M, Kriz P, Kremastinou J, Pangalis A, Blackwell CC, Maiden MC. Phenotypic and genotypic approaches to characterization of isolates of *Neisseria meningitidis* from patients and their close family contacts. *J Clin Microbiol*. 2001;39:1235-40.

44. Tzeng YL, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microb Infect*. 2000;2:687-700.

45. van Duren M, Brandtzaeg P, van der Meer, JWM. Update on Meningococcal Disease with Emphasis on Pathogenesis and Clinical Management. *Clin Micro Rev*. 2000;13:144-66.

46. Vedros NA. Development of meningococcal serogroups. In Evolution of meningococcal disease. *Edited by*: NA Vedros. CRC Press, Boca Raton, FL. Pp. 33-37. 1987002E.

47. Wang JF, Caugant DA, Morelli G, Koumaré B, Achtman M. Antigenic and Epidemiologic Properties of the ET-37 Complex of *Neisseria meningitidis*. *J Infect Dis*. 1993;167:1320-9.

48. Wedege E, Høiby EA, RosenQvist E, Frøholm LO. Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, dot-blotting and ELISA. *J Med Microbiol*. 1990;31:195-201.

49. Whalen CM, Hockin JC, Ryan A, Ashton F. The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992. Emergence of a virulent clone of *Neisseria meningitidis*. *JAMA*. 1993;273:390-4.

50. Yakubu DE, Abadi FJR, Pennington TH. Molecular typing methods for *Neisseria meningitidis*. *J Med Microbiol*. 1999;48:1055-64.

51. Yakubu DE, Pennington TH. Epidemiological evaluation of *Neisseria meningitidis* serogroup B by pulsed-field gel electrophoresis. *FEMS Immunol Med Microbiol*. 1995;10:185-90.

52. Zerouali K, Castelli P, Van Looveren M, El Mdaghri N, Boudouma M, Benbachir M, Nicolas P. Use of multilocus sequence typing and pulsed-field gel electrophoresis for the study of serogroup B *Neisseria meningitidis* isolates from Casablanca (Morocco). *Path Biol*. 2006;54:166-70.

Chapter III

Association between Hyperinvasive Clones of *Neisseria meningitidis*, but not Invasive Meningococcal Disease, and an Integrated Bacteriophage among Isolates Collected in the United States

Introduction

Invasive meningococcal disease (IMD) is a rare, serious disease caused by the gram-negative, diplococcal bacteria, *Neisseria meningitidis*. The most common manifestations of IMD include bacterial meningitis, meningococcemia, and bacteremic pneumonia; septic arthritis and pericarditis occur rarely [23, 27, 34]. Despite appropriate antibiotic therapy, 10 to 15% of IMD cases are fatal and long-term, debilitating sequelae, such as limb amputation, hearing loss, and/or mental retardation, afflict an additional 10-15% of survivors [9, 19, 26, 27]. Until 2000, the overall U.S. annual incidence rate of sporadic IMD – comprising 98% of all cases – had remained stable for over 50 years at 0.9-1.5 cases per 100,000 persons, with periods of hyperendemic or epidemic IMD occurring in roughly 10-year cycles having attack rates several times higher [13, 26, 27].

As the cornerstone of public health laboratory surveillance programs, isolates of *N. meningitidis* are typically defined by serogroup, the antigenic expression of polysaccharide capsule by the organism. Thirteen serogroups have been identified globally, although only four have been commonly seen among meningococcal isolates in the U.S.: B, C, W-135, and Y [7, 16, 35]. However, serogroup does not fully capture the genotypic and phenotypic diversity of the organism and other laboratory techniques have been developed to better elucidate its molecular epidemiology [21]. For instance, the full

serologic profile (serogroup:serotype:serosubtype), the multilocus enzyme electrophoresis (MLEE)-derived electrophoretic type (ET), and the multilocus sequence typing (MLST)-derived sequence type (ST) have been used to describe the dynamic global epidemiology of strains over periods of years and decades [10, 12, 20, 30, 38, 40].

The ST-32/ET5 clonal complex, mainly comprised of serogroup B isolates, emerged in Norway in 1974 and spread throughout Europe, South Africa, Cuba, and South America through the late 1970's and 1980's [5, 29]. The ET-37/ST-11 complex of serogroup C strains was responsible for meningococcal outbreaks in the 1960s, 1970s and 1990s throughout the United States, Canada, and Europe and continues to represent almost all serogroup C IMD isolates, worldwide [3, 25, 26, 32, 37, 39]. More recently, a dramatic increase in serogroup Y IMD occurred in the United States in the late 1990s, with strains tending to belong to the ST-23/ET-508 complex [8, 18, 22, 26, 33].

Exposure to *N. meningitidis* is necessary, but not sufficient, to cause IMD. *N. meningitidis* is transmitted from person-to-person through respiratory droplet spread and the organism typically establishes an asymptomatic colonization state in the nasopharynx. The mechanism by which *N. meningitidis* invades the bloodstream and causes disease has not been well elucidated, although several epidemiological risk factors for invasive disease have been described: and a lack of protective immunity against *N. meningitidis* is the most significant host factor related to IMD; smoking, crowded living conditions, and co-circulating pathogens (i.e. influenza) are important environmental risk factors; and expression of polysaccharide capsule is considered the primary and most important meningococcal virulence factor due to its antibactericidal properties, although the roles of

bacterial pili, outer membrane proteins, and iron-acquisition systems have also been investigated [11, 14, 17, 24, 26, 27, 28, 31, 33, 36].

In 2005, Bille *et al.* reported a potential new virulence factor by describing a chromosomally integrated bacteriophage, termed Meningococcal Disease Associated Island [2]. In their analyses, this element was significantly associated with IMD, even after controlling for known hyperinvasive meningococcal clones in a multivariate statistical analysis. However, over one third of the isolates tested in that study were from the same invasive clonal complexes – the majority of which contained the MDA phage – potentially biasing the interpretation of the presence of this element found in invasive disease isolates. Additionally, isolates used in that study were of a limited number of clonal complexes and did not include any from the ST-23/ET-508 complex commonly seen in the United States.

Through polymerase chain reaction (PCR) amplification and detection of the integrated bacteriophage, the goal of this study was to determine if the integrated bacteriophage was associated with IMD among a diverse collection of invasive and carriage isolates of *N. meningitidis* collected in the United States. We hypothesize that the integrated bacteriophage represents a genetic element acquired by certain meningococcal clones before global spreading globally, rather than a novel meningococcal virulence factor.

Materials and Methods

Meningococcal Strains and Study Population

The Michigan Department of Community Health (MDCH) requests that clinical laboratories submit isolates of *N. meningitidis* recovered from normally-sterile sites to the Bureau of Laboratories for further serogroup characterization by slide agglutination, as part of laboratory surveillance for IMD [38]. The 69 invasive isolates included in this analysis were received by MDCH between Jan 1, 2002 and December 31, 2003. An additional 81 carriage isolates obtained from the 1998 Georgia Carriage Study were included, for a total isolate study population of 150 isolates [18]. Three genome sequenced strains, Z2491, MC58, and FAM18, were included as control isolates.

For the purpose of evaluating the association between clonality and the presence of the MDA phage, serogroup B and serogroup C strains were classified as "hyperinvasive" clones; serogroup Y, nongroupable, or autoagglutinating strains were classified as non-hyperinvasive clones.

PCR Amplification and Detection

Chromosomal DNA was purified from meningococcal isolates grown overnight on GC plates at 37°C under 5% CO_2 using a modified QIAamp DNA mini kit (QIAGEN) and stored at 4°C in sterile MQH_2O. To initially test for the presence of the bacteriophage, polymerase chain reaction (PCR) was performed using the Taq Extender system (Stratagene, California). The reaction mixture (50 µl) contained template chromosomal DNA (1 µg); AmpliTaq reaction buffer; dATP, dCTP, dGTP and dTTP (0.2 mM each)(Roche); forward and reverse primers (0.37 µM each); 0.05 units of Taq

Extender (Stratagene) and 0.05 units of AmpliTaq DNA polymerase (Roche, Indiana). Three reactions were performed per isolate in triplicate: detection of the full length MDA phage using MDA-F/MDA-R2 (8 Kb), the 5' region using MDA-F/1-R (1.5 Kb) and the 3' region using MDA-R2/7-F (3 Kb), as illustrated in Figure 3-1. The primer sequences used are essentially as described in Bille *et al*., with the exception of MDA-R2 (5'-CAGATGATATGTTGCCCGTCAAC -3') [2]. The PCR conditions used were as follows: 2 min at 94°C, followed by 14 amplification cycles of 15 s at 98°C, 20 s at 48°C and 8 min at 68°C, then by 13 amplification cycles of 15 s at 98°C, 20 s at 48°C and *n* min at 68°C (*n* increased linearly from 8 min at cycle 1 to 22 min at cycle 14). Finally, the reactions were held for 10 min at 72°C.

Southern hybridization

Standard Southern blotting conditions were used to confirm the presence of the MDA phage in selected isolates. The 390 bp probe was designed to hybridize within the *Mlu*I sites in the 3' end of the 8 Kb phage, resulting in an ~2.4 Kb fragment. The probe was amplified using primers RH32 (5'-CAGGTTCAAAAATCCCTG-3') and RH33 (5'-ATGTTCTGTTGCCGCCG-3') from the serogroup A *N. meningitidis* strain Z2491. The probe was labeled with DIG-dUTP in the amplification PCR using a ratio of 1:6 (DIG-dUTP:dTTP) (PCR DIG Probe Synthesis kit, Roche, Indiana).

Demographic and Clinical Information

Invasive disease due to *N. meningitis* is reportable to the local and state public health departments, per the Michigan Communicable Disease Rules (325.173).

Demographic (sex and age) and clinical (manifestation and outcome of disease) information was obtained by reviewing individual case report forms corresponding to the isolates included in the analysis.

Statistical analysis

Univariate analyses were conducted to investigate the association between the presence of the MDA phage and invasive collection source, serogroup, hyperinvasive strain type, and select demographic and clinical disease information. A multivariable logistic regression model included serogroup and isolate source as independent variables. All statistical analyses were conducted using SAS statistical software (Version 9.1; SAS Institute; Cary, NC).

Results

Table 3-1 displays the distribution of isolate serogroup by collection source. Serogroup Y was most common overall, comprising half of all isolates analyzed, with no significant difference between invasive and carriage isolates ($p=0.416$). Serogroup B was the second most common serogroup identified, also with no significant difference in proportion between invasive and carriage isolates ($p=0.522$). In contrast, serogroups C and W-135 were positively associated with an invasive collection source ($p<0.0001$ and $p=0.024$, respectively), while non-groupable and autoagglutinating isolates were associated with carriage ($p<0.0001$).

Of the 150 total isolates analyzed, the MDA phage was detected through PCR amplification in 54 (36%). Thirty (43%) invasive isolates and 24 (30%) carriage isolates

contained the MDA phage (Figure 3-2). Although the proportion of invasive isolates containing the phage was higher than that among carriage isolates, this difference was not significant (Odds Ratio [OR] = 1.8; 95% Confidence Interval [CI] 0.93-3.6).

The presence of the MDA phage was associated with serogroup. Ninety-one percent of serogroup B isolates (n=21) and 84% of serogroup C isolates (n=16) contained the phage. This contrasts with serogroups Y, W-135, and non-groupable isolates, in which the phage was found in 9% (n=7), 20% (n=1), and 31% (n=8) of isolates, respectively. Compared to serogroup Y, serogroup B (OR 102; CI 19.7-528.9); serogroup C isolates (OR 52, CI 12.1-222.6); and non-groupable isolates (OR 6, CI 1.7-17.8) were positively associated with the bacteriophage (Table 3-2). Hyperinvasive clones – serogroup B and C isolates, combined – were more likely to contain to contain the phage that non-hyperinvasive clones (OR 6.2, CI 2.8-13.8). In the multivariable logistic model, the association between the presence of the bacteriophage and serogroup remained significantly associated; that between the presence of the phage and collection source remained non-significant (Table 3-3).

Associations between the presence of the MDA phage and select demographic and clinical characteristics of disease among the 69 invasive isolates from Michigan are shown in Table 3–4. The presence of the phage was not statistically associated with sex or young age (less than five years) of the patient or fatal outcome of disease. Clinical manifestation of disease was significantly associated with the presence of the phage, with isolates from pneumonia cases being less likely than bacteremia or meningitis isolates to contain the phage (p=0.01). In an analysis of clinical manifestation by serogroup, with serogroup Y as the referent category, serogroup B was associated with meningitis (OR 7,

CI 1.5-35.2). All pneumonia isolates were serogroup Y. No other significant associations were detected. Due to small numbers, a multivariate analysis of the association between presence of the phage and clinical manifestation of disease controlling for serogroup was not conducted.

Discussion

The 150 isolates on which analyses was presented here must be considered in context as a subset of 403 isolates (211 invasive and 192 carriage) from a larger study by Hobb *et al.*, sharing the same research objective in studying the occurrence of the integrated bacteriophage among a large, diverse collection of meningococcal isolates (Unpublished data). Both studies failed to demonstrate an association between invasive meningococcal isolates and the presence of the 8 kb integrated bacteriophage, in contrast to the previous study by Bille *et al*. While Hobb *et al.* found a significant crude association between the presence of the phage and collection source, likely due to the larger sample size than analyzed for this report, the phage was also found to be associated with serogroup in a similar pattern as presented here. In multivariate analysis controlling for serogroup, the phage was no longer associated with isolate collection source. While not specifically addressed by Hobb *et al.*, the proportion of isolates containing the bacteriophage in these analyses did not differ by age (younger than five years of age versus five years and older), sex clinical manifestation, or outcome of disease. These findings are in contrast to those reported by Bille [2]. That the presence of the bacteriophage was associated with clonal types in both studies supports our hypothesis

that the MDA phage represents a genetic element acquired by certain strains and not a virulence factor required for invasive disease.

The main limitation of this study was a lack of information on the clonal strain type (i.e. ET or ST) of Michigan invasive isolates. The use of serogroup in this study as a proxy for a more discriminating designation raises the question of the extent to which misclassification of strains may have biased these results. By looking at isolates in the Hobb *et al.* collection for which sequence typing information is available, it seems the conclusion drawn by our results is unlikely to change and may represent an underestimate of the true association between the presence of the MDA phage and clonal complex. Among invasive isolates with a known ST, serogroups Y and C tended to be the most clonal: 16 of 16 (100%) serogroup Y isolates with known ST were ST-23/ET-508 and 48 of 50 (96%) serogroup C isolates with known ST were ST-11/ET-37. Serogroup B tended to be more diverse, with seven sequence types identified among 14 isolates. However, 10 isolates (71%) belonged to one of three hyperinvasive lineages (ST-8/Cluster A4: 3 isolates; ST-11/ET-37: 1 isolate; ST-32/ET-5: 3 isolates; and ST-44/Lineage 3: 3 isolates). Overall, considering serogroup B and C isolates to be hyperinvasive and serogroup Y to be non-hyperinvasive would have accurately described the clonality for 93% of the isolates. Invasive strains tend to be clonal in nature than carriage strains, in terms of the number of serogroups, serotypes, ETs, and STs identified [1, 4, 6, 41]. Therefore, it is likely that use of serogroup as a marker of lineage would have resulted in a greater proportion of carriage isolates being incorrectly identified as hyperinvasive and our estimate would reflect an underestimate of the true association between the presence of the phage and clonal designation.

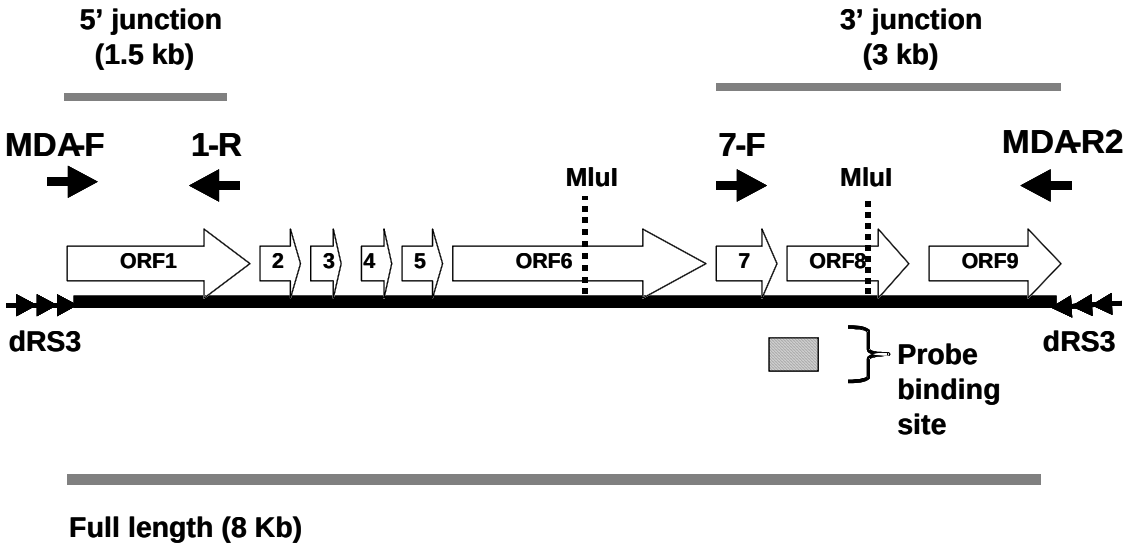
This is supported by Hobb *et al.* in an analysis of the presence of the bacteriophage in relation to sequence type, regardless of isolate serogroup. Isolates belonging to one of four identified hyperinvasive clone lineages, ST-8, ST-11/ET-37, ST-32/ET-5, ST-41/ST-44, 82% of which carried the integrated bacteriophage, were 16.9 times (95% CI 7.9-36.3) more likely to have the phage than other clones. In contrast, only 4% of ST-23/Cluster A isolates were positive for the phage by PCR. A large genomic study in which DNA-DNA hybridization found only certain meningococcal genomic groups were associated with the phage also supports these conclusion (Unpublished data).

Given the current predominance of ST-23/ET-508 serogroup Y strains in the United States, one would expect an inherent meningococcal virulence factor to be carried by this population of organisms. That the integrated genetic element is shared by isolates belonging to defined clonal groupings and not all invasive disease isolates may signify only that it was acquired by certain clones before spread throughout the human population. While our data do not exclude the possibility that the MDA phage contributes to invasive disease by enhancing the virulence of the hyperinvasive lineages with which it is associated, further work will be necessary to elucidate the mechanism whereby this could occur.

As exemplified by the successful development of vaccines targeting the polysaccharide capsule and outer membrane proteins of *N. meningitidis*, the identification of meningococcal virulence factors has driven research into agent-directed interventions to prevent IMD [7, 15]. The publication of the Bille article, heralding the identification of a new meningococcal virulence factor, was well-received due to the potential role for this new target for primary, anti-meningococcal interventions. The findings presented through

analyses in this study, failing to support the role of the integrated bacteriophage as a necessary virulence factor, are relevant less for their direct impact on understanding the epidemiology of *N. meningitidis* – the development of an assay to study the clonal spread of this genetic element is unlikely – or the control of IMD than their role in preventing the unnecessary expenditure of resources to further characterize and develop interventions against this molecular entity. The identification and research of more promising targets – such as additional outer membrane proteins, bacterial adhesin molecules that bind to host receptors, resulting in meningococcal colonization, or endotoxin that contributes to meningococcal pathophysiology – will be needed to further reduce the occurrence of IMD through agent-based interventions.

Figure 3-1: Genetic Organization of the Integrated Bacteriophage from the Serogroup A *N. meningitidis* Strain, Z2491.

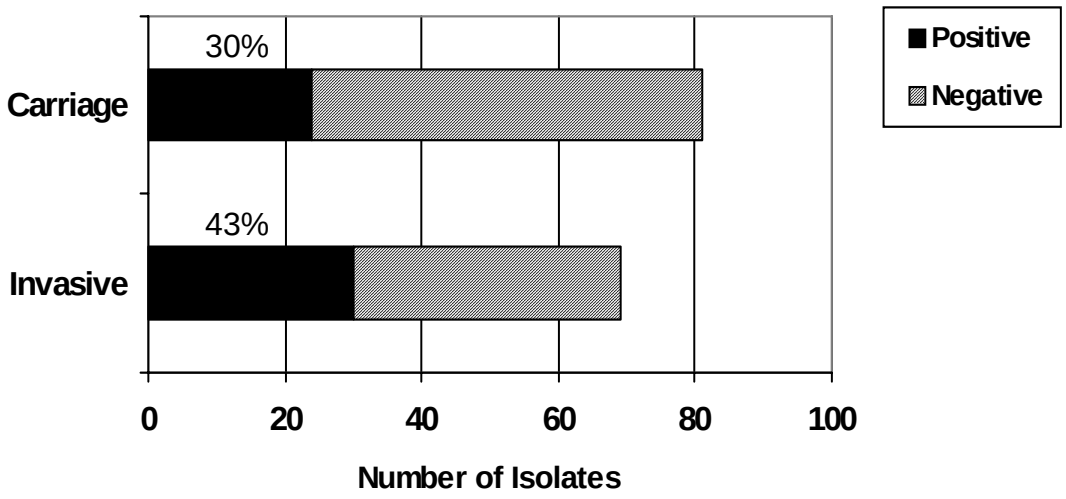


Solid arrows indicate the primer binding sites for each of the three PCR sets used to determine the presence or absence of the MDA phage in this collection of isolates. Dotted lines indicate the position of *Mlu*I restriction enzyme sites and the striped box represents the probe-binding site for Southern hybridization. dRS3 (represented by small arrows at each end of the element) are the 20 bp inverted repeat sequences constituting the chromosomal point of insertion of the element.

Table 3-1: Distribution of 69 Invasive and 81 Carriage Isolates, by Serogroup.

Serogroup	Invasive N (%)	Carriage N (%)	Total
A	1 (100)	0 (0)	1
B	12 (52)	11 (48)	23
C	19 (100)	0 (0)	19
W135	5 (100)	0 (0)	5
Y	32 (43)	43 (57)	75
Z	0 (0)	1 (100)	1
Auto / NG	0 (0)	26 (100)	26
Total	69 (46)	81 (54)	150

Figure 3-2: Distribution of *N. meningitidis* Isolates, by Collection Source and Presence of the MDA Phage, as Detected through PCR Amplification.



The solid region of each bar indicates the number of isolates testing positive, with the percentage of the total noted above the bar.

Table 3-2: Univariate Logistic Regression Analysis of the Association between Serogroup and the Presence of the Integrated Bacteriophage.

Serogroup	Odds Ratio	95% Confidence Interval
Y	1.0	-
W-135	2.4	0.24 – 24.8
NG	5.6	1.7 – 17.8
C	51.8	12.1 – 222.6
B	102.0	19.7 – 528.9
Other	1.9	0.2 – 19.1

Table 3-3: Multivariable Logistic Regression Analysis of the Association between Collection Source and Serogroup and the Presence of the Integrated Bacteriophage.

