Implications of Differential Age Distribution of Disease-Associated Meningococcal Lineages for Vaccine Development

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New vaccines targeting meningococci expressing serogroup B polysaccharide have been developed, with some being licensed in Europe. Coverage depends on the distribution of disease-associated genotypes, which may vary by age. It is well established that a small number of hyperinvasive lineages account for most disease, and these lineages are associated with particular antigens, including vaccine candidates. A collection of 4,048 representative meningococcal disease isolates from 18 European countries, collected over a 3-year period, were characterized by multilocus sequence typing (MLST). Age data were available for 3,147 isolates. The proportions of hyperinvasive lineages, identified as particular clonal complexes (ccs) by MLST, differed among age groups. Subjects <1 year of age experienced lower risk of sequence type 11 (ST-11) cc, ST-32 cc, and ST-269 cc disease and higher risk of disease due to unassigned STs, 1- to 4-year-olds experienced lower risk of ST-11 cc and ST-32 cc disease, 5- to 14-year-olds were less likely to experience ST-11 cc and ST-269 cc disease, and ≥25-year-olds were more likely to experience disease due to less common ccs and unassigned STs. Younger and older subjects were vulnerable to a more diverse set of genotypes, indicating the more clonal nature of genotypes affecting adolescents and young adults. Knowledge of temporal and spatial diversity and the dynamics of meningococcal populations is essential for disease control by vaccines, as coverage is lineage specific. The nonrandom age distribution of hyperinvasive lineages has consequences for the design and implementation of vaccines, as different variants, or perhaps targets, may be required for different age groups.

*N*eisseria *meningitidis*, the meningococcus, is a pathogen of global significance that causes sporadic cases and periodic epidemics and pandemics of meningitis and septicemia. The disease is associated with high mortality rates and severe sequelae in many patients who recover. Disease rates vary with age, with the highest rates for children and young adults (1–3). However, the meningococcus is usually carried asymptomatically in the nasopharynx of approximately 10% of the human population (4–7). Carriage rates in the population also vary with age, being lowest among infants and young children and rising to a peak among adolescents and young adults (8–10).

Twelve immunochemically distinct meningococcal polysaccharide capsules have been described (12), corresponding to meningococcal serogroups, but most disease is caused by meningococci expressing serogroups A, B, C, Y, W, and X (13). Worldwide invasive meningococcal serogroup distributions vary with region (3, 13); serogroups A, W, and X predominate in Africa, particularly in the "meningitis belt" region (14), whereas most disease in

Western Europe is associated with meningococci expressing serogroup B and C capsules. Serogroups B and C also predominate in North and South America and in high-income countries such as New Zealand and Australia (3, 13, 15, 16). Serogroup Y disease has emerged recently as a public health concern in the United States and Canada (17, 18) and more recently in Europe (3, 11, 19, 20). The emergence of serogroup Y-associated lineages in disease and carriage populations underlines the dynamic nature of meningo-

coccal epidemiology and population biology. These changes have implications for vaccine development and implementation.

There has been a decline in meningococcal disease incidence in Europe since 1999, decreasing from 1.9 cases per 100,000 individuals in 1999 to 0.73 cases per 100,000 individuals in 2010 (21). This decline is due in part to implementation of the meningococcal C conjugate (MCC) vaccine in a number of European countries. However, this cannot account for reductions in serogroup B disease incidence, which may be attributable to natural fluctuations. With new protein-based substitute serogroup B vaccines such as Bexsero (22) being licensed in Europe, it is important to monitor changes in the meningococcal population, particularly as herd immunity effects for this type of noncapsular vaccine are largely unknown. Outer membrane protein-based vaccines have been implemented previously in Europe, but that was in response to single-clone outbreaks (23, 24). The diversity of genotypes in a setting of endemicity represents a challenge for vaccine development and implementation. Detailed characterization of disease and carriage isolates, including the age distribution of disease-associated lineages, is essential for the improvement of disease prevention and control strategies.

MATERIALS AND METHODS

European meningococcal disease isolates. Bacterial samples were obtained from European reference laboratories over 3 years, i.e., 2000 to 2002. A structured sampling program was undertaken to ensure a representative sample; laboratories that received ≤80 disease isolates per year submitted all isolates, and laboratories that processed >80 disease isolates per year sent every third isolate, with the exception of the England and Wales Meningococcal Reference Unit, which sent every tenth isolate, as the unit received >1,000 samples per year. A total of 4,183 samples were received and processed by the European Meningococcal MLST Centre (EMMC) (25), and multilocus sequence typing (MLST) was completed for 4,048 of the samples.

MLST and sequence assembly. MLST was performed as described previously (26, 27). Separation of the labeled extension products was carried out on a 3700 or 3730 capillary DNA analyzer (Applied Biosystems). Assembly and editing of MLST sequence data generated were carried out using STARS (http://sourceforge.net/projects/stars) and Staden software, with Pregap 4 version 1.3 and Gap version 4.7 (28). For each isolate, sequences for each of the seven loci were assigned allele numbers through interrogation of the *Neisseria* MLST database (http://pubmlst .org/neisseria). Allelic profiles were assigned a sequence type (ST) and a clonal complex (cc) using the database.

