Immunisation against meningococcal meningitis has a long history, which has passed through several phases: the studies by Flexner, extraction of the polysaccharide capsule, the development of monovalent and multivalent conjugate vaccines, the outer membrane vesicle vaccines up to the development of effective and safe vaccines for meningococcal B invasive disease through the application of the techniques of molecular biology and reverse vaccinology.

The new available vaccines are Bexsero® and Trumenba®. Bexsero® has been approved and is available in Europe, the USA, Canada, Australia and Chile, and is currently under review in Brazil for the prevention of MenB invasive disease in subjects ≥2 months.

Trumenba® is currently approved only in the USA, for use in adolescents and young adults.

At present, the greatest obstacle to the extensive use of these vaccines in industrialised countries is the high cost and the need to administer multiple doses in infants. However, in some European countries and in some Italian Regions, strategies (free and active call) to fight the disease through vaccination (Bexsero®) are already in place.

Meningococcal B vaccination strategies and their practical application in Italy

R. Gasparini, D. Amicizia, P.L. Lai, D. Panatto
Department of Health Sciences, Genoa University, Italy; Inter-University Centre of Research on Influenza and other Communicable Infections (CIRI-IT)

Key words
Meningococcal B vaccine • Vaccination • Italy

Summary

Introduction

Conjugate vaccines against Haemophilus influenzae type b, Streptococcus pneumoniae and Meningococcus C have dramatically reduced cases of bacterial meningitis in the industrialised nations. However, meningitis type B continues to be a threat to children and adolescents worldwide.

Unlike other serogroups, Neisseria meningitidis B (MenB) disease cannot be prevented by polysaccharide vaccines. The reason for this lies in the chemical structure of the MenB capsule, which contains units identical to some human polysaccharides (human foetal neural cells) and, therefore, determine immunological tolerance [1]. Consequently, research into an effective MenB vaccine has focused on subcapsular antigens, outer membrane vesicles (OMVs). OMVs were successfully used to control specific outbreaks. OMVs are proteoliposomes that contain several different molecular components out of which the porin protein, PorA, is the principle antigenic source of bactericidal antibodies. The limitations of these vaccines are that effectiveness tends to be limited to strains containing the same PorA protein (serosubtype-specific), limiting its use to strain-specific outbreaks and they often elicited a scant immune response in young children [2].

In the last years, the vaccine industry has overcome this difficulty, and a MenB multicomponent vaccine – 4CMenB – Bexsero® – has been developed. This vaccine has been licensed in Europe and other developed countries for the prevention of MenB invasive disease in subjects ≥2 months. In addition, a vaccine containing two variants of factor H binding protein (fhbp) of the complement has been approved by the Food and Drug Administration in the USA (Trumenba®) for use in individuals 10 through 25 years of age.

These advances have prompted some authors to wonder whether we are witnessing “the beginning of the end for invasive MenB disease” [1].

In order to eliminate an infectious disease, the first essential requirement is undoubtedly the availability of safe and effective vaccines. However, strategic planning of the application of the vaccines which have become available is equally important.

This overview examines the policies of vaccination with new meningococcal B vaccines, particularly 4CMenB, in Italy.

Natural history of MenB infections

Meningococci have their natural and unique survival niche in humans. This fundamental biological fact implies that N. meningitidis has acquired several mechanisms for cohabitation with the human organism [3]. Only in particular conditions of frailty of the human host or in certain environmental situations is the microorganism able to manifest its aggressiveness, leading to meningococcal diseases and even death [4, 5].
From sero-epidemiological studies [6, 7] we have learnt much about the biology of this microorganism. In the blood of infants, bactericidal antibodies against Neisseriae are present as a result of the passage of maternal antibodies through the placenta during pregnancy. However, as this protection wanes early, infants are soon exposed to the risk of infection. Indeed, the percentage of infants and children who show bactericidal antibodies grows until the age of about four years. Subsequently, antibody titres decline until adolescence, before increasing again. This pattern is in line with the fact that the two main peaks of morbidity of the disease are seen in subjects under 4 years of age (particularly in infants under 1 year) and in young adults. These epidemiological observations are of primary importance in understanding the spread of the microorganism in the environment around the infant and adolescent. The greatest risk to the infant is engendered by premature contact with virulent strains of N. meningitidis, as has been shown by mathematical modelling [8]. Indeed, the risk of MenB invasive disease has been calculated to be 400 times higher in such cases than in the case of non-early contact.