	Adjusted Odds Ratio	95% Confidence Interval
Serogroup		
Y	1.0	-
W-135	3.4	0.3 – 42.6
NG	4.5	1.3 – 15.9
C	73.1	12.7 – 421.3
B	114.1	20.7 – 630.5
Other	1.7	0.2 – 17.2
Collection Source		
Carriage	1.0	-
Invasive	0.6	0.1 – 2.4

Table 3-4: Univariate Analysis of the Presence of the Integrated Bacteriophage and Select Demographic and Clinical Variables.

	Phage (+) N (%)	Phage (-) N (%)	Total	χ^2 p-value
Male	11	17	28	0.66
Female	17	21	38	
Age <5 yrs	2	6	8	0.32
Age ≥5 yrs	24	31	55	
Expired	3	2	5	0.47
Survived	19	25	44	
Bacteremia	15	14	29	0.01
Bacteremia and Meningitis	0	5	5	
Meningitis	11	6	17	
Bacteremic Pneumonia	0	5	5	

References:

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

2. Bille E. Zahar JR. Perrin A. Morelle S. Kriz P. Jolley KA. Maiden MC. Dervin C. Nassif X. Tinsley CR. A chromosomally integrated bacteriophage in invasive meningococci. *J Exp Microbiol*. 2005;201:1905-13.

3. Boras A, Jeren T, Sacchi CT, Schmink S, Boxinovic D, Barsic B, Rosenstein NE, Popovic T. Establishment of an Active Laboratory-Based Surveillance for Bacterial Meningitidis in Croatia and Molecular Characterization of *Neisseria meningitidis* Isolates Causing Meningococcal Disease That Were Collected in the Year 2000, the First Year of Activity. *J Clin Microbiol*. 2004;42:1803-6.

4. Broud DD, Griffiss JM, Baker CJ. Heterogeneity of Serotypes of *Neisseria meningitidis* that Cause Endemic Disease. *J Infect Dis*. 1979;140:465-70.

5. Caugant DA, Frøholm LO, Bøvre K, Holten E, Frasch CE, Mocca LF, Zollinger WD, Selander RK. Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease. *Proc Natl Acad Sci USA*. 1986;83:4927-31.

6. Caugant DA, Kristiansen BE, Froholm LO, Bovre K, Selander RK. Clonal Diversity of *Neisseria meningitidis* from a Population of Asymptomatic Carriers. *Infect Immun*. 1988;56:2060-8.

7. Centers for Disease Control and Prevention. Prevention and Control of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2005;54(No. RR-7).

8. Centers for Disease Control and Prevention. Serogroup Y Meningococcal Disease – Illinois, Connecticut, and Selected Areas, United States, 1989-1996. MMWR 1996;45:1010-3.

9. Edwards MS, Baker CJ. Complications and sequelae of meningococcal infections in children. *J Pediatr*. 1981;99:540-5.

10. Enright MC, Spratt BG. Multilocus sequence typing. *Trends Microbiol*. 1999;7:482-7.

11. Fischer M, Hedberg K, Cardosi P,et al. Tobacco smoke as a risk factor for meningococcal disease. *Pediatr Infect Dis J*. 1997;16:979-83.

12. Frasch CE, Zollinger WD, Poolman JT. Serotype Antigens of *Neisseria meningitidis* and a Proposed Scheme for Designation of Serotypes. *Rev Infect Dis*. 1985;7:504-510.

13. Fraser DW, Thornsberry C, Feldman RA. Trends in meningococcal disease. *J Infect Dis*. 1972;125:443-6.

14. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med*. 1969;129:1307-26.

15. Holst J. Strategies for development of universal vaccines against meningococcal serogroup B disease: the most promising options and the challenges evaluating them. *Human Vaccines*. 2007;3:290-4.

16. Jackson LA, Wenger JD. Laboratory-based surveillance for meningococcal disease in selected areas, United States, 1989-1991. In: CDC surveillance summaries (June). MMWR 1993;42(no. SS-2):21-30.

17. Kallstrom H, Liszewski MK, Atkinson JP, Jonsson AB. Membrane cofactor protein (MCP or CD46) is a cellular pilus receptor for pathogenic *Neisseria*. *Mol Microbiol*. 1997;25:639-47.

18. Kellerman SE, McCombs K, Ray M, Baughman W, Reeves MW, Popovic T, Rosenstein NE, Farley MM, Blake P, Stephens DS, and the Georgia Emerging Infections Program. Genotype-Specific Carriage of *Neisseria meningitidis* in Georgia Counties with Hyper- and Hyposporadic Rates of Meningococcal Disease. *J Infect Dis*. 2002;186:40-8.

19. Kirsch EA, Barton RP, Kitchen L, Giroir B. Pathophysiology, Treatment and Outcome of Meningococcemia: A Review and Recent Experience. *Pediatr Infect Dis J*. 1996;15:967-79.

20. Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zerth K, Caugant DA, Feavers IM, Achtman M, Spratt BG. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA*. 1998;95:3140-5.

21. Maiden MCJ, Feavers IM. Meningococcal typing. *J Med Microbiol*. 1994;40:157-8.

22. McEllistrem MC, Kolano JA, Pass MA, Caugant DA, Mendelsohn AB, Pacheco AGF, Shutt KA, Razeq J, Harrison LH, and Maryland Emerging Infections Program. Correlating Epidemiologic Trends with the Genotypes Causing Meningococcal Disease, Maryland. *Emerg Infect Dis*. 2004;10:451-6.

23. Paret G, Keller N, Barzilai A, Zemach M, Guttman D, Vardi A, Shatzberg G, Cohen H, Barzilay Z. Invasive Meningococcal Disease: Patient and Strain Characteristics Set New Challenge for Prevention and Control. *Infection*. 1999;27:261-4.

24. Pereiró I, Díez-domingo J, Segarra L, Ballester A, Albert A, Morant A. Risk factors for invasive disease among children in Spain. *J Infect*. 2004;48:320-9.

25. Raymond NJ, Reeves M, Ajello G, Baughman W, Gheesling LL, Carlone GM, Wenger JD, Stephens DS. Molecular Epidemiology of Sporadic (Endemic) Serogroup C Meningococcal Disease. *J Infect Dis*. 1997;176:1277-84.

26. Rosenstein NE, Perkins BA, Stephens DS, Lefkowitz L, Cartter ML, Danila R, Cieslak P, Shutt KA, Popovic T, Schuchat A, Harrison LH, Reingold AL. The Changing Epidemiology of Meningococcal Disease in the United States, 1992-1996. *J Infect Dis*. 1999;180:1894-901.

27. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal Disease. *NEJM*. 2001;344:1378-88.

28. Schryvers AB, Stojiljkovic I. Iron acquisition systems in the pathogenic *Neisseria*. *Mol Microbiol*. 1999;32:1117-23.

29. Schwartz B, Moore PS, Broome CV. Global Epidemiology of Meningococcal Disease. *Clin Microbiol Rev*. 1989;2(Supp);S118-24.

30. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of Multilocus Enzyme Electrophoresis for Bacterial Population Genetics and Systematics. Appl Environ Microbiol. 1986;51:873-84.

31. Taha MK, Deghmane AE, Antignac A, Zarantonelli ML, Larribe M, Alonso JM. The duality of virulence and transmissibility in *Neisseria meningitidis*. *Trends Microbiol*. 2002;10:376-82.

32. Tsang RSW, Squires SG, Tam TWS. Characterization of *Neisseria meningitidis* strains isolated from invasive meningococcal disease cases in Canada in 2001. *Can J Microbiol*. 2003;49:633-8.

33. Tzeng YL, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microb Infect*. 2000;2:687-700.

34. van Duren M, Brandtzaeg P, van der Meer, JWM. Update on Meningococcal Disease with Emphasis on Pathogenesis and Clinical Management. *Clin Micro Rev*. 2000;13:144-66.

35. Vedros NA. Development of meningococcal serogroups. In Evolution of meningococcal disease. *Edited by*: NA Vedros. CRC Press, Boca Raton, FL. Pp. 33-37. 1987002E

36. Vogel U, Frosch M. Mechanisms of neisserial serum resistance. *Mol Microbiol*. 1999;32:1133-9.

37. Wang JF, Caugant DA, Morelli G, Koumaré B, Achtman M. Antigenic and Epidemiologic Properties of the ET-37 Complex of *Neisseria meningitidis*. *J Infect Dis*. 1993;167:1320-9.

38. Wedege E, Høiby EA, RosenQvist E, Frøholm LO. Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, dot-blotting and ELISA. *J Med Microbiol*. 1990;31:195-201.

39. Whalen CM, Hockin JC, Ryan A, Ashton F. The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992. Emergence of a virulent clone of *Neisseria meningitidis*. *JAMA*. 1995;273:390-4.

40. Yakubu DE, Abadi FJR, Pennington TH. Molecular typing methods for *Neisseria meningitidis*. *J Med Microbiol*. 1999;48:1055-64.

41. Yazdankhah SP, Kriz P, Tzanakaki G, Kremastinou J, Kalmusova J, Musilek M, Alvestad T, Jolley KA, Wilson DJ, McCarthy ND, Caugant DA, Maiden MCJ. Distribution of Serogroups and Genotypes among Disease-Associated and Carried Isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *J Clin Microbiol*. 2004;42:5146-53.

Chapter IV

Effects of Increasing Broad-Spectrum Antibiotic Use on the Epidemiology of Invasive Meningococcal Disease among Children Younger than Five

Introduction

Invasive meningococcal disease (IMD) is a rare, serious disease caused by the gram-negative, diplococcal bacteria, *Neisseria meningitidis*. The most common manifestations of IMD include bacterial meningitis, meningococcemia, and bacteremic pneumonia; septic arthritis and pericarditis occur rarely [47, 57, 70]. Despite appropriate antibiotic therapy, 10 to 15% of IMD cases are fatal and long-term, debilitating sequelae, such as limb amputation, hearing loss, and/or mental retardation, afflict an additional 10-15% of survivors [21, 35, 56, 57]. The incidence of IMD in the United States is highest among infants, peaking as high as 15.9 cases per 100,000 in those aged 4-5 months of age [56].

Exposure to *N. meningitidis* is necessary, but not sufficient, to cause IMD. *N. meningitidis* is transmitted from person-to-person through respiratory droplet spread. The highest risk of IMD occurs immediately after the acquisition of a new, pathogenic *N. meningitidis* strain, although the organism usually establishes an asymptomatic colonization state in the nasopharynx [45]. The overall prevalence of meningococcal colonization in the population has been estimated at roughly 10%, although in semi-closed communities, such as among military recruits, colonization rates can exceed 60% [7, 8, 37, 68]. The mechanisms by which *N. meningitidis* invades the bloodstream and

causes disease have not been well elucidated, although several epidemiological risk factors have been described: expression of polysaccharide capsule is considered the primary and most important meningococcal virulence factor due to its antibactericidal properties; smoking, crowded living conditions, and co-circulating pathogens (i.e. influenza) are important environmental risk factors; and a lack of protective immunity against *N. meningitidis* is the most significant host factor related to IMD [25, 29, 48, 56, 57, 64, 69].

Over one half of newborns display protective levels of anti-meningococcal immunity, due to the transplacental passage of maternal antibodies to the infant [29, 30]. The level of anti-meningococcal immunity quickly wanes after birth, reaching a nadir among infants six to 12 months of age, after which it increases in an age-dependent manner through adulthood [29, 30, 69]. The acquisition of natural anti-meningococcal immunity occurs as a natural response to nasopharyngeal colonization with *N. meningitidis* or related, commensal species, such as *N. lactamica* [28, 30, 46]. Among children younger than five years of age, the prevalence of *N. meningitidis* is low (0.4-2.1%), whereas colonization with *N. lactamica* occurs within the first few months of life and peaks between 18 and 24 months of age, when 20-25% of children may be colonized with this organism [7, 28, 33]. Consequently, the latter may be more important than *N. meningitidis* in driving the acquisition of anti-meningococcal immunity among children in this age group [7, 28, 30].

That over 98% of all IMD cases in the U.S. are sporadic is a testament to the effectiveness of the public health response to IMD – namely, the provision of antibiotic prophylaxis to eradicate the carriage of potentially virulent meningococci from the

nasopharynx of close case contacts [56]. While rifampin has long been the accepted standard for prophylaxis of contacts, ceftriaxone, ciprofloxacin, and azithromycin are approved alternatives, with comparable efficacy (97%) and duration (two to three weeks) of nasopharyngeal eradication [1, 3, 15, 17, 18, 20, 27, 31, 52, 60, 61]. Penicillin, while the accepted standard for treatment of IMD, is not effective at reducing nasopharyngeal carriage of *N. meningitidis* and individuals receiving a treatment course of penicillin must also receive an antibiotic to eradicate the organism from the nasopharynx [1, 6, 31, 49, 59].

Throughout the 1990's, largely to promote the judicious use of antibiotics in light of increasing antibiotic resistance, overall rates of antibiotic prescriptions in children declined [19, 24, 32, 36, 42, 43, 44, 65]. Indeed, prescriptions for cephalosporins, penicillin, and erythromycin decreased by 28%, 43%, and 76%, respectively [24, 43]. Yet, despite this overall trend, increases in the prescription of broad spectrum antibiotics (BSA), which are effective at eradicating nasopharyngeal colonization of *N. meningitidis*, were seen [42]. In particular, the use of second-generation macrolides, 75% of which is azithromycin, increased between 241% and 388% from 1992-2000 [43, 63]. Although this class of drugs constituted only 8% of antibiotics dispensed among children less than six years of age in 2000, 60% of these prescriptions were used as an initial treatment regimen, most commonly for otitis media or upper respiratory tract infections [24, 63].

This increasing population use of BSA for non-prophylactic purposes, through its effect of reducing the prevalence of colonization of *N. meningitidis* and *N. lactamica*, may have implications for the epidemiology of IMD. For instance, reducing the prevalence of circulating pathogenic organisms would be expected to result in a lower

incidence of IMD. However, to the extent that increasing BSA use reduces the prevalence of colonizing organisms among children in this age group, the normal process of acquisition of anti-meningococcal immunity may be disrupted, thereby resulting in a greater proportion of individuals at risk for IMD. While the literature has hinted at negative consequences of the disrupting *N. lactamica* colonization, and thereby the development of anti-meningococcal immunity, within the context of an acute IMD control strategy, the same process has not been studied on a population level and in the context of changing antibiotic prescribing patterns [16].

Through the development of an agent-based model (ABM), the goal of this research was to consider if, and to what extent, increases in the population use of BSA among children younger than five years of age impacted the epidemiology of IMD [5]. Specifically, we aimed to construct a model that accurately captured the essential epidemiological components and natural history of IMD, through which we could assess the association between increasing population BSA use and the prevalence of colonization, proportion of children acquiring natural immunity, and occurrence of invasive disease among children younger than five years of age.

Materials and Methods

Classes and Parameters

In addition to the overall model, this ABM consisted of three classes of objects: children, bacteria strains, and contact groups. An overview of defining class parameters and values used for these experiments is shown in Tables 4-1 through 4-4. An overview of the entire model is shown in Figures 4-1 through 4-11.

Initialization and Input

At model setup, two bacterial strains were created (Table 4-3). The duration of colonization for the commensal bacteria strain was 114 days (3.8 months) and that of the pathogenic bacteria strain was 123 days (4.1 months) [28]. The probability of inducing immunity upon acquisition was 0.4 for the commensal strain and 0.67 for the pathogenic strain [30]. Parameters for the probabilities of transmission per contact and of developing invasive disease were obtained from an ordinary differential equation (ODE) model of transmission and disease (Appendix).

Children were then created and added to the model (Figure 4-3), up to the number of children indicated by the model parameter value (Table 4-1). First, individuals were assigned an annual frequency of antibiotic use (regardless of BSA status) based on a probability (p), such that each child could receive no antibiotic prescriptions per year (p=0.28), one (p=0.22), two (p=0.15), three (0.10), or four (0.25) [9, 24]. For this model, antibiotics were assumed to be prescribed at uniform time intervals, calculated as 365 / (annual frequency of antibiotic use) and each child was assigned the time at which they would receive the next dose of antibiotics with a uniform probability distribution from zero to this calculated value. Second, an age was assigned with a uniform probability distribution from 0 to 1825. Third, each child had an age-dependent probability of having already acquired immunity against invasive disease, such that, for a child less than a year, p=0.05; for a one year-old child, p=0.10; for a two year-old child, p=0.15; for a three year-old child, p=0.20; and for a four year-old child, p=0.25 [29]. Fourth, each child had a probability of being colonized with either the commensal (p=0.1056) or pathogenic (0.008595) bacterial strains [67]; for colonized agents, the respective strain type was

assigned, as was the time at which they acquired a colonization state, which was based on a uniform probability distribution from the negative of the strain-specific duration of colonization to 0. Fifth, fifty percent of individuals younger than 183 days of age were considered to have passive immunity from birth [28, 29].

Children were then added to household and child care arrangement contact groups (Figures 4-4 and 4-5). The size of each household (i.e. the number of zero to four year-old children within each household) ranged from one to three, which was assigned with an equal probability to each value. As multiple children in a single household would be expected to be enrolled in similar child care arrangements, a household care status was established, such that each household could be enrolled in a small group (SG; p=0.13), day care center (DCC; p=0.14) or preschool only (PS; p=0.23) (Enrollment in a child care group was also age-dependent: children zero to two years of age enrolled in either SG or DCC would also attend PS at three years of age). Each child was added to a house that was not yet at capacity; once the capacity of a house was reached, a new house was created.

Children added to households participating in a formal child care arrangement were subsequently added to a child care group (Figure 4-5). Children three years of age would be added to PS; those zero to two years of age would be added to either SG or DCC, depending on the child care status of the household. Each child would be added to an open child care group of the correct type; once a group was full, a new group was created with a size of six (SG), 12 (DCC), or 25 (PS) (Personal Communication, Mark Sullivan, MS; Executive Director, Michigan Community Coordinated Child Care Association).

Process Overview

The model proceeded in time steps, each representative of one 'real-world' day (Figure 4-1). At each time step, children entered the model, based on the birth rate parameter (Table 4-1, Figure 4-2). This process was the same as indicated above, except the age of the child was set at zero days, there was zero probability of having acquired immunity at birth, and the child was born susceptible to colonization (Figure 4-3). Second, children who reached their fifth birthday were removed from all contact groups, as well as the model, while those who reached their third birthday and were in a household that participates in a formal child care arrangement were enrolled in PS (Figure 4-6). Third, each child had the chance to update colonization status, and disease and immunity states (with the exception of new acquisition of bacterial colonization), based upon the natural history of colonization and disease (Figure 4-7). Fourth, each child had a probability of receiving a dose of antibiotics which, if BSA, resulted in the loss of colonization and temporary resistance to recolonization (Figure 4-8). Fifth, the transmission of bacterial strains, with subsequent acquisitions of new colonization states, was captured based on the number of contacts each child had with other children in the household, child care arrangement group, the entire community of zero to four year-olds, and other individuals not explicitly represented in the model (Table 4-1; Figure 4-9). Finally, all individuals who acquired a new colonization synchronously updated their colonization state (Figure 4-11) and individuals incremented their age. Each model was run for 3650 time steps, reflective of 10 years of real time. Experimental results were

measured during the observation period – time steps 1826 to 3650 – to reflect the system at equilibrium.

Design Concepts

Emergence

The prevalence of commensal and pathogenic strain colonization, the proportion of children having had a previous episode of commensal colonization, the proportion of children having acquired natural immunity against invasive disease, and the number of cases of invasive disease were emergent properties of the model, dependent upon the characteristics and behaviors of individuals, with regard to bacterial strains and contact groups.

Adaption

Individuals acquired natural immunity against invasive disease in response to being colonized by either commensal or pathogenic bacteria. Development of immunity conferred complete protection against invasive disease upon subsequent acquisitions of the pathogenic strain. Individuals who did not develop immunity during colonization were allowed to develop immunity upon repeat colonization events with equal probabilities.

Stochasticity

The assignment of individual characteristics at initialization (i.e. colonization state) and behaviors during the model run (i.e. acquisition of colonization) were

stochastic processes. For each of these processes, a random number was generated and compared to the respective parameter value to determine the output. For example, when a colonized individual made contact with a susceptible individual, a random number was generated from 0 to 1. If this number was less than the per-contact probability of transmission of that bacterial strain, the susceptible individual became colonized; otherwise it remained susceptible. Since each model run was driven by these stochastic processes, results varied between runs. Final results were based on observations averaged across 100 model runs.

Observations

At the end of each time step during the observation period, the following outcomes were measured: the proportion of individuals colonized by commensal and pathogenic strains, the proportion of individuals having had at least one prior episode of commensal strain colonization, the proportion of individuals having developed protective immunity against invasive disease, and the total number of cases of invasive disease. Each of these outcome measures were provided for the overall population, by year of age, by type of child care arrangement, and by the number of annual doses of antibiotics received.

Sub-models

Transmission

The model included multiple levels of contacts to capture transmission of the strains in the population at each time step (Figure 4-9). First, each individual made

contact with every other individual aged zero to four in their household. Second, each child in a child care group made contact with other children in the child care group, as such: all children in small groups and children in either day care or preschool groups with less than six other children would make contact with all other children in the group; children in day care or preschool groups with greater than six other children would make contact with three. Third, each individual was allowed to initiate contact pairs with all other children in the model, calculated as one half of the number of contacts, based on the user-entered parameter. For each of the pairs initiated, a second individual was chosen at random from the list of all individuals, although one individual could not initiate a pair with the same second individual more than once per time step.

For each contact pair, the colonization states of both individuals were assessed and, for pairs in which one was colonized and the other was susceptible to colonization, a contact event was initiated. In each contact event, the colonizing bacterial strain type was determined. The commensal bacterial strain was acquired by the susceptible individual with probability, $p=0.001855$ and the pathogenic bacterial strain was acquired with probability, $p=0.0002048$ (Appendix). Acquisition of colonization was captured in a temporary variable that allowed for synchronous updating after all individuals had initiated contact events with their selected pairs.

At the beginning of each time step, colonized individuals were assessed and those for whom their length of colonization was greater than the type-specific duration of colonization became uncolonized and susceptible to recolonization.

Invasive Disease

Colonized individual who had acquired a bacterial strain during the previous time step were assessed for development of invasive disease, which occurred with probability, p = 0.006096 (Appendix). Individuals developing disease would recover the following time step and remain unsusceptible to further colonization for 21 time steps, reflective of the rapid entry into the health care system and receipt of antibiotics to eradicate *N. meningitidis* carriage, respectively. Individuals who did not develop invasive disease during the time step following bacterial acquisition were considered to have no risk of developing disease throughout the remaining duration of colonization [45].