Data analysis. MLST data were combined with age information collected separately for the isolates by the European Meningococcal Epidemiology Centre (EMEC)/European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) (29) when such data were available. Multinomial regression analyses were performed using Intercooled Stata 12.0 for Windows (StataCorp, College Station, TX). Simpson's index of diversity (D) was used to determine ST diversity by age group. Calculation of discriminatory indices was performed as described previously (30). The value of the index ranges from 0 to 1, with values nearer to 1 indicating greater diversity. The 95% confidence intervals (CIs) for these indices were calculated as described by Grundmann et al. (31). The evenness value (E) is a measure of the relative abundance of the different genotypes making up the richness, i.e., the number of genotypes in a population sample (such as a country). The value ranges from 0 to 1, with values nearer to 1 indicating more even contributions of the genotypes to the overall sample. According to the method described by Robinson et al. (32), the value is calculated as the ratio of the effective number of clones (Se), in this case STs, to the total number of clones (genotypes) (S), i.e., the richness of the

sample. *Se* and *E* increase as the numbers of isolates of each clone become more equal.

RESULTS

Patient age data were available for 3,226 (87.1%) of the 3,705 EU-IBIS epidemiological data records that were able to be harmonized with the EUMenNet data, and complete MLST data were available for 3,147 of those records (Fig. 1). An overall χ^2 test demonstrated a cc-age effect ($P < 0.001$) (Table 1 and Fig. 2 and 3). The age group of 15 to 24 years and the ST-41/44 cc were used as baselines for multinomial regression analyses. The following significant age effects were observed: (i) subjects <1 year of age experienced lower risk of ST-11 cc disease (relative risk ratio [RRR], 0.29 [95% CI, 0.20 to 0.43]), ST-32 cc disease (RRR, 0.50 [95% CI, 0.35 to 0.74]), and ST-269 cc disease (RRR, 0.42 [95% CI, 0.24 to 0.73]) and higher risk of disease due to unassigned STs (RRR, 2.25 [95% CI, 1.33 to 3.83]); (ii) subjects 1 to 4 years of age experienced lower risk of ST-11 cc disease (RRR, 0.44 [95% CI, 0.31 to 0.60]) and ST-32 cc disease (RRR, 0.68 [95% CI, 0.49 to 0.95]); (iii) subjects 5 to 14 years of age were less likely to experience ST-11 cc disease (RRR, 0.64 [95% CI, 0.48 to 0.86]) and ST-269 cc disease (RRR, 0.49 [95% CI, 0.31 to 0.78]); and (iv) subjects ≥25 years of age were more likely to experience disease due to other ccs (i.e., less common ccs that were grouped together for the purposes of this analysis (see Table 1 and Fig. 2 for details) (RRR, 1.88 [95% CI, 1.29 to 2.74]) and unassigned STs (RRR, 2.05 [95% CI, 1.20 to 3.53]). A number of countries implemented the MCC vaccine during the time period of the study (United Kingdom in 1999, the Republic of Ireland in 2000, and the Netherlands in 2002). To account for the possible effects of the implementation on ST-11 cc disease risk, the regression analysis was repeated without isolates from these countries (and those from 2002 from the Netherlands). The lower risk of ST-11 cc disease remained for subjects <1 year of age (RRR, 0.36 [95% CI, 0.24 to 0.57]) and subjects 1 to 4 years of age (RRR, 0.49 [95% CI, 0.35 to 0.69]), with weaker evidence for subjects 5 to 14 years of age (RRR, 0.74 [95% CI, 0.52 to 1.05]).

There was a range of diversity of STs accounting for disease according to age group, with the lowest diversities found in the 25- to 44-year ($D = 0.899$) and 15- to 24-year ($D = 0.914$) age groups. The highest diversity values were found in the <1-year ($D = 0.973$) and 1- to 4-year ($D = 0.959$) age groups. There was significantly greater diversity among subjects <5 years of age versus all other age groups except the ≥45-year age group. There was also varying evenness of STs among the different age groups. The lowest evenness value was found in the 15- to 24-year age group ($E = 0.081$), and the highest value was found in the <1-year age group ($E = 0.180$). This indicates that, in addition to there being lower diversity among 15- to 24-year-olds, the distribution of genotypes was much less even, demonstrating that disease in that age group was dominated by particular STs and ccs. Other age groups, such as the <1-year age group, had more even distributions of STs associated with disease.