The situation changes during adolescence, when a more promiscuous social life (kissing, sexual contact, frequenting recreational premises such as pubs, etc) exposes subjects to more frequent contact with the microorganism. Indeed, it is precisely in these subjects that Neisseriae find their ideal niches for survival, as demonstrated by studies on carriers [9, 10].

**Epidemiology of MenB infections**

The distribution of the various serogroups of meningococcal pathogens fluctuates considerable. However, serogroup B currently predominates over the other serogroups in Europe, Australia, Canada and Japan. One reason for this predominance is attributable to extensive vaccination with conjugate vaccine against meningococcal serogroup C [11-16]. Of a total of 3463 confirmed cases of invasive meningococcal disease (IMD) reported in 28 EU/EEA countries in 2012, 2078 were caused by serogroup B; this predominance of serogroup B was most pronounced in infants (83% of cases, 8.9 per 100,000) and 1-4-year-olds (9% of cases, 2.9 per 100,000) [17].

The Italian surveillance system of invasive bacterial infections detected 991 cases of invasive meningococcal disease from 2007 to 2012, with an average of 165 cases per year. Information on typing is available for 764/991 cases (77.1%). Serogroup B was the most frequent (455 cases), constituting 59.6% of the cases typed, followed by serogroup C (220 cases) and serogroup Y (59 cases). During the reporting period, a decrease was observed in cases of meningococcal B (from 81 cases in 2007 to 52 in 2012) and C (from 43 cases in 2007 to 34 in 2012), while cases of serogroup Y gradually increased (from 3 in 2007 to 17 in 2012). Furthermore, on analysing the distribution of serogroups by age-group, it was observed that serogroup B was the most frequently isolated in the younger age-groups. Indeed, considering the 762 cases for which age information was available, serogroup B accounted for 81.1% (77/95) of all cases occurring in the first year of life, 66.2% (92/139) of cases in 1-4-year-olds and 70.1% (54/77) in 5-9-year-olds; in the other age-groups it accounted for about 50% of cases [18]. However, as demonstrated by Azzari et al. [19] by means of real-time PCR, in those countries (such as in Italy) [20] where only positive-isolate samples are counted as meningococcal cases, the incidence is largely underestimated. Furthermore, it is well known that culture-based methods have even lower sensitivity than molecular methods when the patient has been treated with antibiotics [21]. In addition, Azzari et al. found in their study that the case fatality rate was 13.2%, which is higher than the 5% rate recently reported in MenB in patients of any age [19, 22].

**History of meningococcal vaccines against MenB**

In the early 1900s, several attempts at using inactivated vaccines containing whole bacterial cells were made [23-27]. However, both these studies and subsequent clinical trials revealed that whole inactivated vaccines were excessively reactogenic. Later vaccines obtained from meningococcal culture filtrate also yielded contradictory results [28, 29]. The first successful meningococcal vaccines were obtained as a result of studies by Gotschlich et al., who were able to extract and purify high-molecular-weight meningococcal polysaccharides at the Walter Reed Army Institute [30, 31]. However, unlike the polysaccharides of serogroups A and C, the polysaccharide of serogroup B did not raise the production of antibodies on account of phenomena of immune tolerance. Thus, studies to prepare a vaccine against meningococcal B shifted to subcapsular antigens. The first and simplest approach was to use the meningococcal outer membrane vesicles containing membrane proteins (OMVs) and lipopolysaccharide (LPS). Four different formulations of these vaccines were used in Cuba, Chile, Norway, New Zealand and France [32]. However, as subcapsular proteins are very variable, these vaccines proved to be of limited use in containing clonal epidemics. Therefore, in order to identify antigens for development of universal MenB vaccine, studies were oriented towards the determination of the entire genome of a pathogenic strain of N. meningitidis type B (MC 58 strain) [33]. Thus, thanks to remarkable advances in bioinformatics and molecular biology, along with the knowledge acquired over the entire genome of MenB, a new science was born – “reverse vaccinology”. Indeed, the computer-assisted screening of the genome of the microorganism enabled the proteins that were the best candidates for a vaccine against MenB to be identified [34]. It was thus possible to identify about 600 open reading frames that were believed to express surface or exported proteins of MenB. Starting from these 600 proteins, it was possible to express 350 in E. coli, which, after being purified, were able to elicit bactericidal an-
Meningococcal B vaccination in Italy