Immunity

Individuals had a probability of developing immunity against invasive disease on day 14 after becoming colonized, such that p=0.4 for individuals colonized with the commensal strain and p=0.67 for those colonized with the pathogenic strain [28, 29, 34, 51, 54, 71]. Upon further episodes of acquisition of the pathogenic strain, immune individuals had no probability of developing disease, whereas the probability of developing invasive disease among the non-immune was unaffected. The probability of the development of immunity was constant and independent of previous episodes of colonization.

Antibiotic Prescribing and the Colonization State

During each time step, individuals were assessed to determine whether or not they were to receive a prescription for antibiotics, based upon the time of next scheduled dose.

If the antibiotic dose received was broad-spectrum (determined stochastically based on the BSA parameter value), any colonizing strain was lost and the individual could not be recolonized for 21 days. No changes to the colonization state or susceptibility to recolonization were made after the administration of non-BSA doses. Once an antibiotic was prescribed, the timing of the next antibiotic dose was recalculated. Upon development of invasive disease, individuals who recovered and were also assumed to have been given appropriate prophylactic antibiotics and could not be recolonized for 21 time steps.

Ordinary Differential Equation (ODE) Models

To inform the ABM transmission model, an ODE model of circulation of two bacterial strains in a population was constructed and equations were solved at equilibrium to determine the strain-specific, per-contact probabilities of transmission and probability of developing invasive disease (Appendix). The ODE model was compared with a sub-model of the ABM (with no immunity) to verify accuracy of the ABM.

The per-contact probability of transmission of the commensal strain was determined by solving the ODE model, assuming the commensal prevalence of colonization among zero to four year-old children was 10.56% and that among those five years and older was 2.4% [67]. The per-contact probability of transmission of the pathogenic strain was determined by solving the ODE model, assuming the pathogenic prevalence of colonization among zero to four year-old children was 0.86% and that among those five years and older was 12.3% [67]. The probability of developing invasive disease was first determined in the absence of immunity, to result in 65 cases of invasive

disease occurring over 1825 ODE days (annual incidence rate of 13 cases per 100,000 among those 0 to 4 years of age) [11]. In later iterations of the ABM, the probability of invasive disease was adjusted to account for the presence of immunity (Appendix).

Statistical Analysis

The ABM was programmed in Java v 6.0 (Sun Microsystems, Inc., Santa Clara, CA, USA) using Eclipse SDK v 3.3.2 (Eclipse Foundation, Inc., Portland, OR, USA) and the Repast Modeling Toolkit v 3 [53]. Statistical analysis was conducted using SAS v. 9.1.3 (SAS Institute, Inc., Cary, NC, USA).

The prevalence of colonization (overall and strain-specific), the proportion of children having had at least one prior episode of commensal colonization, the proportion of children having acquired natural anti-meningococcal immunity, and the five-year incidence of invasive disease per 100,000 were obtained by averaging the results for each of these variables across 100 model runs for each BSA level. We constructed general linear regression models to assess the effect of age, child care arrangement, and annual number of antibiotic doses received (independent variables in each of three respective models) on the outcome measures (dependent variables) in the absence of BSA. Multivariate models with BSA level and age, child care arrangement, and annual number of antibiotic doses received, respectively, as independent variables and the outcome measures, as dependent variables, were developed to determine if the effect of BSA was different within different categories (i.e. interaction). Age, child care arrangement, and annual number of antibiotic doses were modeled as categorical variables; BSA was modeled as continuous.

Results

Overall results for the total and strain-specific prevalences of colonization, the proportions of children having had a previous episode of commensal colonization and having acquired natural immunity, the five-year incidence of invasive disease, and the mean age of invasive disease cases, by percent population BSA use, are shown in Table 4-5.

With no BSA use in the population, the overall prevalence of colonization among children less than five years was 8.66% (95% Confidence Interval [CI] 8.64, 8.68). This included 7.75% (CI 7.74, 7.77) of the population colonized with the commensal strain and 0.909% (CI 0.907, 0.910) colonized with the pathogenic strain. Almost two fifths of the population, 39.58% (CI 39.53, 39.64), had had at least one previous episode of commensal colonization and 19.37% (CI 19.35, 19.39) had developed immunity against invasive disease. Over the five-year simulation period, an average total of 65.8 cases (CI 64.0, 67.7) of invasive disease occurred, corresponding to an equivalent 5-year incidence per 100,000 and an average annual incidence rate of 13.2 cases per 100,000. The mean age of invasive disease cases was 2.5 years (886 days, CI 875, 897).

The prevalence of colonization increased from 6.4% among those less than one year of age to 10.9% among those four years of age; the proportion of children having had at least one prior episode of commensal colonization increased from 10.0% of those less than one to 65.5% of those four years of age; and the proportion of children with immunity against invasive disease increased from 4.8% of those less than one to 31.3% of those four years of age (Figure 4-12). The 5-year incidence of invasive disease was highest among children one year of age (73.5 per 100,000) and lowest among children

four years of age (59.4 per 100,000). All four outcome measures were significantly associated with age, at $p<0.0001$, in a general linear regression model.

All four population outcome measures were also significantly associated with type of child care arrangement at $p<0.0001$ (Figure 4-13). The prevalence of colonization and the proportions of children having had a previous episode of commensal colonization and having acquired natural immunity were lowest among those zero to two years of age, not in child care and those in small group settings; followed by those three to four years of age, not in child care; those in day care centers; and those in preschool. The five-year incidence of invasive disease was highest among those in a small group setting; followed by those zero to two years of age, not in a child care setting; and those in day care, preschool, or three- and four-year olds not in a formal child care arrangement.

The prevalence of colonization ($p=0.95$), the proportion of children with at least one previous episode of commensal colonization ($p=0.56$), the proportion of children with immunity against invasive disease ($p=0.23$), and the five-year incidence of invasive disease ($p=0.25$) were not associated with the annual number of antibiotic doses received when none of these doses were BSA (Figure 4-14).

Increasing BSA use in the population significantly decreased the prevalence of colonization, the proportion of children with at least one prior episode of commensal colonization, and the proportion of children with immunity against invasive disease and significantly increased the five-year incidence of invasive disease and the mean age of invasive disease cases (Table 4.2). Every percentage increase in population BSA use led to a decrease of 0.1% in the overall prevalence of colonization. However, the decrease in colonization per percentage increase in population BSA use, by strain, was

disproportionate: that of the commensal strain decreased 0.099% and that of the pathogenic strain decreased 0.0030%. Consequently, as a proportion of all children colonized, the pathogenic strain increased 0.12% per percentage increase in BSA prescribed. The proportions of children under five who had been previously colonized with the commensal strain, at least once, decreased 0.30% and who had acquired natural immunity against invasive disease decreased 0.11% per unit increase in the percentage of population BSA prescribed. The parameter estimate for the effect of increasing BSA use on all of the above outcome measures was significant in the respective regression models at $p<0.0001$. Additionally, each percentage increase in population BSA use led to 0.082 additional cases of invasive disease and an increase in the mean age of invasive disease cases of 0.80 days ($p=0.041$ and $p=0.0097$, respectively).

Increasing population BSA use impacted the total prevalence of colonization, the proportion of children with natural immunity, and the five-year incidence of invasive disease, by age, child care arrangement, and annual number of antibiotic doses received. At 20% population BSA use, the associations seen by age and child care arrangement were the same as those seen when no BSA were prescribed (data not shown). However, all four outcome measures became significantly associated with the annual number of antibiotic doses received ($p\leq0.024$ for each) (Figure 4-15). The prevalence of colonization and proportions of children having had a previous episode of colonization and having acquired immunity against invasive disease decreased among children having received an increasing number of antibiotic doses, while the highest five-year incidence of invasive disease was seen among those not receiving any doses of antibiotics.

The effect of increasing BSA use on four outcome measures was assessed through a series of multivariate linear regression models. As the effects of the independent variables on the proportions of children having had a previous episode of commensal colonization and having acquired natural immunity were similar, only results for the latter are presented.

Age and increasing population BSA use were independently associated with each of the three outcome measures ($p<0.0001$ for the main effects in the three models). However, greater decreases in the prevalence of colonization with increasing BSA use were seen among older children than among younger children ($p<0.0001$ for the interaction term), while the proportion of children acquiring immunity decreased to a greater extent among younger than older children ($p<0.0001$) (Figure 4-16). While the effect of increasing BSA use on the five-year incidence of invasive disease varied by year of age, this interaction was non-significant ($p=0.066$).

A similar pattern was seen in the with child care arrangement, with respect to the results of the multivariate models. Specifically, type of child care arrangement and increasing BSA use were independently associated with the prevalence of colonization, the proportion of children having acquired immunity, and the five-year incidence of invasive disease ($p\leq0.026$ for the main effects in the three models); and child care arrangement and increasing population BSA use significantly interacted, with respect to the first two outcome measures ($p<0.0001$ for each interaction term), but not the latter ($p=0.15$). A decrease in the prevalence of colonization was greatest among children in day care and preschool, while a decrease in the proportion of children having acquired

immunity was greatest among children in day care or small groups and among zero to two year olds not in a formal child care arrangement (Figure 4-17).

The annual number of antibiotic doses received was independently associated with the prevalence of colonization ($p<0.0001$) and the five-year incidence of invasive disease ($p=0.022$), but not the proportion of children with acquired immunity ($p=0.59$), and increasing population BSA use was independently associated with the prevalence of colonization ($p<0.0001$) and the proportion of children with acquired immunity ($p<0.0001$), but not the five-year incidence of invasive disease ($p=0.27$). In all three models, the independent variables significantly interacted ($p<0.0001$ for each). Consequently, the decrease in the prevalence of colonization and in the proportion of children immune with increasing population BSA use was greater with increasing annual number of antibiotic doses received, while the increase in invasive disease was greatest among those not receiving any antibiotics (Figure 4-18).

Discussion

These results demonstrate the capturing of the essential epidemiological components underlying IMD among children younger than five years of age by the model and, in so doing, represent the mechanism by which increasing non-prophylactic use of BSA among children in this age group may be altering the epidemiology of IMD in the United States. Further, through its effect at decreasing the prevalence of circulating bacterial strains in the population, increasing BSA use may be an emergent environmental risk factor for IMD in the United States, as it may lead to a decrease in the proportion of children acquiring natural immunity and, therefore, an increased occurrence

of invasive disease. These effects, disproportionate by age, child care arrangement, and annual number of antibiotic doses received, may have implications for public health interventions against IMD.

The congruence between experimental results with no BSA use and real-world, empirical observations from prior to their widespread use in the population supports the accurate capturing of the essential epidemiological dynamics of *N. meningitidis* and its related commensal species, *N. lactamica*, by the model. For instance, the prevalence of pathogenic strain colonization in this model, 0.91%, is similar to the 0.4-2.1% prevalence of *N. meningitidis* isolated from children younger than five years in previous studies; the proportion of four year-olds in this model having had at least one episode of commensal strain colonization, 65.5%, is similar to the proportion of children observed to have had at least one episode of *N. lactamica* colonization by age four (59%); and the average increase in immunity against invasive disease for each increasing year of life, 6.6%, and the proportion of four year-olds with immunity against invasive disease, 31.3%, in the model are consistent with previous studies observing a 5% increase in population serum bactericidal activity per year of age and 20-40% of children with serum bactericidal activity against meningococcal strains at their fifth birthday [7, 28, 29]. That the prevalence of colonization was higher among those in a larger day care setting is also consistent with higher meningococcal colonization rates seen in semi-closed communities [68]. The incidence of invasive disease in this model was similar to that noted prior to widespread use of BSA and the general trend of increasing occurrence after birth, followed by a decline, was also seen in our results [10, 56].

One observation requires further consideration: this model found a higher occurrence of invasive disease among children not in a formal child care setting, in contrast to higher incidence rates of IMD noted among those in more crowded settings, such as in homes with more than four people and among military recruits, college students living in dorms, black individuals, and those of low socioeconomic status [14, 28, 48, 56, 57]. However, those situations reflect increased number of contacts among older individuals, among whom the prevalence of potentially pathogenic *N. meningitidis* is high, relative to among those younger than five [67]. With fewer same-age contacts in this model, children not in a formal child care arrangement had increased exposure to the pathogenic strain and decreased immunity, leading to the higher occurrence of disease.

Increasing population BSA use led to a decrease in both overall and strain-specific prevalences of colonization. While decreased meningococcal colonization has been noted after mass prophylaxis with rifampin and ciprofloxacin in the context of IMD control, this was the first study to demonstrate similar effects due to increasing non-prophylactic use of BSA in the population [38]. Reducing the prevalence of colonizing organisms, the driving force in the development of anti-meningococcal immunity within an individual, led to a lower proportion of the population with immunity and an increased occurrence of invasive disease. While this study is the first to investigate this phenomenon, the link between decreased population immunity and changes in the epidemiology of IMD is not without precedent. Periodic waves of hyperendemic or epidemic meningococcal disease, occurring outside of the "meningitis belt" of sub-Saharan Africa in roughly 10-year cycles, are thought to be due to the introduction and spread of a new clonal strain of *N. meningitidis* through a non-immune population [26,

61]. In addition to overall higher incidence, the epidemiology of IMD during these waves differs from sporadic cases occurring during interepidemic periods, in that incidence increases disproportionately among adolescents and young adults – older age groups than are typically affected [4]. Although the underlying processes differ, that these two observations were also reflected in the results strengthen our conclusions.

The decision to include only children younger than five years in the model was made for several reasons. The process of acquisition of anti-meningococcal immunity begins at early ages in children, among whom the frequency of antibiotic use is highest [9, 13]. Consequently, the impact of increasing BSA use on the acquisition of immunity is expected to be most pronounced among this group. Further, empirical observations of antibiotic use, contact patterns, prevalence of *N. meningitidis* and *N. lactamica* colonization, and the proportion of individuals with documented anti-meningococcal immunity are more plentiful in the literature, relative to the availability of comparable information among adults. By better informing parameters, results consist with empirical observations increase the validity of our conclusions.

This decision, however, required the assumption of constant epidemiological dynamics of the commensal and pathogenic strains among those not represented in the model (i.e. those five years and older). From 1995-2002, a significant increase in BSA use among adults was seen [22, 58]. Although they receive fewer antibiotic prescriptions than children, an association between increasing BSA use and decreasing prevalence among those five years and older would not have been captured by our assumption. With regard to the model presented, this would have had two consequences. First, through decreased transmission of organisms to children younger than five, the prevalence of

colonization and proportion of children acquiring immunity among our study population would have decreased to a greater extent than we observed. Second, with the prevalence of colonization of *N. meningitidis* higher among adults than among children younger than five, reducing this potentially-pathogenic organism would be expected to lead to a lower occurrence of IMD, rather than the increase noted here. Until the late 1990s, the incidence of sporadic IMD in the United States had remained constant at 0.9-1.5/100,000 for almost 50 years [56, 57]. From 2000 to 2006, the overall incidence of IMD decreased 63%, from 0.8 to 0.3 cases per 100,000, with a 72% decrease among those younger than five years of age [11, 12]. While this decrease has correlated with increasing population BSA use, the dearth of epidemiological research into this question makes it impossible to assess if, and to what extent, the former is attributable to the latter. However, such a herd immunity effect was documented after routine vaccination with a univalent, serogroup C meningococcal conjugate vaccine in the United Kingdom, supporting this possibility [2, 39, 41, 50].

Two additional model limitations were unlikely to drastically alter our conclusions. First, children in the model are independently assigned an annual number of antibiotic doses received as well as a child care arrangement. In reality children in day care receive antibiotics more frequently than other children [66]. The likely effect of this limitation is an underestimate of the interaction between increasing population BSA use and child care arrangement. Second, while household and child care contact groups add complexity to model contact patterns, they likely do not sufficiently account for variations in contact types and networks throughout the five year age spectrum. Consequently, the steadily increasing commensal colonization rates seen in the model

with increasing age was not reflective of the typical pattern of *N. lactamica* colonization, which peaks between 18 and 24 months of age [7, 28, 33]. However, since having had a previous a previous episode of colonization and having acquired natural immunity against invasive disease reflect cumulative exposure to circulating organisms and since increasing BSA use does not alter contact patterns, the trends also would be expected to hold.

Beyond adding to the discussion of the importance of the judicious use of antibiotics, especially among young children, the impact of increasing BSA use on the epidemiology of IMD has implications regarding public health surveillance and control interventions. Lower population immunity may lead to increased potential for meningococcal epidemics, upon introduction of a new meningococcal clone, and meningococcal clones may persist for longer periods of time in a population with a reduced overall prevalence of colonization, as genetic recombination between different meningococcal strains would be less frequent [23, 40, 62]. Characterization of shifts in the epidemiology of IMD, including molecular characterization of circulating strains, will be of increasing importance in the era of increasing BSA use. Additionally, a tetravalent meningococcal conjugate vaccine (MCV4), licensed for use in the United States in 2005, is currently recommended for routine vaccination of individuals 11-55 years of age and for vaccination of children aged two to 10 years at increased risk of disease [13, 14]. A changing epidemiology of IMD, including the identification of individuals disproportionately affected by the increasing BSA use, may require expanding the definition of children at increased risk of disease and/or recommending routine MCV4 vaccination for younger children.

The results of this model suggest that, at the population level, increasing BSA use represents a novel environmental risk factor for IMD. While this study is an important first step in demonstrating the mechanism by which BSA use may be altering the epidemiology of IMD, additional research is necessary to corroborate these findings.

Appendix

Introduction

Compartmental models, described by ordinary differential equations (ODE), are used in many disciplines to understand underlying processes or to make predictions. In epidemiology, one such model – the Susceptible-Infected-Recovered (SIR) model – is frequently used to represent disease transmission in a population. As ODE models are solvable mathematically, they provide a method to estimate certain parameters describing the model system. Further, comparable ODE and ABM results provide a method of model validation, increasing the confidence in the conclusions drawn by the ABM. In this project, two modified SIR models were developed to inform ABM parameters corresponding to the strain-specific, per-contact probabilities of transmission and the probability of invasive disease and to compare with early ABM iterations to verify, through multiple sources, the accurate capturing of the processes.

Materials and Methods

Model 1: Bacterial Transmission and Invasive Disease

The purpose of the first model was to inform the per-contact probability of commensal and pathogenic strain transmission parameters of the ABM, by capturing the underlying transmission dynamics and invasive disease process in a population without any immunity. The model is depicted in Figure 4-19 and is described with the following equations:

1.1 $$dS_0/dt = \alpha N - \beta_0 S_0 (c_0 * C_0/N + c_1 * \mu_0) - \beta_1 S_0 (c_0 * [C_1 + C_2]/N + c_1 * \mu_1) - \varepsilon S_0 + \gamma_0 C_0 + \gamma_1 C_1 + \gamma_2 C_2$$

1.2 $dC_0/dt = \beta_0 c_0 S_0 C_0/N - C_0(\gamma_0 + \varepsilon) + \beta_0 c_1 S_0 \mu_0$

1.3 $dC_1/dt = (1-\theta)\beta_1 c_0 S_0 (C_1 + C_2)/N - C_1(\gamma_1 + \varepsilon) + (1- \theta)\beta_1 c_1 S_0 \mu_1$

1.4 $dC_2/dt = \theta\beta_1 c_0 S_0 (C_1 + C_2)/N - C_2(\gamma_2 + \varepsilon) + \theta\beta_1 c_1 S_0 \mu_1$

The compartments reflect the numbers of susceptibles, S_0, those colonized with the commensal strain, C_0, and those colonized with the pathogenic strain without, C_1, and with, C_2, invasive disease. Each day, new susceptibles are born into the model at a rate given by the total number of individuals being modeled, N, times the proportion of those individuals entering the model per day, α. The number of susceptibles becoming colonized with the commensal strain each day occurs as a density-dependent rate of the product of the number of susceptibles, S_0, the per-contact probability of transmission of the commensal strain, β_0, and the number of contacts each susceptible makes time the proportion of those contacts that occur with others already colonized with the commensal strain. The latter term includes the number of contacts and proportion of contacts colonized among children younger than five years included in the model, c_0*C_0/N, and among individuals five and older, not included in the model ($c_1*\mu_0$). A similar process occurs with the pathogenic strain, although the per-contact probability of transmission, β_1, and the proportion of contacts colonized with the pathogenic strain among children under five years and among those five and over, $[C_1 + C_2]/N$ and μ_1, respectively, are different and the rate is further governed by the probability of developing invasive disease upon acquisition of the pathogenic strain, θ. Colonized individuals lose colonization and revert to susceptible at a density-dependent rate of the number of colonized individuals in each compartment, multiplied by the respective strain- and disease-specific proportion of colonized individuals losing colonization per day, γ_0, γ_1,

and γ_2. Additionally, individual leave the model at a rate given by the probability of an individual leaving the model per unit time, ε, times the number of individuals in each compartment.

The equations were solved for β_0, β_1, and θ in a community where N=10,000, α=1/1825, c_0=5, c_1=3, S_0=3607/4072, C_0/N=430/4072, $(C_1+C_2)/N$=35/4072, C_2=0.003562, μ_0=127/5367, μ_1=662/5367, γ_0=1/114, γ_1=1/123, γ_2=1, and ε=1/1825 [11, 28, 67]. These values were subsequently used as initial parameter estimates for the ABM. The model was run for 3650 time steps; within each model run, observations for the commensal and pathogenic prevalences of colonization were averaged over time steps 1826-3650, and the total number of invasive disease cases summed over this time period, to represent the model at equilibrium. Final results were obtained by averaging these results over ten model runs.