DISCUSSION

Several protein-based vaccines to prevent serogroup B meningococcal disease are in various stages of development (33), with one, Bexsero, recently being licensed in Europe. Molecular epidemiology has become an essential part of this development and implementation. Much analysis of the distribution of the four antigens
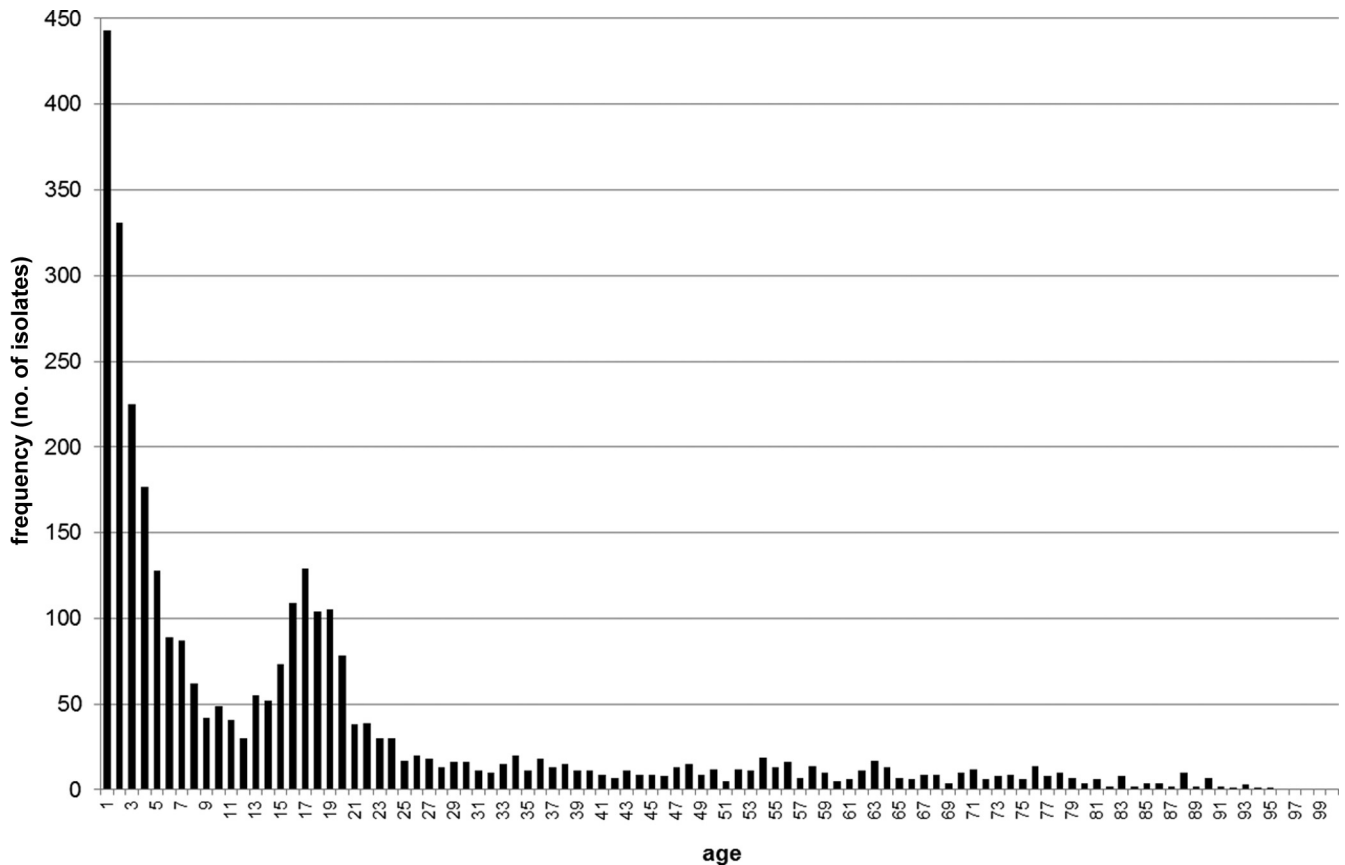
FIG 1 Age distribution of disease isolates in the European meningococcal disease collection.

present in this vaccine has been carried out in recent years, in collections of disease-associated serogroup B-expressing organisms (34–36). Based on the meningococcal antigen typing system (MATS) assay, there is predicted coverage of 78% of disease-associated serogroup B-expressing meningococci in Europe (36). As Bexsero antigen data are not available for this collection, an estimate based on previous studies (34) showing strong associations of antigen and cc would give a baseline coverage of almost 50% for this data set regardless of serogroup (with ST-41/44 cc, ST-32 cc, and ST-8 cc serving as proxies for NHBA-2 and P1.4, fHbp-1.1, and NadA-3.8, respectively). Representative, well-sampled, iso-

late collections such as the EUMenNet collection provide insights into the dynamics of the population biology of the meningococcus and facilitate the planning and implementation of interventions, including immunization programs. Since serogroups and other vaccine antigens are known to be associated with ccs, the differences we observed in the distribution of ccs with age concur with, and extend, previous work in both Europe and North and South America. Studies have indicated a higher prevalence of non-BC serogroups in older age groups, with serogroup B disease being proportionally the greatest among subjects <1 year of age (15, 16, 19, 21, 37–39). The present analysis also indicated that the diver-

TABLE 1 Age distribution among the most common clonal complexes in the European meningococcal disease collection

Clonal complex	No. (%) of isolates per age group					Missing data (no. [%] of isolates)	Total no. of isolates
	<1 yr	1–4 yr	5–14 yr	15–24 yr	≥25 yr		
ST-41/44	143 (14)	253 (25)	155 (15)	161 (16)	138 (14)	164 (16)	1,014
ST-11	49 (5)	131 (15)	132 (15)	189 (21)	183 (20)	219 (24)	903
ST-32	61 (9)	146 (21)	145 (21)	136 (19)	92 (13)	127 (18)	707
ST-8	21 (8)	78 (29)	35 (13)	35 (13)	36 (13)	68 (25)	273
ST-269	20 (8)	63 (25)	22 (9)	54 (21)	39 (15)	58 (23)	256
Others[a]	88 (14)	117 (19)	48 (8)	71 (11)	114 (18)	193 (31)	631
Unassigned	46 (17)	48 (18)	31 (12)	24 (9)	43 (16)	72 (27)	264
Total	428	836	568	670	645	901	4,048