The natural history of meningococcal infections and invasive disease epidemiological trends clearly suggest that: a) it is necessary to protect infants as early as possible; b) it is important to vaccinate adolescents and young adults, who are a risk group, as they constitute the reservoir of the microorganism and can transmit the pathogen to infant siblings. Moreover, in the individual perspective, it is important to reach vulnerable subjects. The best policy would be to vaccinate all subjects from 0 to 18 years of age through an extensive campaign, as suggested by the results achieved in the UK with the conjugate vaccine for meningococcal C [43]. When this is not economically sustainable, it is very important to study the conditions which regulate the spread of the disease. Mathematical models with simplified algorithms can provide the key to obtaining the maximum yield with the minimum of resources. The first question concerns how many subjects the sick person is able to infect [44]. Naturally, this will depend on the characteristics of diffusivity of the pathogen, the number of subjects with whom the patient comes into contact, the number of susceptible, partially susceptible or protected individuals, and the period of time during which the subject is able to spread the disease. It is logical to imagine that, if a large number of subjects are protected, for example through vaccination, the pathogen will have difficulty spreading in the healthy population. If the proportion of vaccinees is high enough, it may be assumed that the patient can infect only a small number of people; if this number falls below 1, there is hope that the disease can be eliminated, as it will be impossible for the microorganism to circulate among humans. The mathematical modellers call this proportion the critical percentage of vaccination coverage. The higher the critical percentage is, the harder it will be to eliminate the disease. Now, in the case of meningitis, this critical percentage is estimated to be not very high. Indeed, to calculate the critical percentage of coverage, it is necessary to know the value of the basic reproductive number \( R_0 \), which has been estimated to be approximately 1.36-1.4 for meningococcal type C. It may be even lower for meningococcal type B [46]. If, however, we imagine that the value of \( R_0 \) is between 1.26-1.4 for MenB, we can calculate [47] that the critical percentage of coverage ranges from 26.5 to 28.6%. Furthermore, during our recent study [9], we did not find the carriage rate for meningococcus C among young adults in a setting where a coverage rate for the conjugate vaccine against \( N. meningitidis \) of serogroup C is 87% among children and 49% among adolescents [48]. It is also important to consider that herd immunity against \( N. meningitidis \) C has been substantially achieved through vaccination with the conjugate vaccine. Indeed, Trotter et al. found that, although the protection provided by the vaccine is, theoretically limited in British infants immunised with conjugate vaccine this protection persisted over time. This was also in agreement with the decrease in meningococcal C carriers among young British adults after the 1999-2000 vaccination campaign [49, 50]. It could therefore be surmised that herd immunity can be effectively induced by MenB vaccine, too. Recently, the World Health Organization (WHO) drew up some criteria for the introduction of a new vaccine. The basic criteria concern: disease burden, efficacy, safety and quality of the vaccine, comparison with other interventions against the disease, economic and financial issues, fiscal impact, financial sustainability, vaccine presentation, supply availability, and programmatic strength [51]. The guidelines defined by the WHO are in line with the criteria of Health Technology Assessment (HTA), which can obviously be applied to vaccines, too. Indeed, HTA is a method of multidisciplinary assessment that deals with analysing the technical, scientific, economic, ethical, legal, social and organizational issues arising from the application of new technologies [52]. Thus, in order to insert a new vaccine into the vaccination schedule (free and active offer by National Health Service), it is necessary to conduct an HTA study [53]. Indeed, vaccines are to be regarded as any other medical technology [52]. In HTA evaluations, cost-effectiveness studies assume great importance. In the specific case of Bexsero®, these have yielded contrasting results and there is still uncertainty as to whether MenB vaccination by means of the 4MenB vaccine should be introduced in developed countries. Indeed, on evaluating the introduction of Bexsero® in England,
Christensen et al. concluded that vaccination would be cost-effective from the National Health Service (NHS) perspective at a cost of £9-£17 per dose [54]. Subsequently, however, after re-evaluating the cost-effectiveness of universal vaccination with Bexsero® in England could be cost-effective with a low vaccine price [55]. By contrast, the results of a study conducted in Italy by Capri et al. demonstrated the cost-effectiveness of vaccination at a cost of €60 per dose, from the societal perspective [56]. However, a study conducted by Tirani et al. in Italy concluded that, from the NHS perspective, the immunisation programme was unlikely to be cost-effective [57].