Model 2: Natural Immunity

The second ODE model built upon the first by incorporating the development of natural immunity against invasive disease into the underlying transmission and disease dynamics. It is depicted in Figure 4-20 and can be described by the following seven equations:

2.1 $$dS_0/dt = \alpha N - \beta_0 S_0(c_0[C_0 + C_3]/N + c_1\mu_0) - \beta_1 S_0(c_0[C_1 + C_2 + C_4]/N + c_1\mu_1) - \varepsilon S_0 + (1-\varphi_0)\gamma_0 C_0 + (1-\varphi_1)\gamma_1 C_1 + \gamma_2 C_2$$

2.2 $$dC_0/dt = \beta_0 S_0(c_0[C_0 + C_3]/N + c_1\mu_0) - C_0(\gamma_0 + \varepsilon)$$

2.3 $$dC_1/dt = (1-\theta)\beta_1 S_0(c_0[C_1 + C_2 + C_4]/N + c_1\mu_1) - C_1(\gamma_1 + \varepsilon)$$

2.4 $$dC_2/dt = \theta\beta_1 S_0(c_0[C_1 + C_2 + C_4]/N + c_1\mu_1) - C_2(\gamma_2 + \varepsilon)$$

2.5 $dS_1/dt = \varphi_0\gamma_0C_0 + \varphi_1\gamma_1C_1 - \beta_0S_1(c_0[C_0 + C_3]/N + c_1\mu_0) - \beta_1S_1(c_0[C_1 + C_2 + C_4]/N + c_1\mu_1) - \varepsilon S_1 + \gamma_3C_3 + \gamma_4C_4$

2.6 $dC_3/dt = \beta_0S_1(c_0[C_0 + C_3]/N + c_1\mu_0) - C_3(\gamma_3 + \varepsilon)$

2.7 $dC_4/dt = \beta_1S_1(c_0[C_1 + C_2 + C_4]/N + c_1\mu_1) - C_4(\gamma_4 + \varepsilon)$

This model is similar to the first model, with additional compartments added to reflect the number of immune individuals susceptible to colonization, S_1, colonized with the commensal strain, C_3, and colonized with the pathogenic strain, C_4. New individuals born into the model lack immunity against invasive disease and enter S_0. Non-immune susceptibles become colonized (and develop invasive disease) at respective rates identical to those described above. However, a proportion of those colonized with the commensal strain will acquire immunity at the end of their episode of colonization, noted by a probability, φ_0, and will revert to S_1. The remainder reverts to S_0. The same holds true among those colonized with the pathogenic strain and without invasive disease, for which the probability of acquiring immunity is given by φ_1. Diseased individuals are assumed to receive antibiotic treatment relatively soon after acquiring colonization, preventing the development of immunity. Therefore, all diseased individuals revert to S_0. Immune susceptibles become colonized at the previously-described, respective rates; invasive disease is not possible after acquiring natural immunity.

The equations were solved for β_0, β_1, and θ in a community where N=10,000, α=1/1825, c_0=5, c_1=3, $(S_0+ S_1)$=3607/4072, $(C_0+ C_3)/N$=430/4072, $(C_1+C_2+C_4)/N$=35/4072, C_2=0.003562, μ_0=127/5367, μ_1=662/5367, γ_0=1/114, γ_1=1/123, γ_2=1, φ_0=0.4, φ_1=0.87, and ε=1/1825 [11, 28, 30, 67]. These values were subsequently used as initial parameter estimates for the ABM. The model was run for 3650 time steps; within each model run, observations for the commensal and pathogenic prevalences of

colonization were averaged over time steps 1826-3650, and the total number of invasive disease cases summed over this time period, to represent the model at equilibrium. Final results were obtained by averaging individual run results over ten model runs.

Results

Results for the determination of model parameters, β_0, β_1, and θ, and a comparison between ODE and ABM Models 1 and 2 are shown in Table 4-6.

Discussion

The development of two ODE models provided parameter estimates for the commensal and pathogenic strain per-contact probabilities of transmission and the probability of developing invasive disease that were subsequently used in the development of the ABM. As the two models differ, with regard to how they represent underlying processes, an ODE model is unlikely to predict the exact outcome of an ABM simulation [55]. That we obtained similar results from both techniques demonstrated that the ODE and ABM models likely represented the same processes, support the verification of the ABM, and increase confidence that the conclusions drawn from the ABM are correct.

Table 4-1: Parameters and Parameter Values for the Overall Model Class.

Parameter	Description	Value
birthRate	Birth Rate of Children into the Model	numChildren/1825
bSPercent	Probability that an antibiotic prescribed is broad-spectrum	0.0 to 0.2[1]
careContacts	Number of contacts made with other children in a child care group	3[2]
commContacts	Number of contacts made with other children included in the model	2[2]
numChildren	Number of Children in the Model	100,000
othContacts	Number of contacts made with individuals not included in the model	3[2]
prevC	Prevalence of commensal strain colonization among those included in the model	430/4072[3]
prevExC	Prevalence of commensal strain colonization among those not included in the model	127/5367[3]
prevExP	Prevalence of pathogenic strain colonization among those not included in the model	662/5367[3]
prevP	Prevalence of pathogenic strain colonization among those included in the model	35/4072[3]
T	Time Step	0 to 3650[4]

[1]Increases in increments of 0.02; results averaged over 100 model runs at each increment.
[2]Unpublished Surveillance Data
[3]Trotter et al. [67]
[4]Increases incrementally through model run

Table 4-2: Parameters and Parameter Values for the Child Class.

Parameter	Description	Value
abxLevel	Number of antibiotic prescriptions received per year	0-4[1]
abxTime	Time step at which antibiotics are prescribed	Varies[2]
age	Age of the child, in days	0 to 1825[3]
bImm	Passive immunity state at birth	True False
cTime	Time step at colonization acquisition	*Assigned as shown in Fig. 4-10*
colState	Colonization state	Susceptible (S) Colonized (C) Resistant (R)
dxState	Disease state	Susceptible (S) Diseased (D)
immState	Acquired immunity state	True False
loseBI	Time step at which child loses birth immunity	91-182[3]
rTime	Time step at which child becomes resistant to colonization	*Assigned as shown in Fig. 4-8*
tempCol	Temporary colonization state variable, for use in synchronous updating of new colonization acquisitions	Susceptible (S) Colonized (C)

[1]Distributed such that the probability of receiving zero antibiotic prescriptions per year is 0.28; one prescription, 0.22; two prescriptions, 0.15; three prescriptions, 0.10; and four prescriptions, 0.25.
[2]The frequency of receiving antibiotic prescriptions (abxFreq) is calculated as 365/abxLevel. At initialization, abxTime is assigned based on a uniform distribution of –abxFreq to 0. During model run, abxTime is updated as shown in Figure 4-8.
[3]Assigned as shown in Figure 4-3. Age increases incrementally through model run.
[4]Uniformly-distributed.

Table 4-3: Parameters and Parameter Values for the Strain Class.

Parameter	Description	Value
sType	Strain type	Commensal (C) Pathogenic (P) none (null)
colDur	Duration of colonization	C: 114[1] P: 123
dxProb	Probability of causing invasive disease	C: 0[2] P: 0.006096
immProb	Probability of inducing immunity, upon colonization	C: 0.4[3] P: 0.67
probTx	Probability of transmission	C: 0.001855[2] P: 0.0002048

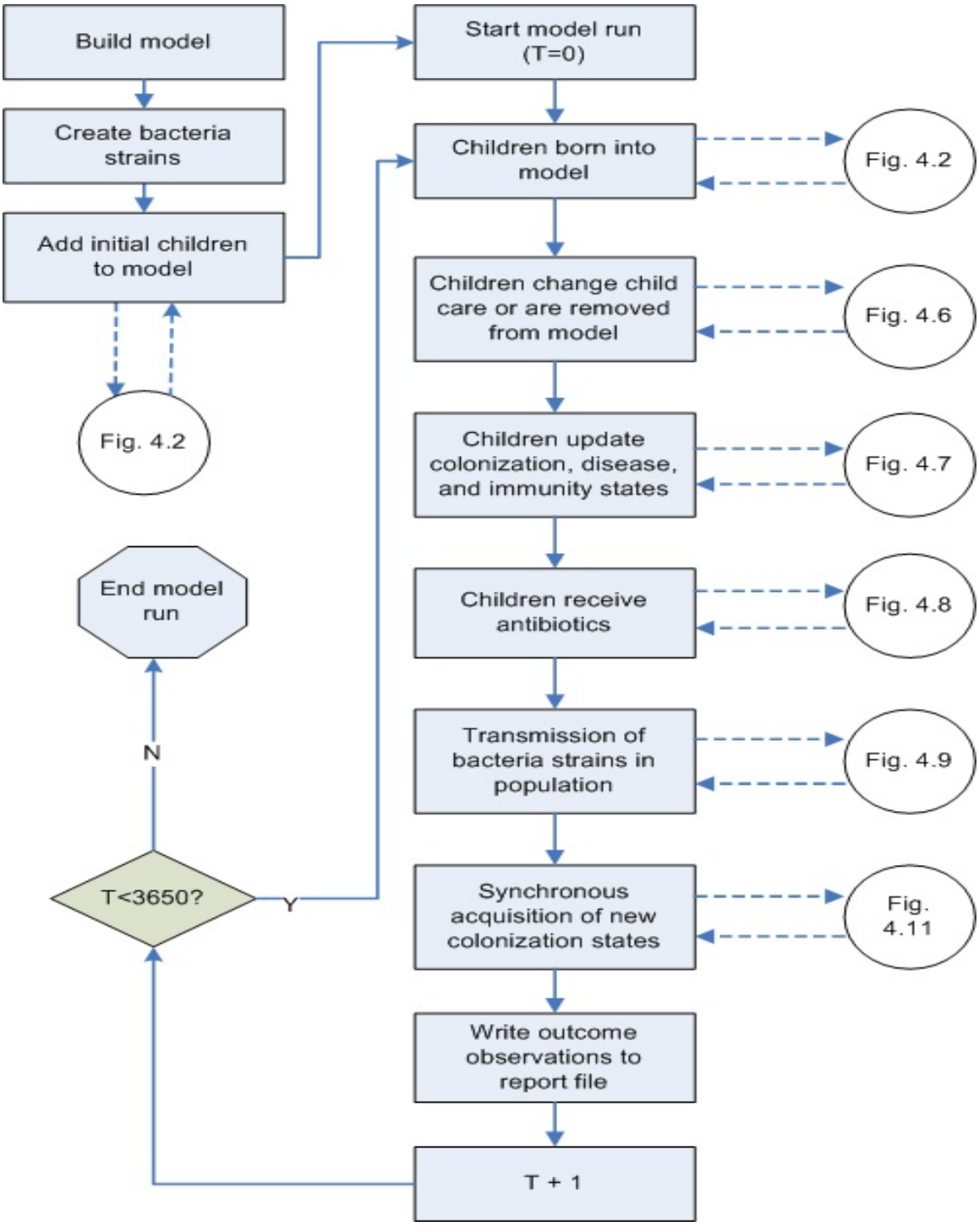
[1]Gold et al. [28]
[2]Determined through ordinary differential equation models – see Appendix.
[3]Golschneider et al. [30]

Table 4-4: Parameters and Parameter Values for the Contact Group Class.

Parameter	Description	Value
type	Type of contact group	Household (HH) Small group (SG) Day Care Center (DCC) Preschool (PS)
careStatus	Child care arrangement (HH only)	SG DCC PS[1]
size	Size of contact group	HH: 1-3[2] SG: 6 DCC: 12; PS 25

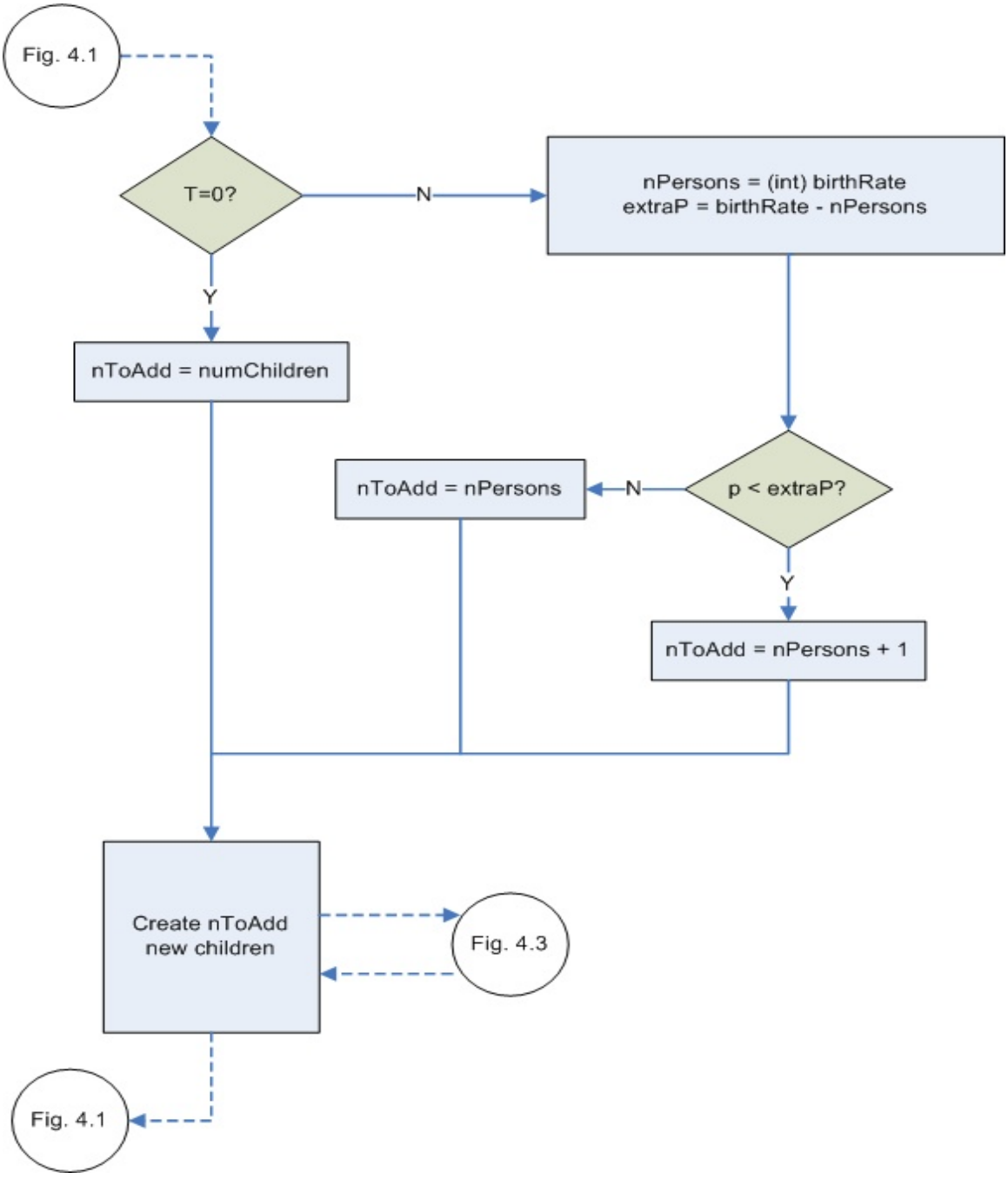
[1]Households are distributed as such: 0.13 assigned to SG; 0.14 assigned to DCC; and 0.23 assigned to PS. As all children in SG and DCC enter PS at three years of age, half of three and four year-olds are in PS.
[2]Uniformly-distributed.

Figure 4-1: Flowchart Depicting the Overview of the Model Process



Model parameters included in this flowchart: model time step (T). Subprocesses of the model are shown in the figures indicated. Additional parameters and parameter values are shown in Tables 4-1 to 4-4.

Figure 4-2: Flowchart Depicting the Process of Calculating the Number of Children to Add to the Model.



Model parameters included in this flowchart: model time step (T); the number of children in the community (numChildren); and the birth rate (birthRate). Parameter values are shown in Tables 4-1 to 4-4. Temporary method variables included in this flowchart: the number of children to be added in a given time step (nToAdd); the number of children to add during the given time step, equal to the integer value of the birth rate (nPersons); the probability of adding an additional child during the time step, calculated as shown (extraP); and a randomly-generated, decimal number for the decision process, with a uniform distribution of 0 to 1 (p).

Model parameters included in this flowchart (Figure 4-3): model time step (T); the prevalence of commensal strain colonization among those included in the model (prevC); and the prevalence of pathogenic strain colonization among those included in the model (prevP). Child parameters include: age of child in days (age), the time step at which antibiotics are prescribed (abxTime); acquired immunity state (immState), colonization state (colState), time step at colonization (cTime), passive immunity state at birth (bImm), and date at which child loses birth immunity (loseBI). Strain parameters include: strain type (sType). Shown values for colState include: susceptible (S) and colonized (C). Shown values for sType include: commensal (C), pathogenic (P), and none (null). Other parameter values are shown Tables 4-1 to 4-4. Temporary method variables include: a random integer representing the date at which the first antibiotic dose will be given (initAbxTime) for those who receive at least one antibiotic prescription per year, which has a uniform distribution from 0 to 365 divided by the number of antibiotic prescriptions received per year (abxLevel); a random integer representing the age for children added at initialization, with a uniform distribution of 0 to 1825 (initAge); a decimal number representing the probability of being immune upon model initialization, by year of age, with values of 0.05, 0.1, 0.15, 0.2, and 0.25 for years 0-4 (initImmProb); an integer representing the date at which a colonized child added at model initialization first became colonized, with a uniform distribution of 0 to -114 for the commensal strain or 0 to -123 for the pathogenic strain (initCTime); a random.integer representing the date at which passive birth immunity will be lost, with a uniform distribution of 91 to 182 (bImmDate); and randomly-generated decimal numbers for decision processes, with distributions of 0 to 1 (p_1-p_3). The values for abxLevel are found in Table 4-2.

Figure 4-3: Flowchart Depicting the Process of Adding New Children to the Model.

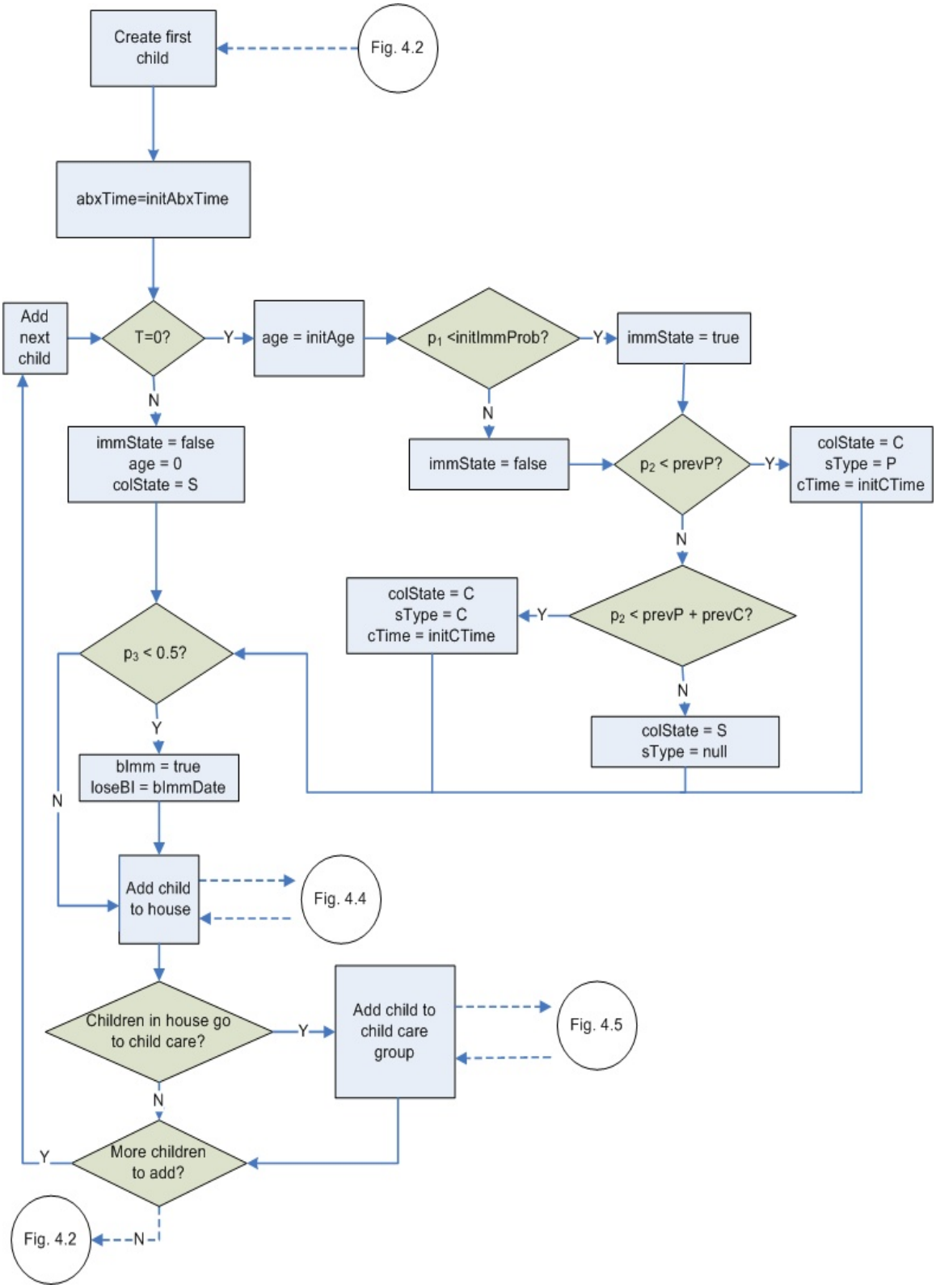
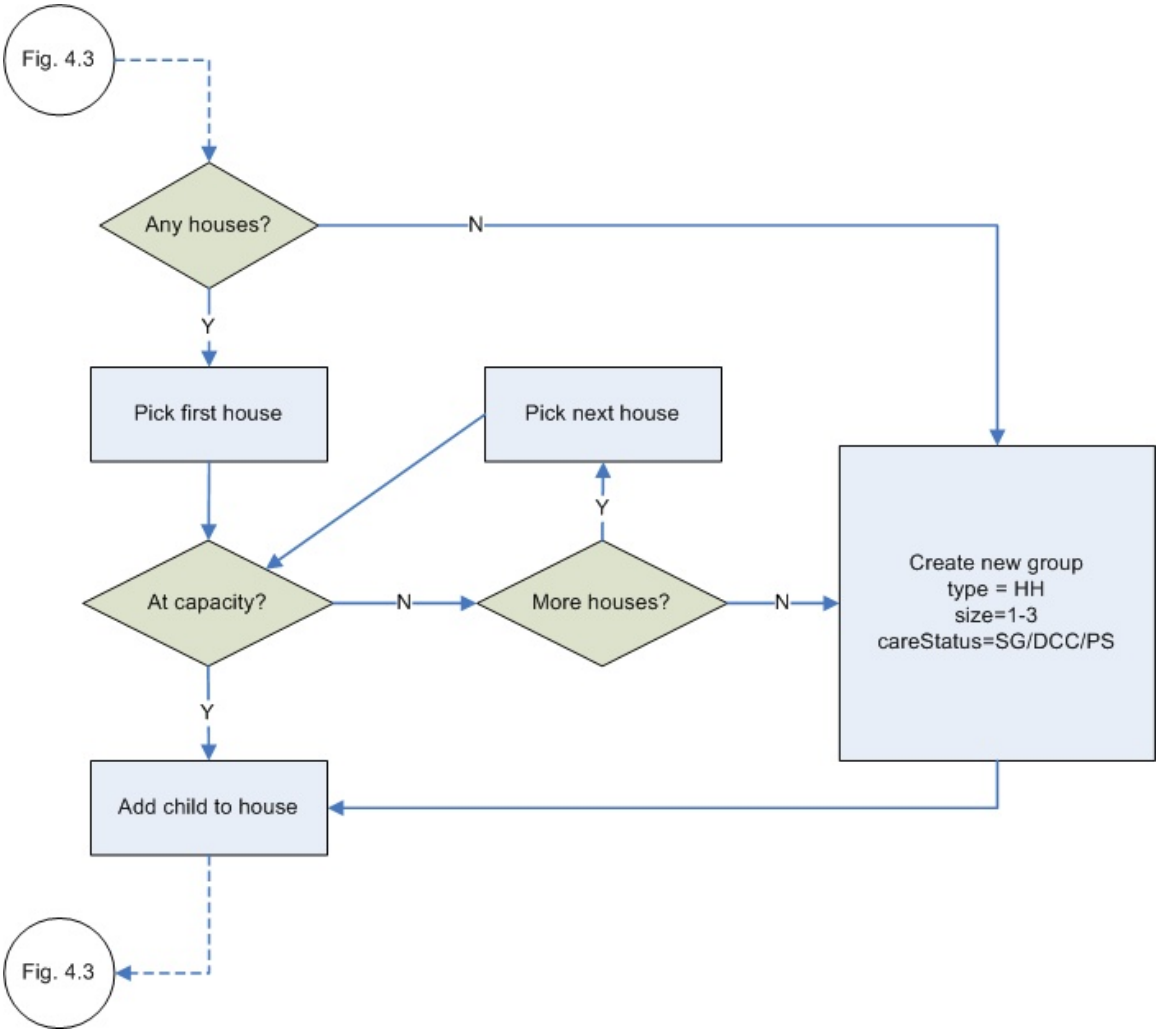


Figure 4-4: Flowchart Depicting the Process of Adding Children to a Household.