[a] 'Other' ccs include ST-213, ST-23, ST-22, ST-60, ST-35, ST-461, ST-162, ST-18, ST-174, ST-334, ST-167, ST-364, ST-254, ST-103, ST-865, ST-231, ST-750, ST-1157, ST-53, ST-5, ST-226, ST-198, ST-212, ST-92, ST-1136, ST-178, ST-282, ST-37, ST-376, ST-1117, ST-116, ST-175, ST-4240/6688, and ST-549 ccs.
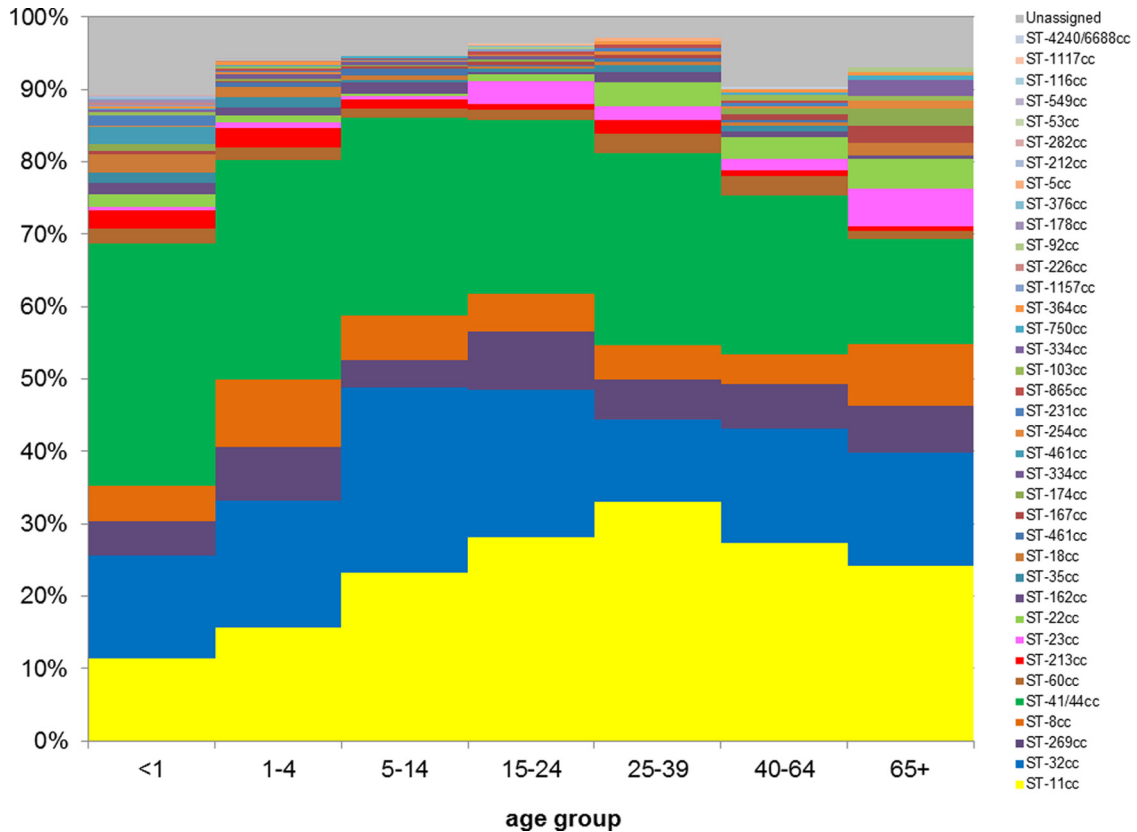
FIG 2 Age distribution of common clonal complexes according to their proportions in the European meningococcal disease collection.

sity of disease-causing meningococci was higher in the youngest and oldest age groups and that ccs other than the major hyperinvasive lineages and unassigned STs were more associated with older age groups.

The relatively lower prevalence of ST-11 cc and ST-269 cc among individuals <14 years of age was consistent with findings in Canada, where there was an association of ST-269 cc in 11- to 40-year-olds and many fewer cases among <1-year-olds (40). Also, a study of meningococcal disease in Poland over 10 years demonstrated a significantly higher frequency of ST-11 cc-associated disease among individuals >5 years of age (41). As with ST-11 cc and ST-269 cc, the serogroup B-associated ST-32 cc was less likely to affect subjects <4 years old. These findings are consistent with the presence of the meningococcal disease-associated (MDA) phage, a candidate virulence factor associated with adolescent disease, in these lineages (42).

The nonrandom variation in age distributions of meningococcal lineages has several consequences. It demonstrates that the different ccs have different phenotypes in terms of disease association and probably carriage. Genotypes that are thought to be comparatively less invasive, along with those not assigned to a cc, were more likely to affect the very young or relatively old (<1 or >65 years of age), which may be due to these cohorts being vulnerable to higher rates of disease caused by less-invasive meningococci, perhaps as a consequence of poorer immune responses. Niche competition with commensal organisms such as *Neisseria lactamica*, which has its highest rates of carriage in 1- to 2-year-olds, may have an influence on meningococcal carriage and thus

potentially disease (43, 44). Immunologically mature individuals such as older adolescents and young adults may more easily clear less-virulent strains but then be more susceptible to more-virulent strains such as ST-11 cc, which may have shorter durations of carriage. Some of these differences may be due to behavioral factors or potential virulence factors such as the MDA phage, which may affect the expression of certain genes. These differences in age associations may have consequences for the design and implementation of vaccines, as different variants or perhaps targets may be required for different age groups. Like other well-studied vaccine candidate antigens, such as PorA and FetA, those included in newly developed vaccines such as Bexsero have associations with clonal complexes (34, 35). Therefore, it is expected that they will also have different age distributions, which may have consequences for vaccine implementation.