We recently carried out a cost-effectiveness study of this issue (article submitted to Human Vaccines & Immunotherapeutics). Our results confirmed that, especially from the societal perspective, the vaccination of Italian infants is cost-effective; the study considered various scenarios and also took into account the fact that cases occurring in Italy are underestimated [19]. It is important to consider that economic studies on vaccinations can have some limitations and they often adopt conservative estimates as not considering the underestimation of cases of illness and considering a short-term duration of protection. It is certain that 4CmenB stimulates the immune memory as it is made from MenB surface proteins. Indeed, it is well known that the protein antigens are much more immunogenic in comparison with the polysaccharidic ones even if the latter are conjugated.

**The offer of MenB vaccination against in Italy**

Eight Italian Regions and one Autonomous Province currently offer free vaccination for MenB to certain groups of people (Fig. 1). While Piedmont and Emilia Romagna offer it only to subjects at risk [58, 59], seven Regions and one autonomous Province offer it actively and free of charge for infants. Basilicata was the first Region to insert it into the childhood vaccination calendar [60]. Subsequently, Puglia, Veneto, Friuli Venezia Giulia, Tuscany, Liguria, Sicilia and the Autonomous Province of Bolzano included it in their vaccination schedules [61-67]. The above-mentioned seven Regions and the Autonomous Province of Bolzano, following the possible vaccine schedules (Tab. I), display slight variations in the age at which vaccination is administered. Indeed, as can be seen in figure 2, most of the Regions start vaccination early, as most cases of MenB invasive disease occur within the first year of life; only Veneto and Friuli Venezia Giulia schedule vaccination to begin at 7 months. With regard to the booster dose, most of the Regions schedule this at the 15th month, while Basilicata and Tuscany provide a booster at the 13th month. For the moment, there is no plan to offer active and free vaccination for teenagers.

![Fig. 1. Italian Regions where MenB vaccine is offer free vaccination (February 2015).](image)

| Age of administration | Primary immunisation | Time interval between doses | Booster dose |
|-----------------------|----------------------|----------------------------|--------------|
| From 2 to 5 months    | 3 doses (0.5 ml)     | At least 1 month           | One dose from 12 to 23 months after primary immunisation |
| From 6 to 11 months   | 2 doses (0.5 ml)     | At least 2 months          | One dose in the 2nd year of life (at least 2 months after primary immunisation) |
| From 12 to 23 months  | 2 doses (0.5 ml)     | At least 2 months          | One dose (from 12 to 23 months after primary immunisation) |
| From 2 to 10 months   | 2 doses (0.5 ml)     | At least 2 months          |              |
| From 11 months        | 2 doses (0.5 ml)     | At least 2 months          |              |
Conclusions

Strategically, infants constitute the first class of subjects to be vaccinated, and the Regions which offer free vaccination are rightly oriented in this direction. Indeed, the incidence rate of meningococcal meningitis in Italian infants under 1 year of age is 3.7 per 100,000, i.e. more than 10 times higher than the overall rate of invasive meningococcal diseases observed in Italy. Furthermore, serogroup B is more frequently detected among infants aged under 1 year, accounting for 65% of the total [68]. Moreover, both in order to better protect (indirectly) new-borns and to achieve the best herd immunity, it would be very useful to vaccinate young adults. It is likely that a similar multi-cohort strategy, even with relatively low coverage rates, could prevent the circulation of MenB.

Finally, the vaccination plans of the Italian Regions that offer vaccination for infants are appropriate to epidemiological reality, although Veneto and Friuli Venezia Giulia should bring forward the time of vaccination.