Contact Group parameters included in this flowchart: type (type), size (size), and child care arrangement (child care). Shown values for type include: household (HH); values for size are uniformly distributed from 1 to 3; values for careStatus are distributed with probability SG (0.13), DCC (0.14), and PS (0.5). Other parameter values can be found in Tables 4-1 to 4-4.

Model parameters included in this flowchart (Figure 4-5) include: model time step (T). Child parameters include: age of child, in days (age). Contact Group parameters include type (type), size (size), and child care arrangement status of households (careStatus). Shown values for type and careStatus are equivalent and include: small group (SG), day care center (DCC), and preschool (PS). Other parameter values are shown in Tables 4-1 to 4-4.

Figure 4-5: Flowchart Depicting the Process of Adding Children to a Child Care Arrangement Group.

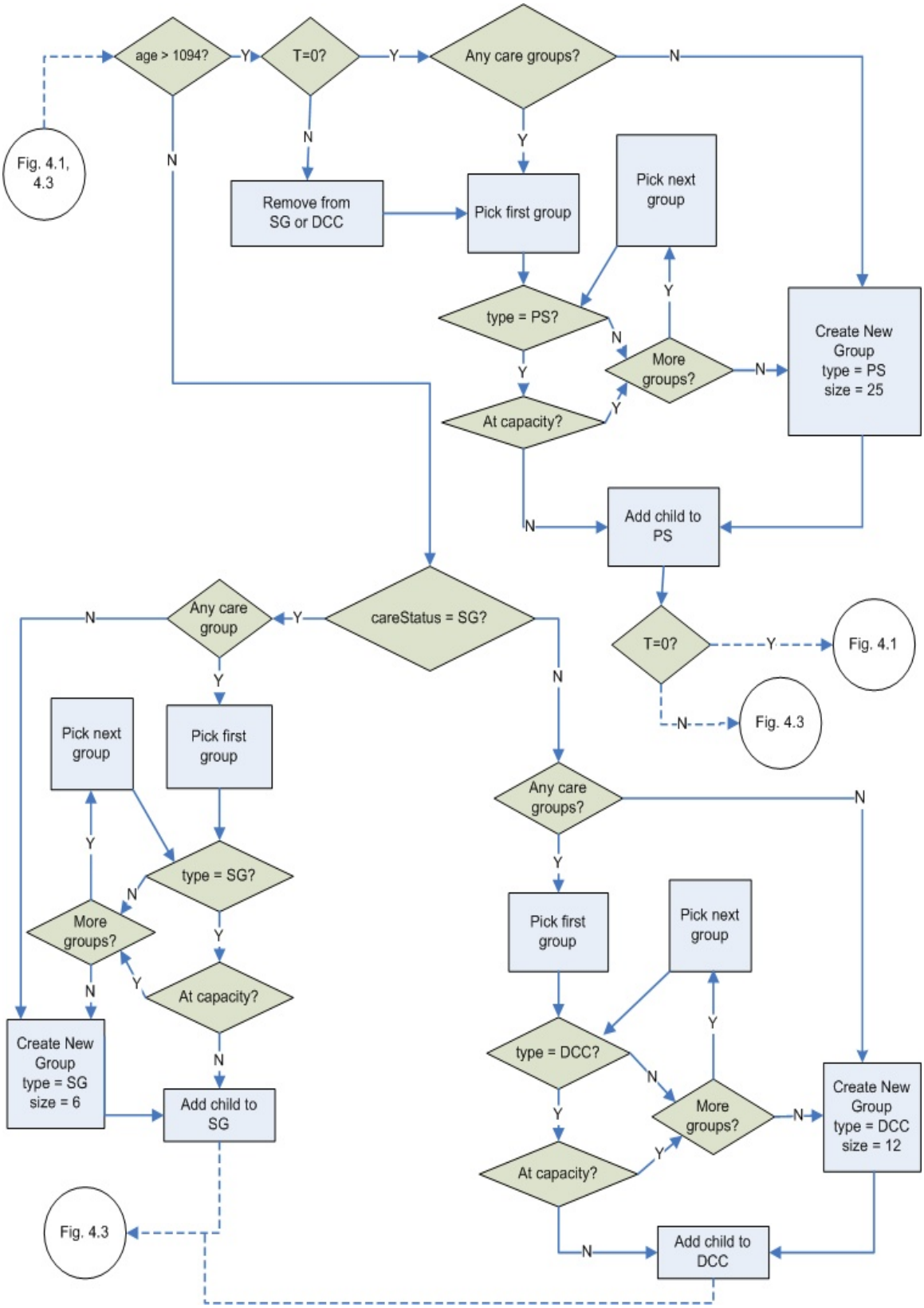
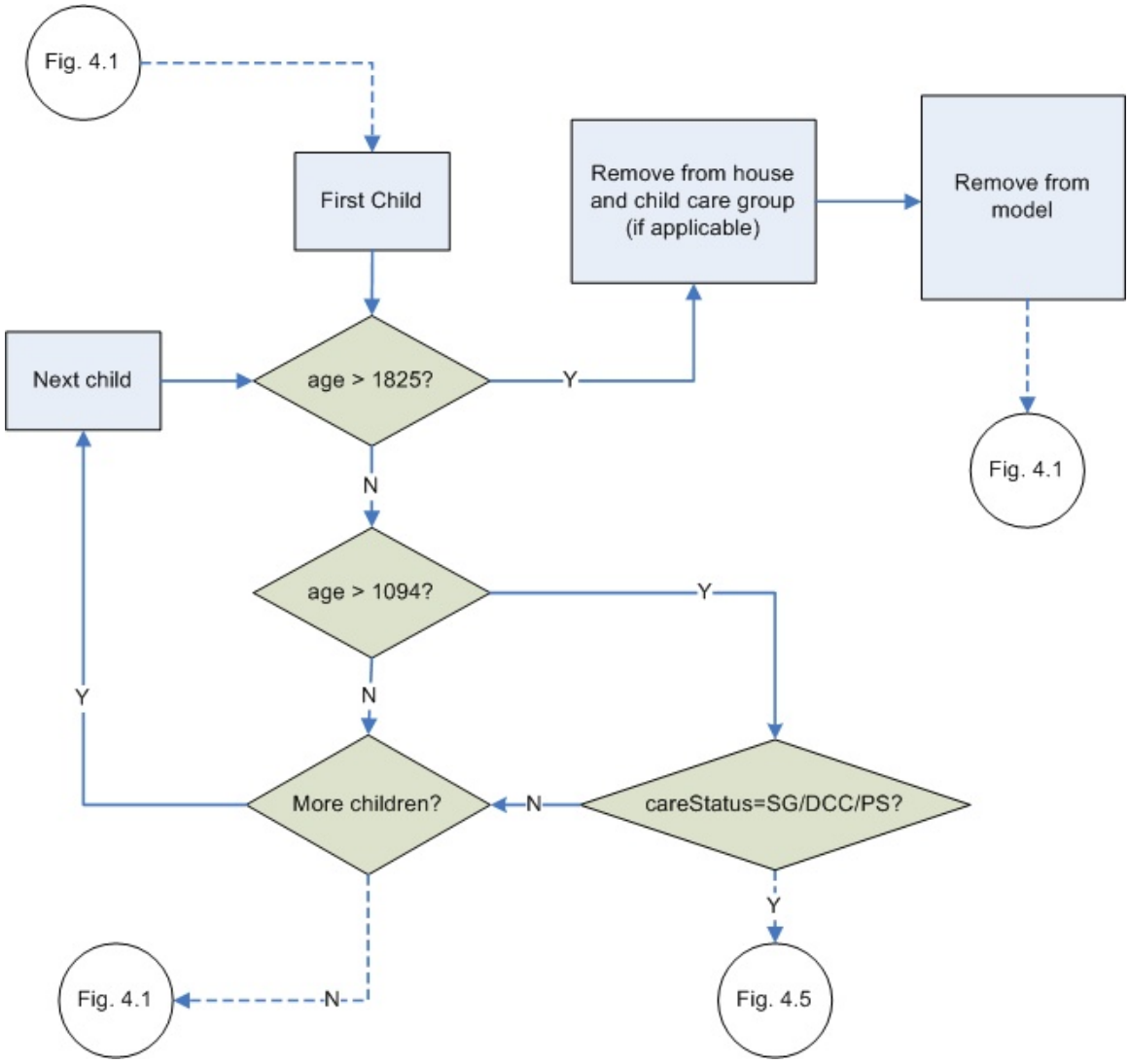


Figure 4-6: Flowchart Depicting the Process of Removing Children from the Model or Changing Child Care Arrangement Status.



Child parameters included in this flowchart include age of child, in days (age). Contact Group parameters include child care arrangement status of households (careStatus). Shown values for careStatus include: small group (SG), day care center (DCC), and preschool (PS). Other parameter values are shown in Tables 4-1 to 4-4.

Model parameters included in this flowchart (Figure 4-7): model time step (T). Child parameters included in this flowchart include: age of child, in days (age); date at which child loses birth immunity (loseBI); passive immunity state at birth (bImm); time at which child becomes resistant to colonization (rTime); colonization state (colState); disease state (dxState); time step at which child became colonized (cTime); and acquired immunity state (immState). Strain parameters included in this flowchart include probability of developing invasive disease (dxProb); the probability of acquiring immunity against invasive disease (immProb); and the duration of colonization (colDur). Shown values for colState include: susceptible (S), colonized (C), and resistant to colonization (R). Shown values for dxState include: susceptible (S) and diseased (D). Other parameter values are shown in Tables 4.1 to 4.4.

Figure 4-7: Flowchart Depicting the Process of Updating Colonization, Disease, and Immunity States During Each Model Time Step.

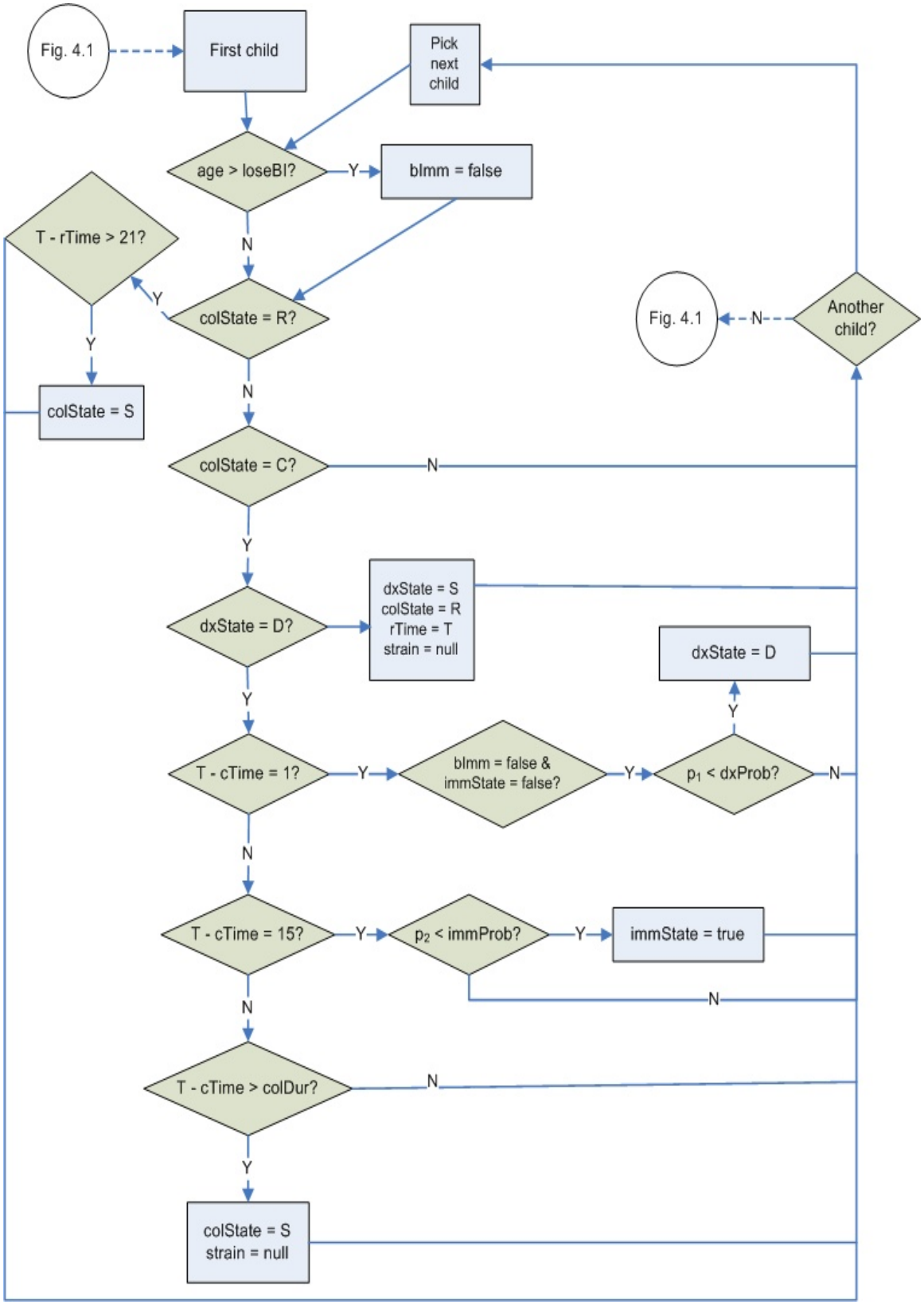
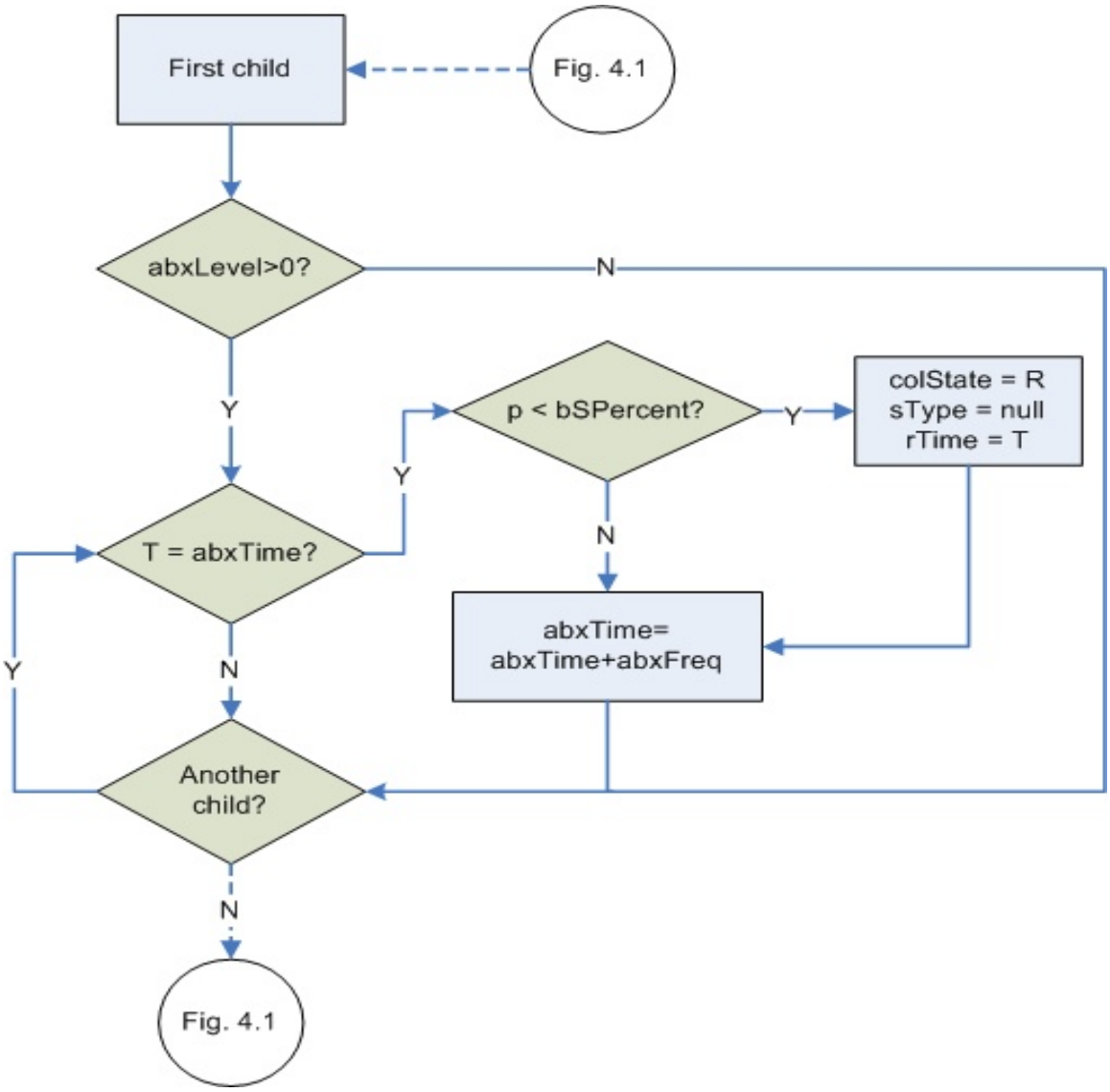


Figure 4-8: Flowchart Depicting the Process of Prescribing Antibiotics During Each Model Step.



Model parameters included in this flowchart: model time step (T) and the probability that a prescribed antibiotic is broad-spectrum (bSPercent). Child parameters included in this flowchart: number of antibiotic prescriptions received per year (abxLevel); time step at which an antibiotic is prescribed (abxTime); colonization state (colState); and time at which child becomes resistant to colonization (rTime). Shown values for colState include: resistant to colonization (R). Other parameter values are shown in Tables 4-1 to 4-4.Temporary method variables included: a randomly-generated decimal number for a decision process, with a distribution of 0 to 1 (p) and the frequency, in days, of receiving antibiotics (abxFreq). The value for abxFreq is calculated as the integer value of 365/abxLevel. Values for abxLevel are shown in Table 4-2.

Model parameters included in this flowchart include (Figure 4-9): model time step (T); the number of contacts made with other children in the child care group (careContacts). Child parameters included in this flowchart include: age of child, in days (age); date at which child loses birth immunity (loseBI); passive immunity state at birth (bImm); time at which child becomes resistant to colonization (rTime); colonization state (colState); disease state (dxState); time at which child became colonized (cTime); and acquired immunity state (immState). Strain parameters included in this flowchart include probability of developing invasive disease (dxProb); the probability of inducing acquired immunity, upon colonization (immProb); and the duration of colonization (colDur). Contact group parameters include: size (size), with subscripts indicating household (HH) or care group (CG) types. Shown values of colState include: susceptible (S), colonized (C), and resistant to colonization (R). Shown values of dxState include: susceptible (S) and diseased (D). Shown values of sType include: commensal (C) and pathogenic (P). Other parameter values are shown in Tables 4-1 to 4-4. Temporary method variables include: the number of contact pairs made with other children in the model (commPairs); a process loop variable to represent the number of child care contacts (nCC); a process loop variable to represent the number of community pairs (nP); a decimal number representing the probability of acquiring commensal colonization from contact with individuals not included in the model (pExC); a decimal number representing the probability of acquiring pathogenic colonization from contact with individuals not included in the model (pExP); and a randomly-generated decimal number for a decision process, with a distribution of 0 to 1 (p). commPairs is calculated as one-half the number of contacts made with other children included in the model (commContacts); pExC is calculated by multiplying the prevalence of commensal colonization among those not included in the model (prevExC) and the number of contacts made with individuals not included in the model (othContacts) and pExP is calculated by multiplying the prevalence of pathogenic colonization among those not included in the model (prevExP) and othContacts. Values for commContacts, prevExC, prevExP, and othContacts are shown in Table 4-1.

Figure 4-9: Flowchart Depicting the Process of Making Contact with Other Children in Households, Child Care Settings, and the Community.