Given the diversity of the meningococcal population, a relatively small number of genotypes are associated with disease. In Europe, five ccs (ST-41/44, ST-11, ST-32, ST-8, and ST-269 ccs) accounted for 77% of the disease isolates. These hyperinvasive lineages are a subset of those observed globally and represent a minority of carried meningococci (45–47). The prevalence of particular hyperinvasive lineages in carriage changes over time, and this is reflected in the rates of disease that they cause. For instance, in the past decade previously rare serogroup Y-associated lineages increased in prevalence, in both disease and carriage, in Europe (3, 11, 19, 20, 48–51). It is therefore necessary to maintain surveillance to identify changes in the distribution of types, including the emergence of new clones and possible capsule-switching events in
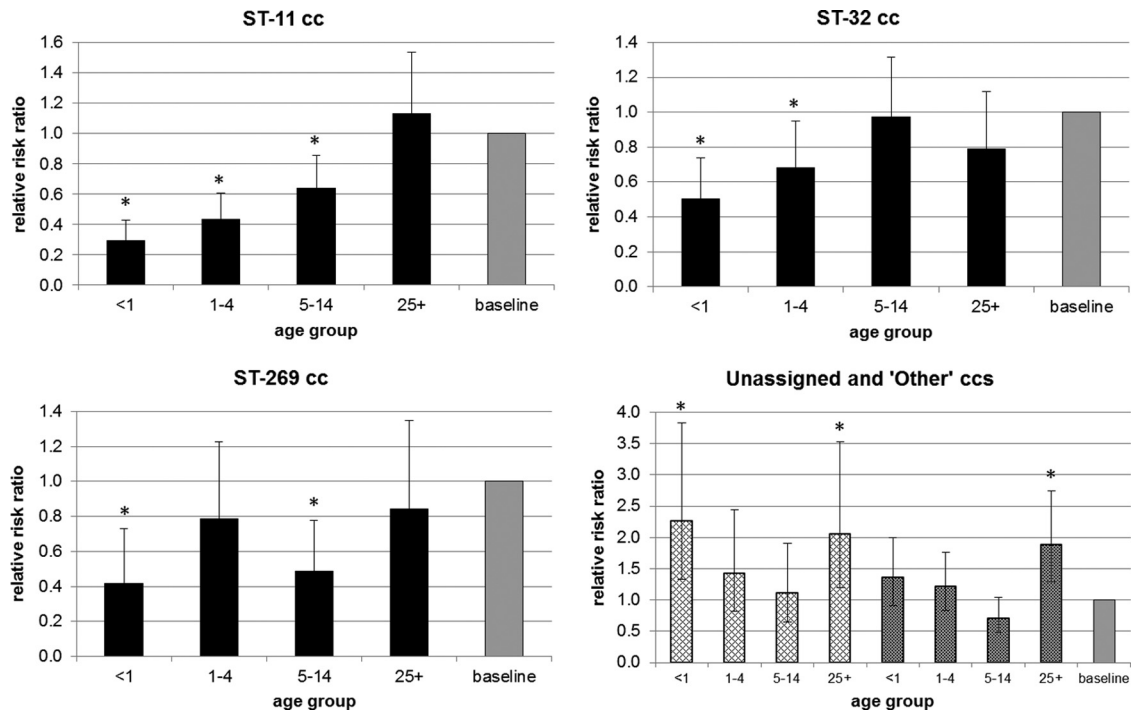
FIG 3 Relative risk ratios (RRRs) of disease by age group for ST-11, ST-32, and ST-269 ccs, unassigned STs (hatched bars), and other ccs (dark gray bars). 'Other' ccs include ST-213, ST-23, ST-22, ST-60, ST-35, ST-461, ST-162, ST-18, ST-174, ST-334, ST-167, ST-364, ST-254, ST-103, ST-865, ST-231, ST-750, ST-1157, ST-53, ST-5, ST-226, ST-198, ST-212, ST-92, ST-1136, ST-178, ST-282, ST-37, ST-376, ST-1117, ST-116, ST-175, ST-4240/6688, and ST-549 ccs. ∗, significant RRR, compared with the baselines of 15 to 24 years and the ST-41/44 cc.

the face of immunization campaigns. This should include monitoring changes in genotype distributions, in disease and carriage, according to age group. Initiatives such as the Meningitis Research Foundation Meningococcus Genome Library are valuable resources that will allow such analyses using the latest molecular epidemiological tools. In the absence of serogroup B conjugate polysaccharide vaccines, the control of meningococcal disease will rely on the implementation of protein-based vaccines, the coverage of which will vary as changes occur in the circulating meningococcal populations over time. Any implementation of new vaccines, such as Bexsero, that target proteins that are derived from particular serogroup B meningococci but may be shared by strains belonging to other serogroups, will therefore require intensive epidemiological surveillance.