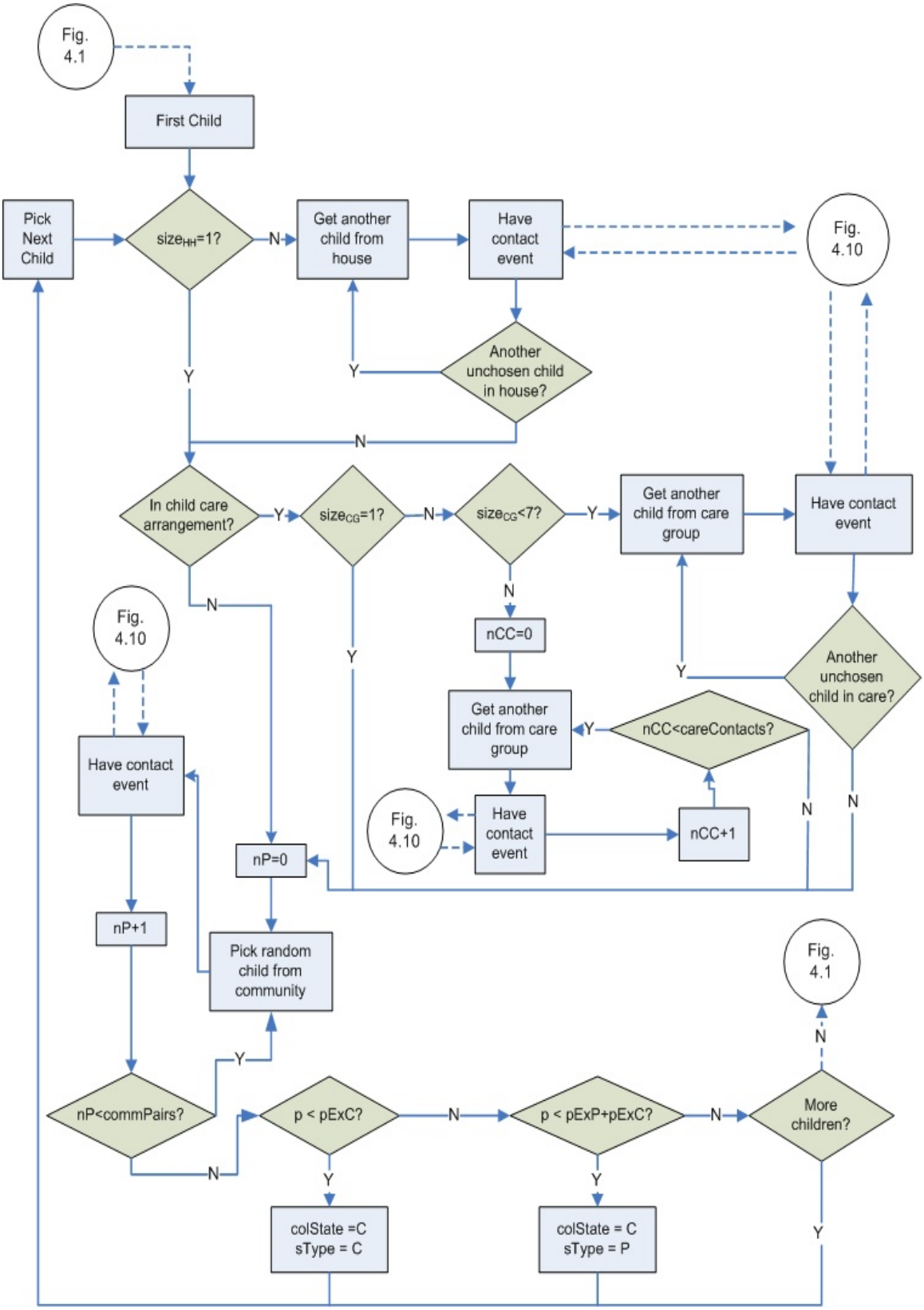
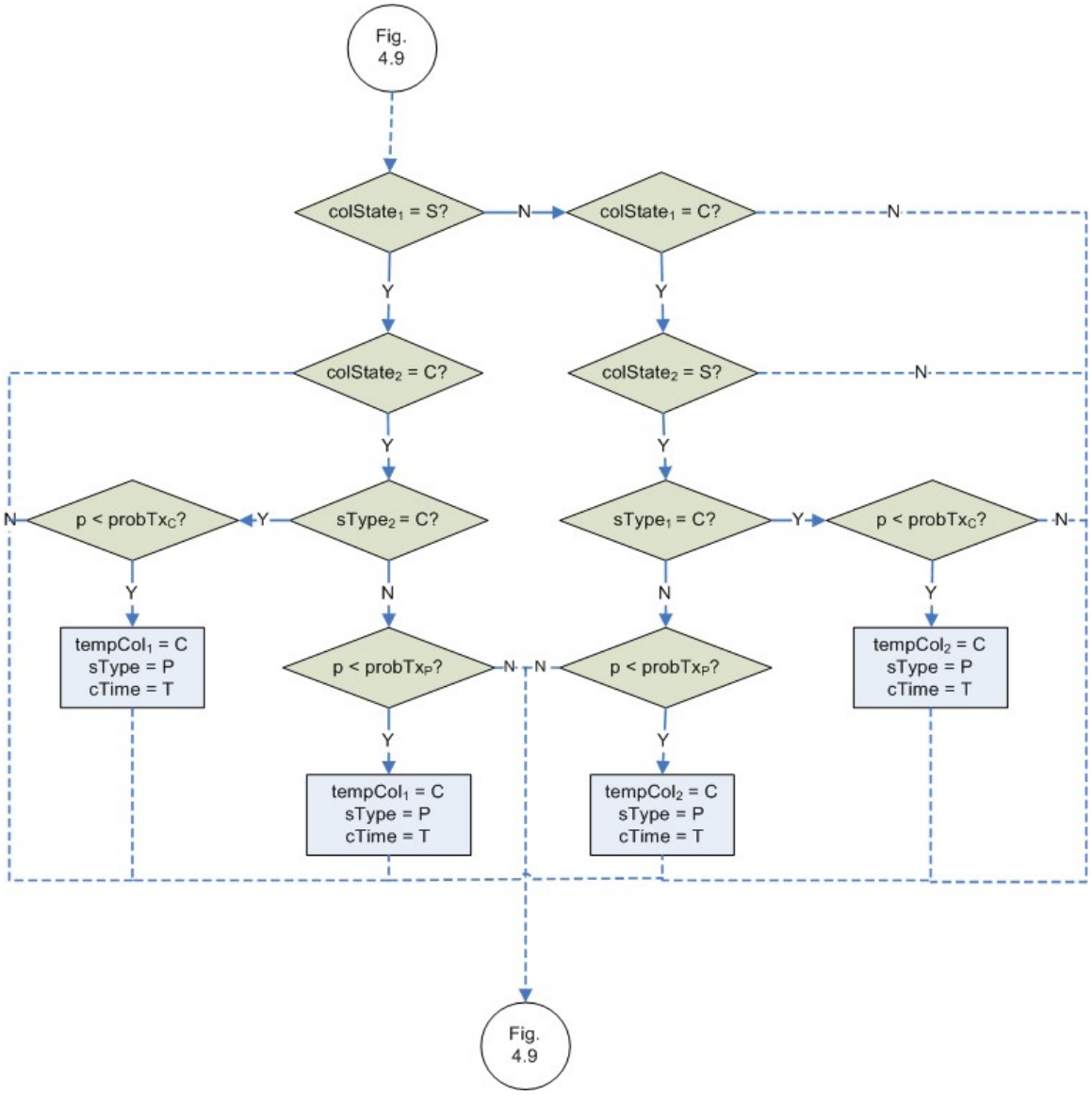
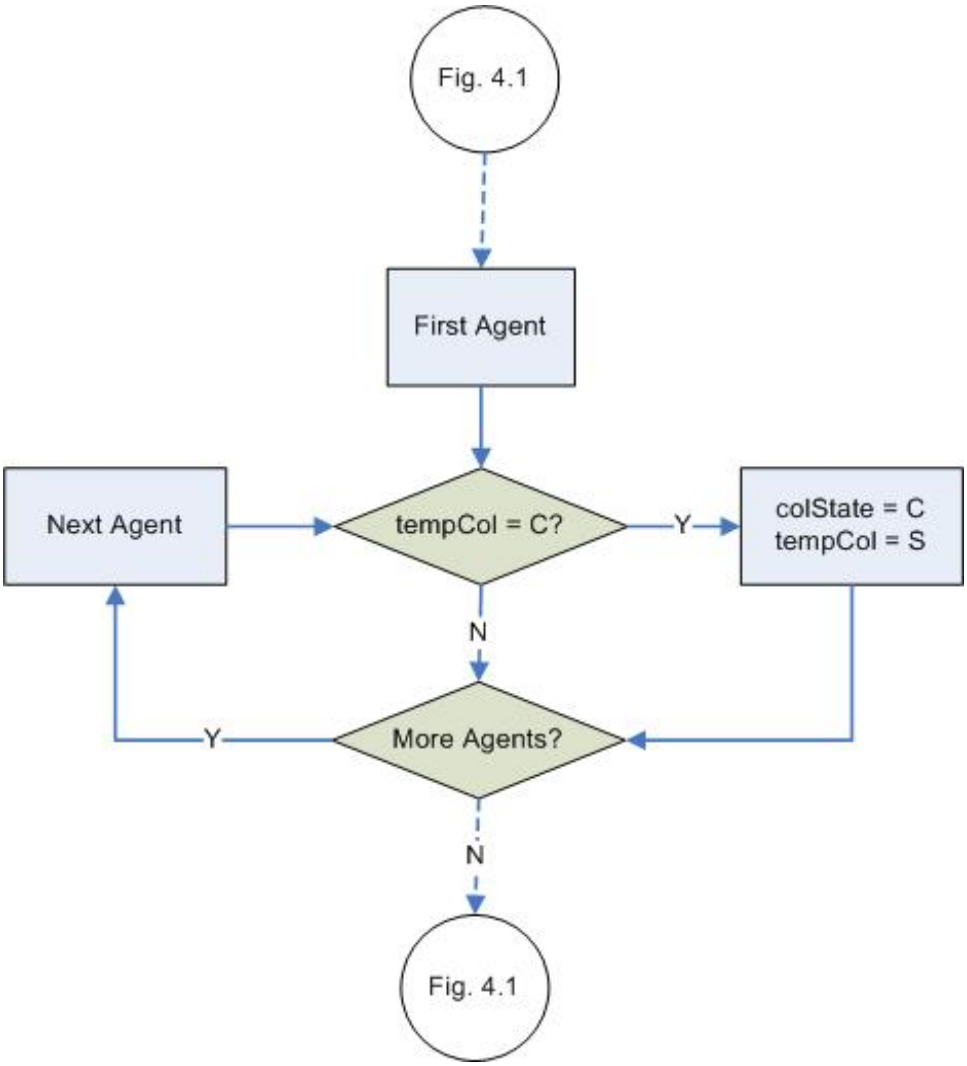


Figure 4-10: Flowchart Depicting the Transmission of Bacteria and Acquisition of Colonization During a Contact Event.



Model parameters included in this flowchart: model time step (T). Child parameters included in this flowchart: colonization state (colState); temporary colonization state for synchronous updating of new colonization acquisition (tempCol); and time step at which child is colonized (cTime). Strain parameters included in this flowchart: strain type (sType) and the probability of transmission (probTx). Shown values of colState include: susceptible (S) and colonized (C); shown values of tempCol include: colonized (C). Subscripts 1 and 2 of colState, tempCol, and sType denote child one and two, respectively. Shown values of sType include: commensal (C) or pathogenic (P). Subscripts C and P of probTx denote commensal and pathogenic strains, respectively. Other parameter values are shown in Tables 4-1 to 4-4. Temporary method variables include: a randomly-generated decimal number with a distribution of 0-1 (p).

Figure 4-11: Flowchart Depicting the Synchronous Updating of Colonization State at the End of Each Model Time Step.

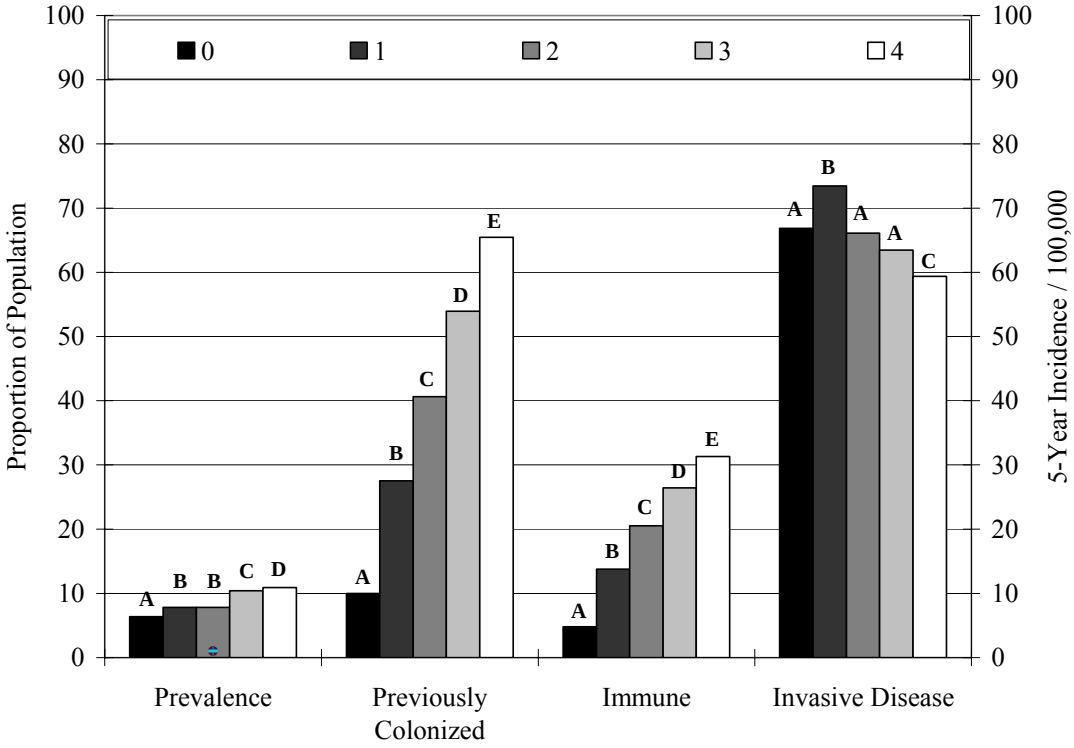


Child parameters included in this flowchart: colonization state (colState) and temporary colonization state for synchronous updating of new colonization acquisition (tempCol). Shown values of colState and tempCol are equivalent and include: susceptible (S) and colonized (C). Other parameter values are shown in Tables 4-1 to 4-4.

Table 4-5: Prevalence of Colonization, Proportion Having Had a Previous Episode of Commensal Colonization, Proportion Having Acquired Natural Immunity, Occurrence of Invasive Disease, and Mean Age of Invasive Disease Cases, by BSA.

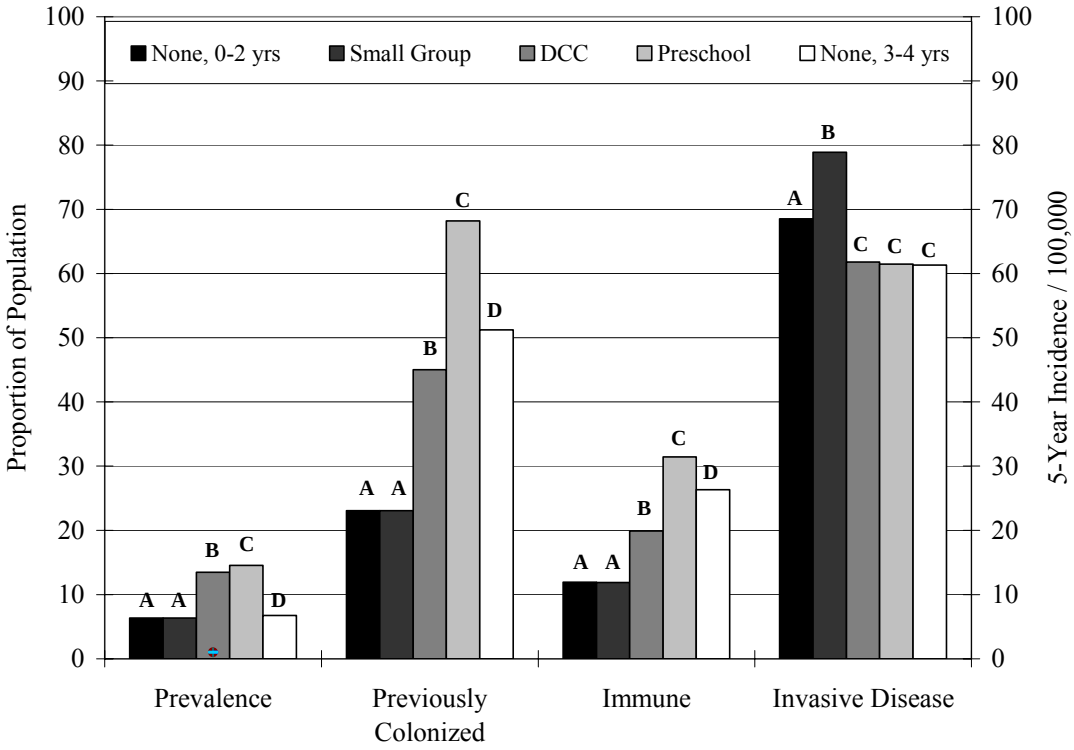
BSA (%)	Prevalence of Colonization, %	Prevalence of Commensal Colonization, %	Prevalence of Pathogenic Colonization, %	Previously Colonized, %	Immune, %	Mean Occurrence of IMD, N	Mean Age of Cases, Days
0	8.7	7.8	0.91	39.6	19.4	65.8	886
2	8.4	7.5	0.90	39.0	19.1	67.6	882
4	8.2	7.3	0.90	38.4	18.9	68.2	893
6	8.0	7.1	0.89	37.7	18.7	66.9	884
8	7.8	6.9	0.89	37.1	18.4	67.6	888
10	7.6	6.7	0.88	36.5	18.2	66.8	889
12	7.4	6.5	0.87	35.9	18.0	66.2	902
14	7.2	6.3	0.87	35.3	17.8	69.0	898
16	7.0	6.1	0.86	34.7	17.5	68.0	898
18	6.8	5.9	0.85	34.2	17.3	68.0	893
20	6.6	5.8	0.85	33.6	17.1	68.6	901

Figure 4-12: Total Prevalence of Colonization, Proportion of Children with At Least One Previous Episode of Colonization, Proportion of Children with Acquired Immunity, and the Five-Year Incidence of Invasive Disease, by Age in Years, BSA=0%.



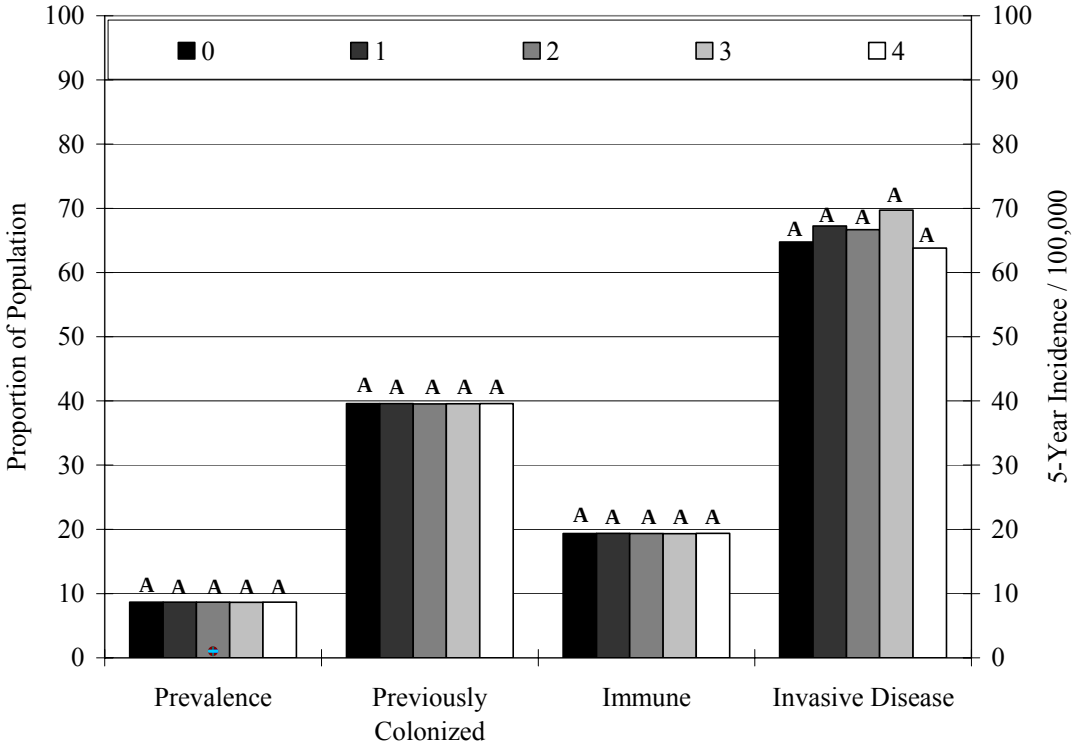
Values for the prevalence of colonization, the proportion of children with at least one previous episode of colonization, and the proportion of children with acquired immunity are plotted along the left axis; values for the five-year incidence of invasive disease are plotted along the right access. Within each outcome measure, letters (i.e. A, B, C…) indicate values which are not significantly different.

Figure 4-13: Total Prevalence of Colonization, Proportion of Children with At Least One Previous Episode of Colonization, Proportion of Children with Acquired Immunity, and the Five-Year Incidence of Invasive Disease, by Child Care Arrangement, BSA=0%.



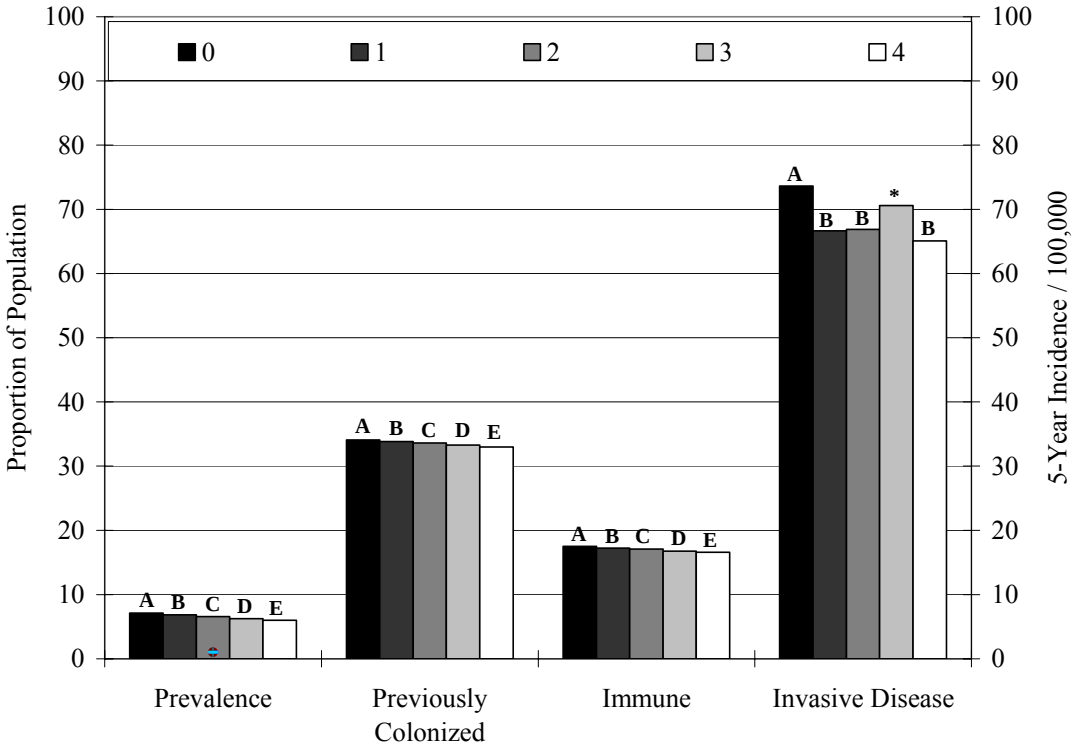
Values for the prevalence of colonization, the proportion of children with at least one previous episode of colonization, and the proportion of children with acquired immunity are plotted along the left axis; values for the five-year incidence of invasive disease are plotted along the right access. Within each outcome measure, letters (i.e. A, B, C…) indicate values which are not significantly different at alpha=0.05.

Figure 4-14: Total Prevalence of Colonization, Proportion of Children with At Least One Previous Episode of Colonization, Proportion of Children with Acquired Immunity, and the Five-Year Incidence of Invasive Disease, by Annual Number of Antibiotic Doses Received, BSA=0%.



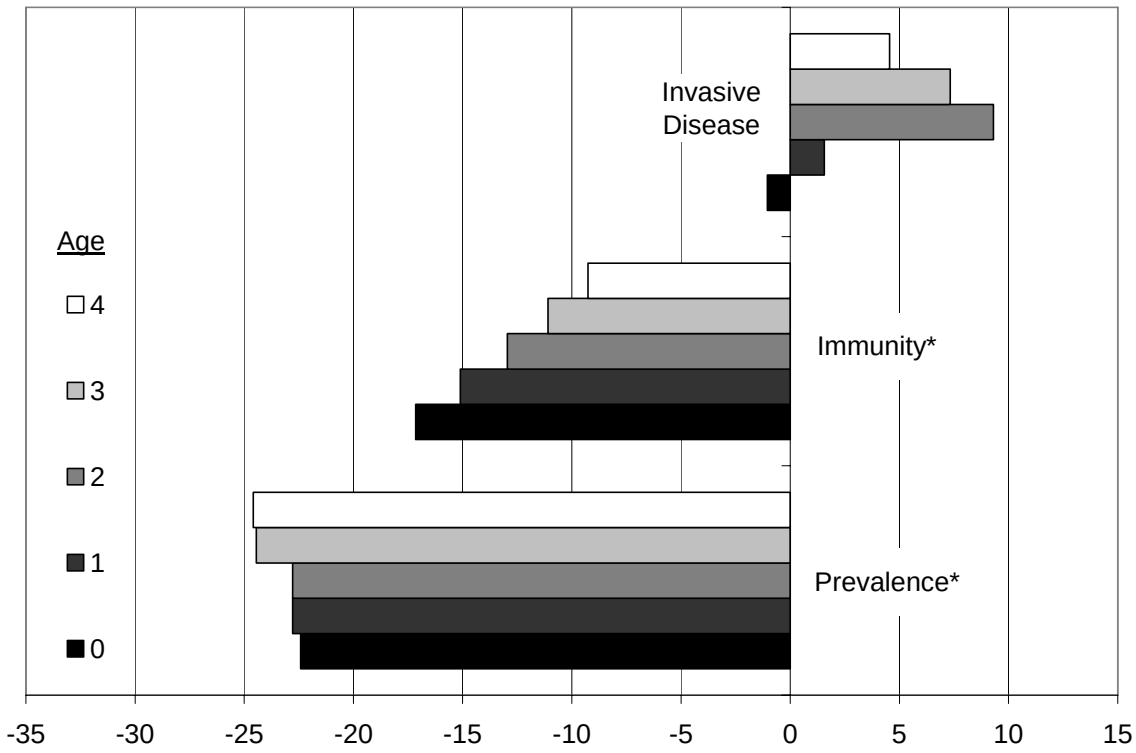
Values for the prevalence of colonization, the proportion of children with at least one previous episode of colonization, and the proportion of children with acquired immunity are plotted along the left axis; values for the five-year incidence of invasive disease are plotted along the right access. Within each outcome measure, letters (i.e. A, B, C…) indicate values which are not significantly different.

Figure 4-15: Total Prevalence of Colonization, Proportion of Children with At Least One Previous Episode of Colonization, Proportion of Children with Acquired Immunity, and the Five-Year Incidence of Invasive Disease, by Annual Number of Antibiotic Doses Received, BSA=20%.



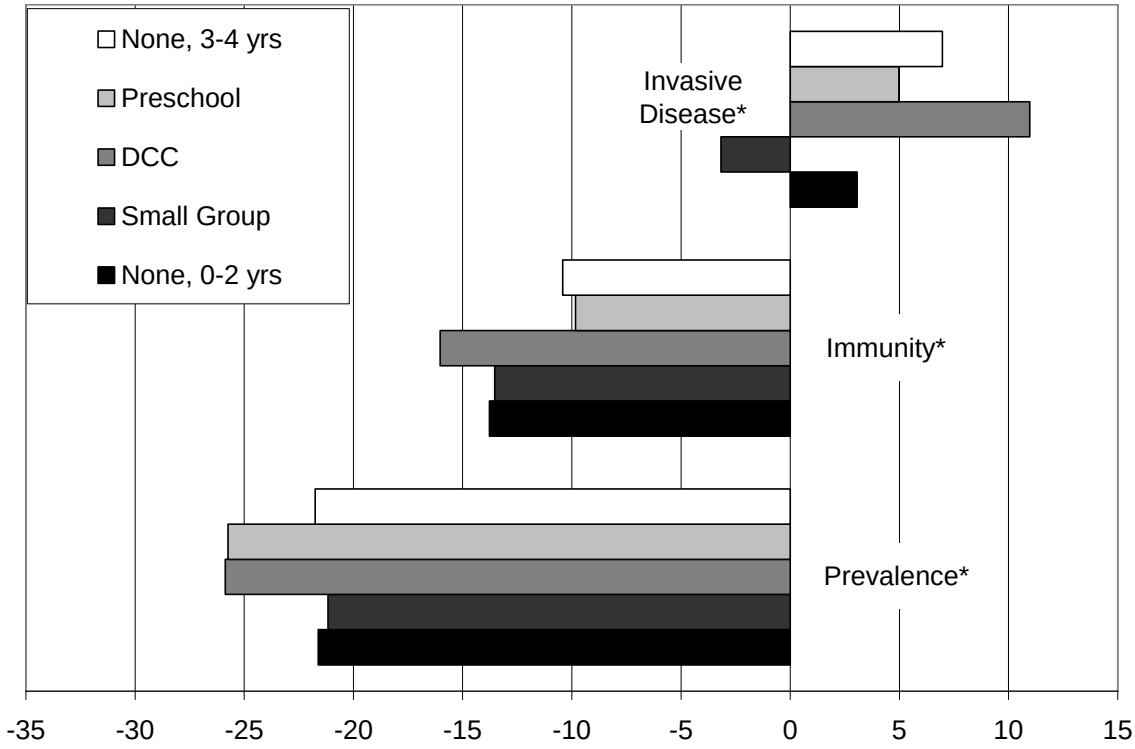
Values for the prevalence of colonization, the proportion of children with at least one previous episode of colonization, and the proportion of children with acquired immunity are plotted along the left axis; values for the five-year incidence of invasive disease are plotted along the right access. Within each outcome measure, letters (i.e. A, B, C…) indicate values which are not significantly different. *Indicates value not significantly different than either A or B.

Figure 4-16: Percent Change in the Total Prevalence of Colonization, Proportion of Children with Acquired Immunity, and Five-Year Incidence of Invasive Disease, by Age in Years, BSA=20% versus BSA=0%.



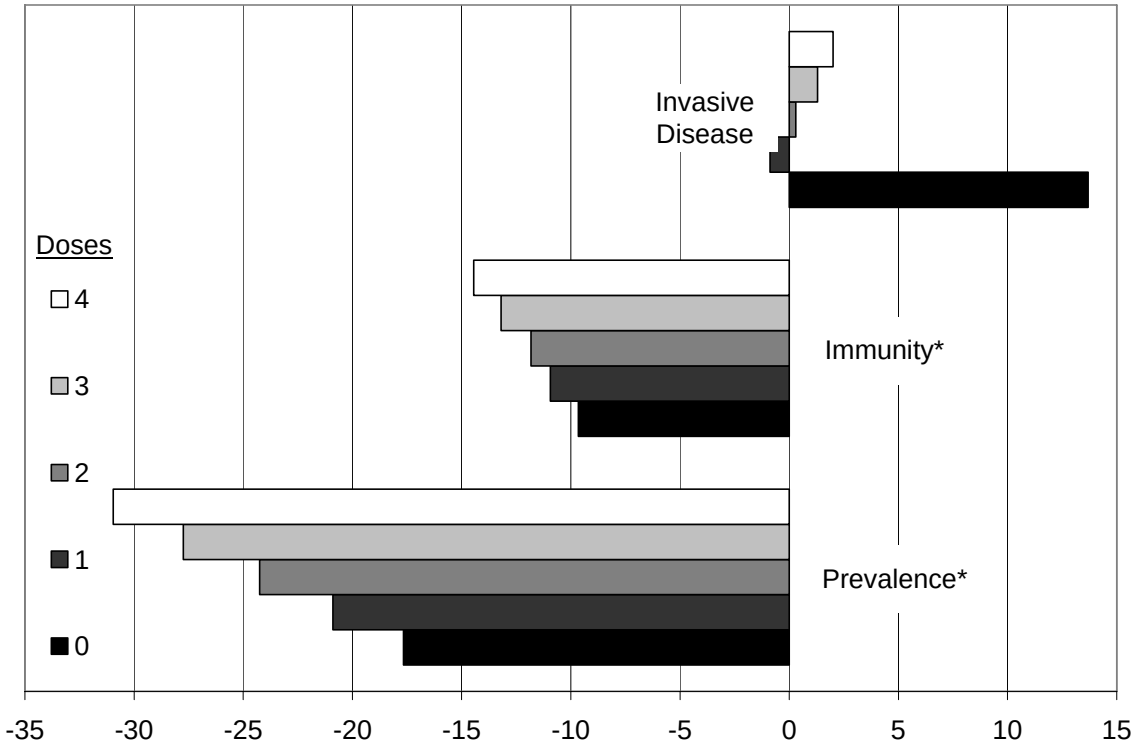
*Indicates a significant interaction term between increasing population BSA use and age in years in a multivariate linear regression model, at $p<0.05$.

Figure 4-17: Percent Change in the Total Prevalence of Colonization, Proportion of Children with Acquired Immunity, and Five-Year Incidence of Invasive Disease, by Child Care Arrangement, BSA=20% versus BSA=0%.



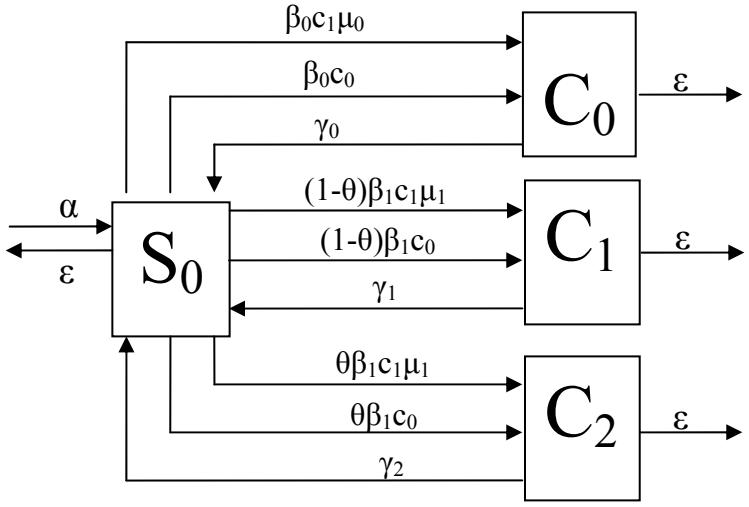
*Indicates a significant interaction term between increasing population BSA use and age in years in a multivariate linear regression model, at $p<0.05$.

Figure 4-18: Percent Change in the Total Prevalence of Colonization, Proportion of Children with Acquired Immunity, and Five-Year Incidence of Invasive Disease, by Annual Number of Antibiotic Doses Received, BSA=20% versus BSA=0%.



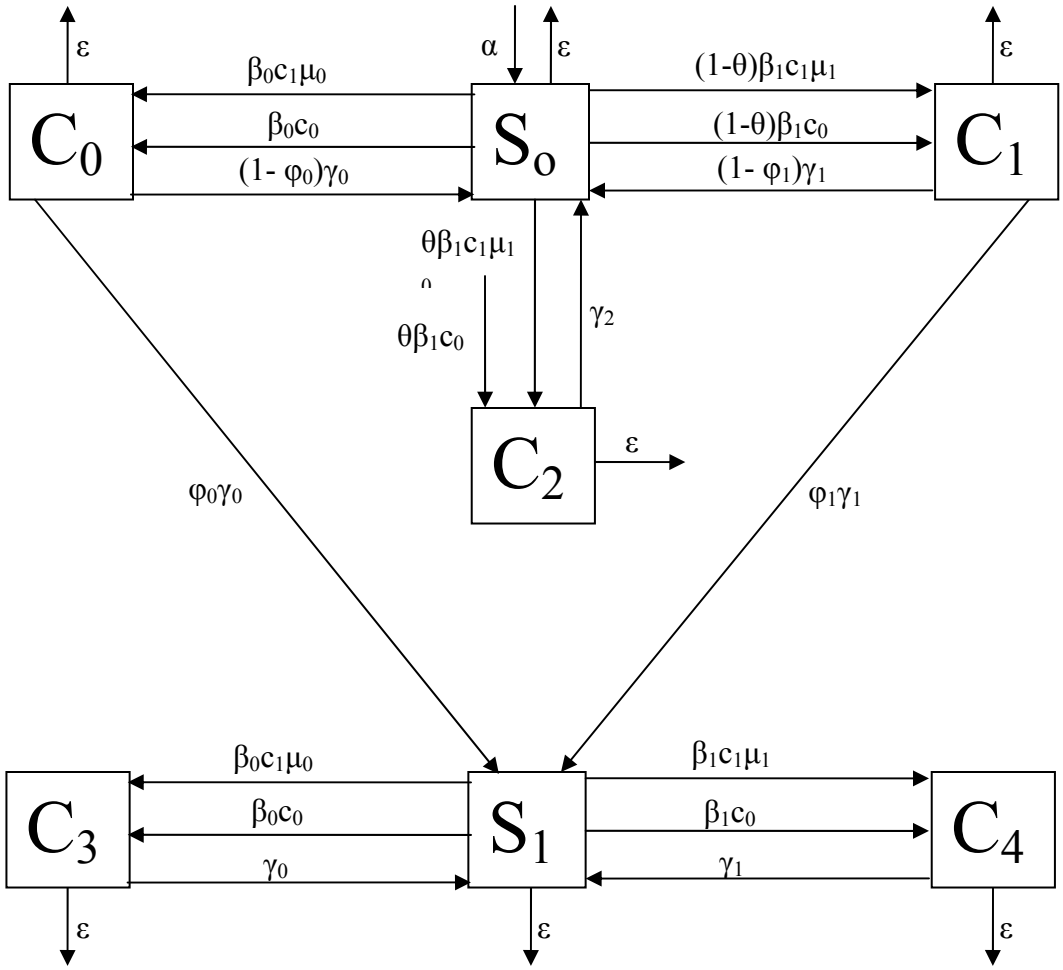
*Indicates a significant interaction term between increasing population BSA use and age in years in a multivariate linear regression model, at $p<0.05$.

Figure 4-19: Ordinary Differential Equation Model Describing the Transmission of Commensal and Pathogenic Strains and the Occurrence of Invasive Disease in a Population Without Immunity.



Compartments represent the numbers of individuals susceptible to colonization (S_0); those colonized with the commensal strain (C_0); those colonized with the pathogenic strain (C_1); and those with invasive disease (C_2). Flows between compartments are determined by the total number of individuals (N); the proportion of individuals entering the model per day (α); the per-contact probabilities of transmission of the commensal (β_0) and pathogenic (β_1) strains; the number of contacts made between other children 0-4 years of age (c_0) and those five years and older (c_1); the proportion of individuals five and older colonized with the commensal (μ_0) and pathogenic (μ_1) strains; the probability of developing invasive disease (θ); the proportion of those losing commensal colonization (γ_0) and pathogenic colonization among those without (γ_1) and with (γ_2) invasive disease per day; and the proportion of individuals leaving the model per day (ε).

Figure 4-20: Ordinary Differential Equation Model Describing the Transmission of Commensal and Pathogenic Strains and the Occurrence of Invasive Disease in a Population with Immunity.



Compartments represent the numbers of individuals susceptible to colonization without (S_0) or with (S_1) immunity; those colonized with the commensal strain without (C_0) or with (C_3) immunity; those colonized with the pathogenic strain without (C_1) or with (C_4) immunity; and those with invasive disease (C_2). Flows between compartments are determined by the total number of individuals (N); the proportion of individuals entering the model per day (α); the per-contact probabilities of transmission of the commensal (β_0) and pathogenic (β_1) strains; the number of contacts made between other children 0-4 years of age (c_0) and those five years and older (c_1); the proportion of individuals five and older colonized with the commensal (μ_0) and pathogenic (μ_1) strains; the probability of developing invasive disease (θ); the proportion of those losing commensal colonization (γ_0) and pathogenic colonization among those without (γ_1) and with (γ_2) invasive disease per day; the probability of acquiring immunity if colonized with the commensal (φ_0) and pathogenic (φ_1) strain; and the proportion of individuals leaving the model per day (ε).

Table 4-6: Parameter Estimates for the Strain-Specific, Per-Contact Probabilities of Transmission and a Comparison of Ordinary Differential Equation (ODE) and Agent Based Model (ABM) Results.

	Model 1		Model 2	
Parameter Estimates*				
β_0	0.001855		0.001855	
β_1	0.0002048		0.0002048	
θ	0.004755		0.006096	
	ODE	ABM	ODE	ABM
Results**				
Total Prevalence (%)	11.4	12.8 (12.6, 13.1)	11.4	12.8 (12.7, 12.9)
Commensal Prevalence (%)	10.6	11.9 (11.7, 12.2)	10.6	11.9 (11.8, 12.1)
Pathogenic Prevalence (%)	0.86	0.89 (0.86, 0.90)	0.86	0.89 (0.87, 0.91)
Number of Cases (N)	6.5	6.3 (4.6, 7.9)	5.5	6.2 (5.7, 6.7)

*Parameter estimates include the per-contact probability of transmission of the commensal strain, β_0, or pathogenic strain, β_1, and the probability of developing invasive disease, θ.

**Results for the ODE model are mathematically determined; results for ABM are shown as Mean (95% Confidence Interval) over ten model runs.

References:

1. American Academy of Pediatrics. Meningococcal Infections. In: Pickering LK, ed. *Red Book: 2006 Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2006; 433-4.

2. Balmer P, Borrow R, Miller E. Impact of meningococcal C conjugate vaccine in the UK. *J Med Microbiol*. 2002;51:717-22.

3. Beam WE, Newberg NR, Devine LF, Pierce WE, Davies JA. The Effect of Rifampin on the Nasopharyngeal Carriage of *Neisseria meningitidis* in a Military Population. *J Infect Dis*. 1971;124:39-46.

4. Broud DD, Griffiss JM, Baker CJ. Heterogeneity of Serotypes of *Neisseria meningitidis* that Cause Endemic Disease. *J Infect Dis*. 1979;140:465-70.

5. Brown DG. 2006. Agent-based models. In H. Geist, ed. *The Earth's Changing Land: An Encyclopedia of Land-Use and Land-Cover Change*. Westport, CT: Greenwood Publishing Group, pp. 7-13.

6. Cartwright K, Reilly S, White D, Stuart J. Early treatment with parenteral penicillin in meningococcal disease. *BMJ*. 1992;305:143-7.

7. Cartwright KA, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and Neisseria lactamica. *Epidemiol Infect*. 1987;99:591-601.

8. Caugant DA, Hoiby EA, Magnus P, Scheel O, Hoel T, Bjune G, Wedege E, Eng J, Froholm LO. Asymptomatic Carriage of *Neisseria meningitidis* in a Randomly Sampled Population. *Journal of Clinical Microbiology*. 1994;32:323-30.

9. Celedón JC, Litonjua AA, Ryan L, Weiss ST, Gold DR. Lack of Association between Antibiotic Use in the First Year of Life and Asthma, Allergic Rhinitis, or Eczema at Age 5 Years. *Am J Respir Crit Care Med*. 2002;166:72-5.

10. Centers for Disease Control and Prevention. 1998. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Neisseria meningitidis*, 1997. Available at: http//www.cdc.gov/ncidod/dbmd/abcs/survreports/mening97.pdf. Accessed: September 27, 2008.

11. Centers for Disease Control and Prevention. 2001. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Neisseria meningitidis*, 2000. Available at: http//www.cdc.gov/ncidod/dbmd/abcs/survreports/mening00.pdf. Accessed: September 27, 2008.

12. Centers for Disease Control and Prevention. 2007. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Neisseria meningitidis*, 2006. Available at: http//www.cdc.gov/ncidod/dbmd/abcs/survreports/mening00.pdf. Accessed: September 27, 2008.

13. Centers for Disease Control and Prevention. Notice to Readers: Recommendation from the Advisory Committee on Immunization Practices (ACIP) for Use of Quadrivalent Meningococcal Conjugate Vaccine (MCV4) in Children Aged 2—10 Years at Increased Risk for Invasive Meningococcal Disease. *MMWR*. 2007;56:12-65-6.

14. Centers for Disease Control and Prevention. Prevention and Control of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 2005;54(No. RR-7).

15. Clark J, Lakshman R, Galloway A, Cant A. Does cefotaxime eradicate nasopharyngeal carriage of *N. meningitidis*. *Arch Dis Child*. 2002;87:449.

16. Davison KL, Andrews N, White JM, Ramsay, ME, Crowcroft NS, Rushdy AA, Kaczmarski EB, Monk PN, Stuart JM. Clusters of meningococcal disease in school and preschool settings in England and Wales: what is the risk? *Arch Dis Child*. 2004;89:256-60.

17. Deal WB, Sanders E. Efficacy of rifampin in treatment of meningococcal carriers. *New Eng J Med*. 1969;281:641-5.

18. Devine LF, Johnson DP, Hagerman CR, Pierce WE, Rhode SL, Peckinpaugh RO. Rifampin: Levels in Serum and Saliva and Effect on The Meningococcal Carrier State. *JAMA*. 1970;214:1055-9.

19. Dowell SF. Principles of judicious use of antimicrobial agents for pediatric upper respiratory infections. *Pediatrics*. 1998;101(Suppl 1):163-84.

20. Dworzack DL, Sanders CC, Horowitz EA, Allais JM, Sookpranee M, Sanders WE, Ferraro FM. Evaluation of Single-Dose Ciprofloxacin in the Eradication of *Neisseria meningitidis* from Nasopharyngeal Carriers. *Antimicrob Agents Chemother*. 1988;32:1740-1.