REFERENCES

1. **Stephens DS, Greenwood B, Brandtzaeg P.** 2007. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. Lancet **369:**2196–2210. http://dx.doi.org/10.1016/S0140-6736(07)61016-2.
2. **Edmond K, Clark AJ, Korczak VS, Sanderson C, Griffiths UK, Rudan I.** 2010. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. Lancet Infect. Dis. **10:**317–328. http://dx.doi.org/10.1016/S1473-3099(10)70048-7.
3. **Halperin SA, Bettinger JA, Greenwood B, Harrison LH, Jelfs J, Ladhani SN, McIntyre P, Ramsay ME, Sáfadi MAP.** 2012. The changing and dynamic epidemiology of meningococcal disease. Vaccine **30**(Suppl 2)**:** B26–B36. http://dx.doi.org/10.1016/j.vaccine.2011.12.032.
4. **Broome CV.** 1986. The carrier state: *Neisseria meningitidis*. J. Antimicrob. Chemother. **18**(Suppl A)**:**25–34.
5. **Cartwright K** (**ed**). 1995. Meningococcal disease. John Wiley & Sons, Chichester, United Kingdom.
6. **Maiden MC.** 2004. Dynamics of bacterial carriage and disease: lessons from the meningococcus. Adv. Exp. Med. Biol. **549:**23–29. http://dx.doi .org/10.1007/978-1-4419-8993-2_5.
7. **Christensen H, May M, Bowen L, Hickman M, Trotter CL.** 2010. Meningococcal carriage by age: a systematic review and meta-analysis. Lancet Infect. Dis. **10:**853–861. http://dx.doi.org/10.1016/S1473-3099 (10)70251-6.
8. **Caugant DA, Høiby EA, Magnus P, Scheel O, Hoel T, Bjune G, Wedege E, Eng J, Frøholm LO.** 1994. Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population. J. Clin. Microbiol. **32:**323–330.
9. **Bogaert D, Hermans PW, Boelens H, Sluijter M, Luijendijk A, Rumke HC, Koppen S, van Belkum A, de Groot R, Verbrugh HA.** 2005. Epidemiology of nasopharyngeal carriage of *Neisseria meningitidis* in healthy Dutch children. Clin. Infect. Dis. **40:**899–902. http://dx.doi.org /10.1086/428351.
10. **Claus H, Maiden MC, Wilson DJ, McCarthy ND, Jolley KA, Urwin R, Hessler F, Frosch M, Vogel U.** 2005. Genetic analysis of meningococci carried by children and young adults. J. Infect. Dis. **191:**1263–1271. http: //dx.doi.org/10.1086/428590.
11. **Whitney AM, Coulson GB, von Gottberg A, Block C, Keller N, Mayer LW, Messonnier NE, Klugman KP.** 2009. Genotypic comparison of invasive *Neisseria meningitidis* serogroup Y isolates from the United States, South Africa, and Israel, isolated from 1999 through 2002. J. Clin. Microbiol. **47:**2787–2793. http://dx.doi.org/10.1128/JCM.00091-09.
12. **Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, Corton C, Care R, Poolman JT, Zollinger WD, Frasch CE, Stephens DS, Feavers I, Frosch M, Parkhill J, Vogel U, Quail MA, Bentley SD, Maiden MCJ.** 2013. Description and nomenclature of *Neisseria meningitidis* capsule locus. Emerg. Infect. Dis. **19:**566–573. http://dx.doi.org/10.3201 /eid1904.111799.

13. **Harrison LH, Trotter CL, Ramsay ME.** 2009. Global epidemiology of meningococcal disease. Vaccine **27:**B51–B63. http://dx.doi.org/10.1016/j.vaccine.2009.04.063.

14. **Caugant DA, Kristiansen PA, Wang X, Mayer LW, Taha MK, Ouedraogo R, Kandolo D, Bougoudogo F, Sow S, Bonte L.** 2012. Molecular characterization of invasive meningococcal isolates from countries in the African Meningitis Belt before introduction of a serogroup A conjugate vaccine. PLoS One **7:**e46019. http://dx.doi.org/10.1371/journal.pone.0046019.

15. **Bettinger JA, Scheifele DW, Le Saux N, Halperin SA, Vaudry W, Tsang R.** 2013. The disease burden of invasive meningococcal serogroup B disease in Canada. Pediatr. Infect. Dis. J. **32:**e20–e25. http://dx.doi.org/10.1097/INF.0b013e3182706b89.

16. **Ibarz-Pavón AB, Lemos AP, Gorla MC, Regueira M, SIREVA Working Group II, Gabastou JM.** 2012. Laboratory-based surveillance of *Neisseria meningitidis* isolates from disease cases in Latin American and Caribbean countries, SIREVA II 2006–2010. PLoS One **7:**e44102. http://dx.doi.org/10.1371/journal.pone.0044102.

17. **McEllistrem MC, Kolano JA, Pass MA, Caugant DA, Mendelsohn AB, Fonseca Pacheco AG, Shutt KA, Razeq J, Harrison LH, Maryland Emerging Infections Program.** 2004. Correlating epidemiologic trends with the genotypes causing meningococcal disease, Maryland. Emerg. Infect. Dis. **10:**451–456.

18. **Harrison LH, Jolley KA, Shutt KA, Marsh JW, O'Leary M, Sanza LT, Maiden MC.** 2006. Antigenic shift and increased incidence of meningococcal disease. J. Infect. Dis. **193:**1266–1274. http://dx.doi.org/10.1086/501371.

19. **Ladhani SN, Lucidarme J, Newbold LS, Gray SJ, Carr AD, Findlow J, Ramsay ME, Kaczmarski EB, Borrow R.** 2012. Invasive meningococcal capsular group Y disease, England and Wales, 2007–2009. Emerg. Infect. Dis. **18:**63–70. http://dx.doi.org/10.3201/eid1801.110901.