21. Edwards MS, Baker CJ. Complications and sequelae of meningococcal infections in children. *J Pediatr*. 1981;99:540-5.

22. Evans CT, Smith B, Parada JP, Kurichi JE, Weaver FM. Trends in antibiotic prescribing for acute respiratory infection in veterans with spinal cord injury and disorder. *J Antimicrob Chemotherapy*. 2005;55:1045-9.

23. Feil EJ, Enright MC, Spratt BG. Estimating the relative contributions of mutation and recombination to clonal diversification: a comparison between *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Res Microbiol*. 2000;151:465-9.

24. Finkelstein JA, Stille C, Nordin J, Davis R, Raebel MA, Roblin D, Go AS, Smith D, Johnson CC, Kleinman K, Chan KA, Platt R. Reduction in Antibiotic Use Among US Children, 1996-2000. *Pediatrics*. 2003;112:620-7.

25. Fischer M, Hedberg K, Cardosi P,et al. Tobacco smoke as a risk factor for meningococcal disease. *Pediatr Infect Dis J*. 1997;16:979-83.

26. Fraser DW, Thornsberry C, Feldman RA. Trends in meningococcal disease. *J Infect Dis*. 1972;125:443-6.

27. Girgis N, Sultan Y, Frenck R, El-Gendy A, Farid, Z, Mateczun A. Azithromycin compared with rifampin for eradication of nasopharyngeal colonization by *Neisseria meningitidis*. *Pediatr Infect Dis J*. 1998;17:816-9.

28. Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in Infants and Children. *J Infect Dis*. 1978;137:112-21.

29. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med*. 1969;129:1307-26.

30. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. *J Exp Med*. 1969;129:1327-48.

31. Guttler RB, Counts GW, Avent CK, Beaty HN. Effect of Rifampin and Minocycline on Meningococcal Carrier Rates. *J Infect Dis*. 1971;124:199-205.

32. Halasa NB, Griffin MR, Zhu Y, Edwards KM. Decreased number of antibiotic prescriptions in office-based settings from 1993-1999 in children less than five years of age. *Pediatr Infect Dis J*. 2002;21:1023-8.

33. Harrison LM, Morris JA, Telford DR, Brown SM, Jones K. The nasopharyngeal bacterial flora in infancy: effects of age, gender, season, viral upper respiratory tract infection and sleeping position. *FEMS Immunol Med Microbiol*. 1999;25:19-28.

34. Jones GR, Christodoulides M, Brooks JL, Miller AR, Cartwright KA, Heckels JE. 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*. 1998;178:451-9.

35. Kirsch EA, Barton RP, Kitchen L, Giroir B. Pathophysiology, Treatment and Outcome of Meningococcemia: A Review and Recent Experience. *Pediatr Infect Dis J*. 1996;15:967-79.

36. Kozyrskyj AL, Carrie AG, Mazowita GB, Lix LM, Klassen TP, Law BJ. Decrease in antibiotic use among children in the 1990s: not all antibiotics, not all children *CMAJ*. 2004;171:133-8.

37. Kremastinou J, Tzanakaki G, Levidiotou S, Markou F, Themeli E, Voyiatzi A, Psoma E, Theodoridou M, Blackwell CC. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in northern Greece. *FEMS Immunol Med Microbiol*. 2003;39:23-9.

38. Lepe JA, Salcedo C, Alcala B, Vazquez JA. [Evolution of *Neisseria meningitidis* sensitivity to various antimicrobial drugs over the course of chemoprophylaxis during an epidemic outbreak]. [Spanish] *Enfermedades Infecciosas y Microbiologia Clinica*. 2006;24:608-12.

39. Maiden MCJ, Ibarz-Pavon AB, Urwin R, et al. Impact of Meningococcal Serogroup C Conjugate Vaccines on Carriage and Herd Immunity. *J Infect Dis*. 2008;197:737-43.

40. Maiden MCJ. Population genetics of a transformable bacterium: The influence of horizontal genetic exchange on the biology of *Neisseria meningitidis*. *FEMS Microbiol Let*. 1993; 112:243-50.

41. Maiden MC, Stuart JM; UK Meningococcal Carriage Group. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. *Lancet*. 2002;359-1829-31.

42. Mainous AG 3rd, Hueston WJ, Davis MP, Pearson WS. Trends in antimicrobial prescribing for bronchitis and upper respiratory infections among adults and children. *Am J Pub Health*. 2003;93:1910-4.

43. McCaig LF, Besser RE, Hughes JM. Antimicrobial Drug Prescriptions in Ambulatory Care Settings, United States, 1992-2000. *Emerg Infect Dis*. 2003;9:432-7.

44. McCaig LF, Besser RE, Hughes JM. Trends in Antimicrobial Prescribing Rates for Children and Adolescents. *JAMA*. 2002;287:3096-3102.

45. Meyers LA, Levin BR, Richardson AR, Stojiljkovic I. Epidemiology, hypermutation, within-host evolution and the virulence of *Neisseria meningitidis*. *Proc Biol Sci*. 2003;270:1667-77.

46. Oliver KJ, Reddin KM, Bracegirdle P, Hudson MJ, Borrow R, Feavers IM, Robinson A, Cartwright K, Gorringe AR. *Neisseria lactamica* Protects against Experimental Meningococcal Infection. *Infect Immun*. 2002;70:3621-6.

47. Paret G, Keller N, Barzilai A, Zemach M, Guttman D, Vardi A, Shatzberg G, Cohen H, Barzilay Z. Invasive Meningococcal Disease: Patient and Strain Characteristics Set New Challenge for Prevention and Control. *Infection*. 1999;27:261-4.

48. Pereiró I, Díez-domingo J, Segarra L, Ballester A, Albert A, Morant A. Risk factors for invasive disease among children in Spain. *J Infect*. 2004;48:320-9.

49. Purcell B, Samuelsson S, Hahné SJM, Ehrhard I, Heuberger S, Camaroni I, Charlett A, Stuart JM. Effectiveness of antibiotics in preventing meningococcal disease after a case: systematic review. *BMJ*. 2004;328:1339-43.

50. Ramsay ME, Andrews NJ, Trotter CL, Kaczmarski EB, Miller E. Herd immunity from meningococcal serogroup C conjugate vaccination in England: database analysis. *BMJ*. 2003;326:365-6.

51. Reller LB, MacGregor RR, Beaty HN. Bactericidal antibody after colonization with *Neisseria meningitidis*. *J Infect Dis*. 1973;127:56-62.

52. Renkonen OV, Sivonen A, Visakorpi R. Effect of Ciprofloxacin on Carrier Rate of *Neisseria meningitidis* in Army Recruits in Finland. *Antimicrob Agents Chemother*. 1987;31:962-3.

53. ROAD, 2005, *Repast*. Repast home page, Repast Organization for Architecture and Design, Chicago, IL; available at: http: //repast.sourceforge.net. Accessed: September 27, 2008.

54. Robinson K, Neal KR, Howard C, Stockton J, Atkinson K, Scarth E, Moran J, Robins A, Todd I, Kaczmarski E, Gray S, Muscat I, Slack R, Ala'Aldeen DAA. Characterization of humoral and cellular immune responses elicited by meningococcal carriage. *Infect Immun*. 2002;70:1301-9.

55. Robinson S. Simulation Model Verification and Validation: Increasing the Users' Confidence. *Proc 1997 Winter Simulation Conference*. 1997;53-59. Available at: http://cscs.umich.edu/~rlr/CSCS530/uploads//Main/robinson-1997.pdf. Accessed: November 14, 2008.

56. Rosenstein NE, Perkins BA, Stephens DS, Lefkowitz L, Cartter ML, Danila R, Cieslak P, Shutt KA, Popovic T, Schuchat A, Harrison LH, Reingold AL. The Changing Epidemiology of Meningococcal Disease in the United States, 1992-1996. *J Infect Dis*. 1999;180:1894-901.

57. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal Disease. *NEJM*. 2001;344:1378-88.

58. Roumie CL, Halasa NB, Grijalva CG, Edwards KM, Zhu Y, Dittus RS, Griffin MR. Trends in Atntibiotic Prescribing for Adults in the United States—1995-2002. *J Gen Intern Med*. 2005;20:697-702.

59. Sanders E, Deal WB. Prevention of meningococcal infections. *J Infect Dis*. 1970;121:449-51.

60. Schaad UB. Chemoprophylaxis for the prevention of bacterial meningitis. *Infection*. 1984; 12 Suppl 1:S65-71.

61. Schwartz B, Moore PS, Broome CV. Global Epidemiology of Meningococcal Disease. *Clin Microbiol Rev*. 1989;2(Supp);S118-24.

62. Smith JM, Smith NH, O'Rourke M, Spratt BG. How clonal are bacteria? *Proc Natl Acad Sci USA*. 1993;90:4384-8.

63. Stille CJ, Andrade SE, Huang SS, Nordin J, Raebel MA, Go AS, Chan KA, Finkelstein JA. Increased Use of Second-Generation Macrolide Antibiotics in Children in Nine Health Plans in the United States. *Pediatrics*. 2004;114:1206-11.

64. Taha MK, Deghmane AE, Antignac A, Zarantonelli ML, Larribe M, Alonso JM. The duality of virulence and transmissibility in *Neisseria meningitidis*. *Trends Microbiol*. 2002;10:376-82.

65. Thorpe JM, Smith SR, Trygstad TK. Trends in Emergency Department Antibiotic Prescribing for Acute Respiratory Tract Infections. *Ann Pharmacother*. 2004;38:928-35.

66. Thrane N, Olesen C, Mortensen JT, Sondergaard C, Schonheyder HC, Sorensen HT. Influence of Day Care Attendance on the Use of Systematic Antibiotics in 0- to 2-Year-Old Children. *Pediatrics*. 2001;107:E76.

67. Trotter CL, Gay NJ, Edmunds WJ. The natural history of meningococcal carriage and disease. *Epidemiol Infect*. 2006;134:556-566.

68. Tyski S, Grzybowska W, Dulny G, Berthelsen L, Lind I. Phenotypical and genotypical characterization of *Neisseria meningitidis* carrier strains isolated from Polish recruits in 1998. *Eur J Clin Microbiol Infect Dis*. 2001;20:350-3.

69. Tzeng YL, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microb Infect*. 2000;2:687-700.

70. van Duren M, Brandtzaeg P, van der Meer, JWM. Update on Meningococcal Disease with Emphasis on Pathogenesis and Clinical Management. *Clin Micro Rev*. 2000;13:144-66.

71. Yazdankhah SP, Kriz P, Tzanakaki G, Kremastinou J, Kalmusova J, Musilek M, Alvestad T, Jolley KA, Wilson DJ, McCarthy ND, Caugant DA, Maiden MCJ. Distribution of Serogroups and Genotypes among Disease-Associated and Carried Isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *J Clin Microbiol*. 2004;42:5146-53.

Chapter V

Conclusion

The occurrence of IMD is the result of a complex interaction of host, agent, and environmental risk factors within both individuals and populations. While the provision of antibiotic prophylaxis to close case contacts – the historical standard public health response – has been largely effective at preventing additional cases, its success is without regard to the epidemiology of the underlying pathogen, *N. meningitidis*. The development of primary public health measures against IMD (i.e. the meningococcal conjugate vaccine), however, requires a greater understanding of the dynamics of *N. meningitidis* within the population and agent, host, and environmental factors related to invasive disease. The goal of this dissertation research was to achieve a greater understanding of meningococcal epidemiology and factors related to invasive disease.

In the mid-1990s, Oregon experienced an IMD epidemic due to serogroup B strains. During that period, the overall incidence of serogroup B disease doubled from baseline, that among individuals 15-19 years of age was 13 times higher, and 70% of all isolates tested by MLEE were a single ET clone [4]. The goal of the first study was to use PFGE to better characterize this epidemic. The aims were to demonstrate the concordance of PFGE with previous strain typing techniques in identifying the circulating epidemic clonal strain; to assess whether the PFGE profile of isolates recovered from cases occurring during a later epidemic period were more diverse than that of isolates recovered from cases occurring during the peak epidemic, thus supporting

the hypothesis that the epidemic was due to the introduction and spread of a clonal strain in the population; and to describe the relatedness of PFGE patterns from cases with an known epidemiological link and those occurring within a narrow spatiotemporal frame. PFGE was completed on 221 Oregon serogroup B meningococcal isolates, including 129 isolates from peak of the epidemic (January 1, 1994 to December 31, 1996) and 92 isolates from a later epidemic period (January 1, 2001 to December 31, 2003), on which previous strain typing by serologic assay, MLEE, and/or MLST had been performed.

PFGE and strain typing, by MLEE, MLST, or phenotypic profile, differentiated between the circulating epidemic clonal strain and non-epidemic strains with an observed agreement of 95%. That 89% of peak epidemic isolates were identified as one PFGE group, Group I, including 48% of isolates that shared one PFGE pattern, and that these isolates were 98% similar provide evidence of that the serogroup B epidemic in Oregon was due to the introduction and spread of one strain through the Oregon population. The exchange of genetic material between the epidemic strain and other circulating *Neisseria* species would be expected to increase the diversity of bacterial population over the course of the epidemic, a theory supported by our increase in the number of distinguishable PFGE patterns, decrease in the proportions of NEME-003 and PFGE Group I isolates, increase in the proportions of possibly-related and unrelated strains, and decrease in the similarity of Group I isolates between the peak and later epidemic periods. Finally, the majority of clustered isolates analyzed were distinguishable by one or more molecular methods, demonstrating that Oregon serogroup B IMD epidemic was largely due to an increased number of sporadic cases rather than an increase in localized community outbreaks.

In 2005, Bille *et al.* reported a potentially new virulence factor by describing a chromosomally integrated bacteriophage, termed Meningococcal Disease Associated (MDA) Island, which was significantly associated with IMD even after controlling for known hyperinvasive meningococcal clones [1]. However, over one third of the isolates tested in that study were from the same invasive clonal complexes and did not include serogroup Y, ST-23/ET-508 complex strains, common in the United States. The goal of the second study was to describe the association between the MDA phage and IMD using a diverse collection of invasive and carriage *N. meningitidis* isolates collected in the United States. Amplification and detection of the MDA phage was conducted by PCR assay of 69 invasive isolates obtained from the Michigan Department of Community Health laboratory and 81 carriage isolates obtained from a 1998 Georgia Carriage Study; Southern blot confirmed the presence of the phage in select isolates. This isolate study population was a subset of 403 isolates (211 invasive and 192 carriage) from a larger, congruent study by Hobb *et al* (Unpublished data).

In contrast to Bille *et al.*, these results failed to demonstrate an association between invasive meningococcal isolates and the presence of the 8 kb integrated, MDA bacteriophage [1]. While we found a significant crude association between the presence of the phage and collection source, the phage was found to be associated with serogroup B and C strains and not serogroup Y strains. Consequently, in multivariate analysis controlling for serogroup, the phage was no longer associated with isolate collection source. The association between the presence of the phage and hyperinvasive clones was consistent with the Bille *et al*. findings. As ST-23, serogroup Y strains became a major cause of sporadic IMD in the United States in the 1990s, one would expect a virulence

factor related to invasiveness to be carried by a high proportion of these organisms. That our results do not show this is consistent with the hypothesis that the MDA phage represents a genetic element acquired by certain strains prior to clonal spread, rather than a virulence factor required for invasive disease.

By reducing the prevalence of colonization of *N. menignitidis* and *N. lactamica*, the increasing population use of BSA for non-prophylactic purposes, especially among children younger than five years of age, may impact the epidemiology of IMD. For instance, reducing the prevalence of circulating pathogenic organisms would be expected to result in a lower incidence of IMD. However, to the extent that increasing BSA use reduces the prevalence of colonizing organisms among children in this age group, the normal process of acquisition of anti-meningococcal immunity in the population may be disrupted, thereby resulting in a greater proportion of individuals at risk for IMD. Through the development of a stochastic, agent-based, simulation model, the goal of the third study was to consider if, and to what extent, increases in the population use of BSA among children younger than five years of age impacted the prevalence of colonization (overall and pathogenic and commensal strain-specific), the proportion of the population with protective immunity, and the occurrence of invasive disease among children within this age group.

The model results demonstrated the accurate capturing of the essential epidemiological components underlying IMD among children younger than five years of age and, in so doing, suggest that increasing BSA use be considered an emergent environmental risk factor for IMD. Specifically, through its effect at decreasing the prevalence of circulating bacterial strains in the population, increasing BSA use led to a

decrease in the proportion of children acquiring natural immunity and, therefore, an increased occurrence of invasive disease. While this study is the first to investigate this phenomenon in its entirety, a decreased prevalence of meningococcal colonization was noted after a mass chemoprophylaxis campaign. Additionally, increased IMD occurrence – in the form of periodic IMD epidemics – has been noted due do to the introduction of a new meningococcal clone in an immunologically-naïve population. These two reports support the conclusions of the model. While the decision to include only children younger than five years in the model led to a more accurate model, increasing the validity of our conclusions, it likely led to an underestimate of the effect of increasing population BSA on decreases in the prevalence of colonization and proportion of children acquiring immunity.

The results presented from all three studies have implications for public health meningococcal surveillance and control activities. First, by characterizing the molecular epidemiology to a greater extent than serogroup alone, PFGE can better differentiate between sporadic and epidemiologically linked IMD cases and, as such, can be used by public health professionals to decide whether or not to undertake a more aggressive public health intervention, such as mass vaccination or chemoprophylaxis campaigns. Thus, PFGE should be considered as one component of a laboratory-based meningococcal surveillance program. Second, as exemplified by the development of vaccines against the polysaccharide capsule, the identification of meningococcal virulence factors has driven research into agent-directed interventions to prevent IMD. The failure of our results to support the role of the integrated bacteriophage as a necessary virulence factor highlights the need for continued research to identify more

promising targets for primary public health interventions intended to further reduce the occurrence of IMD. Third, the tetravalent meningococcal conjugate vaccine (MCV4) is currently recommended for routine vaccination of individuals 11-55 years of age and for individuals two to 10 years of age at increased risk of disease [2, 3]. Changes to these recommendations, such as routine vaccination for younger children or redefining groups at increased risk of disease, may need to be considered upon additional corroborating evidence from epidemiological studies in the context of increasing population BSA use.

In addition to this knowledge and implications derived from this research, however, future directions in the development and implementation of public health IMD measures will be dictated by their feasibility and cost, as well as the goals of the funding institution (i.e. government or academia). For instance, PFGE is a low-cost assay ($50-$100 per isolate) and the technology is available by most, if not all, state public health laboratories in the United States. However, it is not routinely done by public health laboratories, as it does not typically influence the decision-making process for the institution of anti-meningococcal public health interventions. Even in instances where institutional or organizational IMD outbreaks are suspected, measures such as administration of MCV4 or mass chemoprophylaxis are undertaken based on serogroup information. Other molecular techniques (i.e. DNA sequencing or DNA hybridization experiments) do have more promise with regard to the identification of novel agent virulence factors that may ultimately serve as a target for anti-meningococcal vaccines. They are, however, orders of magnitude more expensive; are available in fewer and mainly academic laboratories, due to the increased technological capacity required; are not always successful (as illustrated by the research presented in Chapter III); and require

further research and development to translate identified factors into effective vaccines. Further characterization of epidemiological risk factors for IMD may be more feasible than some of these laboratory techniques, particularly in the ten states currently conducting active surveillance for IMD through the Centers for Disease Control and Prevention Emerging Infections Program (EIP), as medical chart review is already being conducted on each reported IMD case within each surveillance area and the sites have already provided an infrastructure for conducting further research studies, such as the MCV4 vaccine effectiveness study. Two challenges facing such epidemiological research into the specific effect of increasing BSA use, however, are the low occurrence of disease – requiring the recruitment, training, and funding of non-EIP surveillance sites and potentially long enrollment periods to obtain a sufficient sample size – and the difficulty in obtaining an accurate measurement of antibiotic use history.

In light of these considerations and the current profile of IMD in the United States, it is my opinion that priority should be given to better characterization of isolates of *N. meningitidis* using PFGE and further epidemiological research into the impact of increasing population BSA use on IMD epidemiology. Increased molecular characterization of isolates would directly assist public health professionals by identifying potential clusters of IMD that may help better inform decisions on when to institute appropriate public health control measures. For instance, over the past two years in Oregon, three potential institutional clusters of IMD were identified by serogroup determination (Unpublished data). Mass chemoprophylaxis campaigns were instituted in all three clusters. Subsequent PFGE characterization of isolates from the clusters revealed two to consist of isolates with indistinguishable PFGE patterns, with the third consisting

of dissimilar isolates. More fully characterizing isolates on a real-time basis could have conserved resources that were spent to control the third cluster. Indirectly, PFGE may also help public health professionals identify new strains of *N. meningitidis* and, thus, new IMD epidemic periods. Considering the low assay cost and low burden of disease, PFGE characterization of isolates should be feasible.

Additionally, despite the challenges noted, priority should be given to conducting further epidemiological research into the impact increasing population BSA use may be having on the epidemiology of IMD. After remaining stable for approximately 50 years, the annual incidence of IMD has decreased to historic lows over the past eight. The role of increasing BSA as a causal factor in this decline needs to be determined, especially with the potential consequences of increasing population susceptibility to IMD – and therefore meningococcal epidemics – and resulting in certain individuals with a higher IMD risk. Currently available resources – MCV4 in particular – may help prevent against an increase in IMD, rendering the ultimate cost lower. Although a national research initiative may be neither practical nor cost feasible, the network of ten EIP sites may serve as an effective venue through which to better characterize this effect.

At this point, due to the low occurrence of disease, high cost of technology, and limited resource capacity, further research into meningococcal virulence factors leading to sporadic disease is unlikely to be a priority for government funding and may only remain of importance to pharmaceutical research and development programs. However, the extra effort to obtain PFGE results and collect additional epidemiological information, at least in the 10 EIP states, could make a substantial contribution to the field, with limited funds.

References:

1. Bille E. Zahar JR. Perrin A. Morelle S. Kriz P. Jolley KA. Maiden MC. Dervin C. Nassif X. Tinsley CR. A chromosomally integrated bacteriophage in invasive meningococci. *J Exp Microbiol*. 2005;201:1905-13.

2. Centers for Disease Control and Prevention. Notice to Readers: Recommendation from the Advisory Committee on Immunization Practices (ACIP) for Use of Quadrivalent Meningococcal Conjugate Vaccine (MCV4) in Children Aged 2—10 Years at Increased Risk for Invasive Meningococcal Disease. *MMWR*. 2007;56:12-65-6.

3. Centers for Disease Control and Prevention. Prevention and Control of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 2005;54(No. RR-7).

4. Diermayer M, Hedberg K, Hoesly F, Fischer M, Perkins B, Reeves M, Fleming D. Epidemic Serogroup B Meningococcal Disease in Oregon: The Evolving Epidemiology of the ET-5 Strain. *JAMA*. 1999;281:1493-7.