20. **Broker M, Jacobsson S, Kuusi M, Pace D, Simoes MJ, Skoczynska A, Taha MK, Toropainen M, Tzanakaki G.** 2012. Meningococcal serogroup Y emergence in Europe: update 2011. Hum. Vaccin. Immunother. **8:**1907–1911. http://dx.doi.org/10.4161/hv.21794.

21. **European Centre for Disease Prevention and Control.** 2013. Annual epidemiological report 2012. Reporting on 2010 surveillance data and 2011 epidemic intelligence data. ECDC, Stockholm, Sweden. http://dx.doi.org/10.2900/76137.

22. **Serruto D, Bottomley MJ, Ram S, Giuliani MM, Rappuoli R.** 2012. The new multicomponent vaccine against meningococcal serogroup B, 4CMenB: immunological, functional and structural characterization of the antigens. Vaccine **30**(Suppl 2):B87–B97. http://dx.doi.org/10.1016/j.vaccine.2012.01.033.

23. **Bjune G, Gronnesby JK, Høiby EA, Closs O, Nokleby H.** 1991. Results of an efficacy trial with an outer membrane vesicle vaccine against systemic serogroup B meningococcal disease in Norway. NIPH Ann. **14:**125–130.

24. **Caron F, du Chatelet IP, Leroy JP, Ruckly C, Blanchard M, Bohic N, Massy N, Morer I, Floret D, Delbos V, Hong E, Revillion M, Berthelot G, Lemee L, Deghmane AE, Benichou J, Levy-Bruhl D, Taha MK.** 2011. From tailor-made to ready-to-wear meningococcal B vaccines: longitudinal study of a clonal meningococcal B outbreak. Lancet Infect. Dis. **11:**455–463. http://dx.doi.org/10.1016/S1473-3099(11)70027-5.

25. **Brehony C, Jolley KA, Maiden MC.** 2007. Multilocus sequence typing for global surveillance of meningococcal disease. FEMS Microbiol. Rev. **31:**15–26. http://dx.doi.org/10.1111/j.1574-6976.2006.00056.x.

26. **Jolley KA, Kalmusova J, Feil EJ, Gupta S, Musilek M, Kriz P, Maiden MC.** 2000. Carried meningococci in the Czech Republic: a diverse recombining population. J. Clin. Microbiol. **38:**4492–4498.

27. **Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A. **95:**3140–3145. http://dx.doi.org/10.1073/pnas.95.6.3140.

28. **Staden R.** 1996. The Staden sequence analysis package. Mol. Biotechnol. **5:**233–241. http://dx.doi.org/10.1007/BF02900361.

29. **Trotter CL, Chandra M, Cano R, Larrauri A, Ramsay ME, Brehony C, Jolley KA, Maiden MC, Heuberger S, Frosch M.** 2007. A surveillance network for meningococcal disease in Europe. FEMS Microbiol. Rev. **31:**27–36. http://dx.doi.org/10.1111/j.1574-6976.2006.00060.x.

30. **Hunter PR, Gaston MA.** 1988. Numerical index of discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.

31. **Grundmann H, Hori S, Tanner G.** 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J. Clin. Microbiol. **39:**4190–4192. http://dx.doi.org/10.1128/JCM.39.11.4190-4192.2001.

32. **Robinson DA, Sutcliffe JA, Tewodros W, Manoharan A, Bessen DE.** 2006. Evolution and global dissemination of macrolide-resistant group A streptococci. Antimicrob. Agents Chemother. **50:**2903–2911. http://dx.doi.org/10.1128/AAC.00325-06.

33. **Dull PM, McIntosh ED.** 2012. Meningococcal vaccine development—from glycoconjugates against MenACWY to proteins against MenB: potential for broad protection against meningococcal disease. Vaccine **30**(Suppl 2):B18–B25. http://dx.doi.org/10.1016/j.vaccine.2012.01.062.

34. **Bambini S, Piet J, Muzzi A, Keijzers W, Comandi S, De Tora L, Pizza M, Rappuoli R, van de Beek D, van der Ende A, Comanducci M.** 2013. An analysis of the sequence variability of meningococcal fHbp, NadA and NHBA over a 50-year period in the Netherlands. PLoS One **8:**e65043. http://dx.doi.org/10.1371/journal.pone.0065043.

35. **Lucidarme J, Comanducci M, Findlow J, Gray SJ, Kaczmarski EB, Guiver M, Vallely PJ, Oster P, Pizza M, Bambini S, Muzzi A, Borrow R.** 2010. Characterization of *fHbp*, *nhba* (*gna2132*), *nadA*, *porA*, and sequence type in group B meningococcal case isolates collected in England and Wales during January 2008 and potential coverage of an investigational group B meningococcal vaccine. Clin. Vaccine Immunol. **17:**919–929. http://dx.doi.org/10.1128/CVI.00027-10.

36. **Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, Caugant DA, Kriz P, Abad R, Bambini S, Carannante A, Deghmane AE, Fazio C, Frosch M, Frosi G, Gilchrist S, Giuliani MM, Hong E, Ledroit M, Lovaglio PG, Lucidarme J, Musilek M, Muzzi A, Oksnes J, Rigat F, Orlandi L, Stella M, Thompson D, Pizza M, Rappuoli R, Serruto D, Comanducci M, Boccadifuoco G, Donnelly JJ, Medini D, Borrow R.** 2013. Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. Lancet Infect. Dis. **13:**416–425. http://dx.doi.org/10.1016/S1473-3099(13)70006-9.

37. **EU-IBIS Network.** 2007. Invasive *Neisseria meningitidis* in Europe 2006. Health Protection Agency, London, United Kingdom.

38. **Rosenstein NE, Perkins BA, Stephens DS, Lefkowitz L, Cartter ML, Danila R, Cieslak P, Shutt KA, Popovic T, Schuchat A, Harrison LH, Reingold AL.** 1999. The changing epidemiology of meningococcal disease in the United States 1992–1996. J. Infect. Dis. **180:**1894–1901. http://dx.doi.org/10.1086/315158.

39. **Hellenbrand W, Elias J, Wichmann O, Dehnert M, Frosch M, Vogel U.** 2013. Epidemiology of invasive meningococcal disease in Germany, 2002–2010, and impact of vaccination with meningococcal C conjugate vaccine. J. Infect. **66:**48–56. http://dx.doi.org/10.1016/j.jinf.2012.09.008.

40. **Zhou J, Lefebvre B, Deng S, Gilca R, Deceuninck G, Law DK, De Wals P, Tsang RS.** 2012. Invasive serogroup B *Neisseria meningitidis* in Quebec, Canada, 2003 to 2010: persistence of the ST-269 clone since it first emerged in 2003. J. Clin. Microbiol. **50:**1545–1551. http://dx.doi.org/10.1128/JCM.06835-11.

41. **Skoczyńska A, Wacœko I, Kuch A, Kadłubowski M, Gołębiewska A, Foryœ M, Markowska M, Ronkiewicz P, Wasiak K, Kozińska A, Matynia B, Hryniewicz W.** 2013. A decade of invasive meningococcal disease surveillance in Poland. PLoS One **8:**e71943. http://dx.doi.org/10.1371/journal.pone.0071943.

42. **Bille E, Ure R, Gray SJ, Kaczmarski EB, McCarthy ND, Nassif X, Maiden MC, Tinsley CR.** 2008. Association of a bacteriophage with meningococcal disease in young adults. PLoS One **3:**e3885. http://dx.doi.org/10.1371/journal.pone.0003885.

43. **Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M.** 1978. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. J. Infect. Dis. **137:**112–121. http://dx.doi.org/10.1093/infdis/137.2.112.

44. **Bennett JS, Griffiths DT, McCarthy ND, Sleeman KL, Jolley KA, Crook DW, Maiden MC.** 2005. Genetic diversity and carriage dynamics of *Neisseria lactamica* in infants. Infect. Immun. **73:**2424–2432. http://dx.doi.org/10.1128/IAI.73.4.2424-2432.2005.

45. **Caugant DA.** 1998. Population genetics and molecular epidemiology of *Neisseria meningitidis*. APMIS **106:**505–525. http://dx.doi.org/10.1111/j.1699-0463.1998.tb01379.x.

46. **Watkins ER, Maiden MC.** 2012. Persistence of hyperinvasive meningococcal

strain types during global spread as recorded in the PubMLST database. PLoS One **7:**e45349. http://dx.doi.org/10.1371/journal.pone.0045349.

47. **Yazdankhah SP, Kriz P, Tzanakaki G, Kremastinou J, Kalmusova J, Musilek M, Alvestad T, Jolley KA, Wilson DJ, McCarthy ND, Caugant DA, Maiden MC.** 2004. Distribution of serogroups and genotypes among disease-associated and carried isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. J. Clin. Microbiol. **42:**5146–5153. http://dx.doi.org/10.1128/JCM.42.11.5146-5153.2004.

48. **Thulin Hedberg S, Toros B, Fredlund H, Olcen P, Molling P.** 2011. Genetic characterisation of the emerging invasive *Neisseria meningitidis* serogroup Y in Sweden, 2000 to 2010. Euro Surveill. **16:**pii=19885. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19885.

49. **Jounio U, Saukkoriipi A, Bratcher HB, Bloigu A, Juvonen R, Silven-noinen-Kassinen S, Peitso A, Harju T, Vainio O, Kuusi M, Maiden MC, Leinonen M, Kayhty H, Toropainen M.** 2012. Genotypic and phenotypic characterization of carriage and invasive disease isolates of *Neisseria meningitidis* in Finland. J. Clin. Microbiol. **50:**264–273. http://dx.doi.org/10.1128/JCM.05385-11.

50. **Fazio C, Neri A, Sofia T, Carannante A, Caporali MG, Salmaso S, Mastrantonio P, Stefanelli P.** 2009. Characterisation of *Neisseria meningitidis* C strains causing two clusters in the north of Italy in 2007 and 2008. Euro Surveill. **14:**pii=19179. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19179.

51. **Ala'aldeen DA, Oldfield NJ, Bidmos FA, Abouseada NM, Ahmed NW, Turner DP, Neal KR, Bayliss CD.** 2011. Carriage of meningococci by university students, United Kingdom. Emerg. Infect. Dis. **17:**1762–1763. http://dx.doi.org/10.3201/eid1709.101762.

52. **Jolley KA, Maiden MC.** 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics **11:**595. http://dx.doi.org/10.1186/1471-2105-11-595.