References

[1] Snape MD, Pollard AJ. The beginning of the end for serogroup B meningococcus? Lancet 2013;381:785-7.
[2] Panatto D, Amicizia D, Lai PL, et al. New versus old meningococcal group B vaccines: how the new ones may benefit infants & toddlers. Indian J Med Res 2013;138:835-46.
[3] Gasparini R, Amicizia D, Lai PL, et al. Neisseria meningitidis, pathogenetic mechanisms to overcome the human immune defences. J Prev Med Hyg 2012;53:50-5.
[4] Notarangelo LD, Schumaker RF. Vaccinazione antimeningococcica. Area Pediatrica 2003;4:38-44.
[5] Bartolozzi G, Azzari C, Bona G, et al. Meningococco. Dalla vaccinazione d’emergenza alla prevenzione programmata. Medica Editoria e Diffusione Scientifica. Milano, 2011.
[6] Trotter C, Borrow R, Andrews NE, et al. Seroprevalence of meningococcal serogroup C bacterial antibody in England and Wales in the pre-vaccination era. Vaccine 2003;21:1094-8.
[7] Gasparini R, Rizzetto R, Sasso T, et al. Seroprevalence of bacterial antibody against Neisseria meningitidis serogroup C in pre-vaccinal era: the Italian epidemiological scenario. Vaccine 2009;27:3435-8.
[8] Guzzetta G, Manfredi P, Gasparini R, et al. On the relationship between meningococcal transmission dynamics and disease: remarks on humoral immunity. Vaccine 2009;27:3429-34.
[9] Gasparini R, Comando M, Amicizia D, et al. Molecular and serological diversity of Neisseria meningitidis carrier strains isolated from Italian students aged 14 to 22 years. J Clin Microbiol 2014;52:1901-10.
[10] Christensen H, May M, Bowen L, et al. Meningococcal carriage by age: a systematic review and meta-analysis. Lancet Infect Dis 2010;10:853-6.
[11] Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Net-
work. Neisseria meningitidis, 1998-2009. http://www.cdc.gov/abc/reports-findings/surv-reports.html. Accessed June 19, 2015.

[12] European Centre for Disease Prevention and Control Surveillance Report: Surveillance of invasive bacterial diseases in Europe 2008/2009. Stockholm, Sweden: ECDC; 2011.

[13] Organización Panamericana de la Salud. Informe Regional de SIREVA II, 2009: datos por país y por grupos de edad sobre las características de los aislamientos de Streptococcus pneumoniæ. Haemophilus influenzae Neisseria meningitidis en procesos invasores. Washington, DC: Vigilancia Sanitaria, Prevención y Control de Enfermedades (HSD); 2010.

[14] Takahashi H, Karoki T, Watanabe Y, et al. Characterization of Neisseria meningitidisisolates collected from 1974 to 2003 in Japan by multitocus sequence typing. J Med Microbiol 2004;53:657-62.

[15] Australian Government – Department of Health. Immunize Australia Program. Meningococcal disease. http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/immunise-meningococcal Accessed June 19, 2015.

[16] Dang V, Jamieson FE, Wilson S, et al. Epidemiology of serogroup B invasive meningococcal disease in Ontario, Canada, 2000 to 2010. BMC Infect Dis 2012;29:12;2002.

[17] European Centre for Disease Prevention and Control. Surveillance of invasive bacterial diseases in Europe, 2012. Stockholm: ECDC; 2015.

[18] Rota MC, Bella A, D’Angelo F, et al. Vaccinazione anti- meningococco B: dati e evidenze disponibili per l’introduzione in nuovi nati e adolescenti. Roma: Istituto Superiore di Sanità; 2015. (Rapporti ISTISAN 15/12).

[19] Azzari C, Canessa C, Lippi F, et al. Distribution of invasive meningococcal B disease in Italian pediatric population: implications for vaccination timing. Vaccine 2014;32:1187-91.

[20] Istituto Superiore di sanità – Sorveglianza MIB. A report on antimeningitis vaccination and observations on 1938-40. Proc Roy Soc Med 1916;10:44-60.

[21] Rota MC, Bella A, D’Angelo F, et al. Vaccinazione anti-meningococco B: dati e evidenze disponibili per l’introduzione in nuovi nati e adolescenti. Roma: Istituto Superiore di Sanità; 2015. (Rapporti ISTISAN 15/12).

[22] Resti M, Micheli A, Moriondo M, et al. Comparison of the effect of antibiotic treatment on the possibility of diagnosing invasive pneumococcal disease by culture or molecular methods: a prospective, observational study of children and adolescents with proven pneumococcal infection. Clin Ther 2009;31:1266-73.

[23] Ladhani SN, Flood JS, Ramsay ME, et al. Invasive meningococcal disease in England and Wales: implications for the introduction of new vaccines. Vaccine 2012;30:3710-6.

[24] Flexner S. The results of serum treatment in thirteen hundred cases of epidemic meningitis. J Exp Med 1913;17:533.

[25] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of human antibodies. J Exp Med 1969;129:1307-26.

[26] Sophian A, Balck J. Prophylactic vaccination against epidemic meningitis. Jama 1912;59:527-32.

[27] Greenwood M. The outbreak of cerebrospinal fever at Salisbury in 1914-15. Proc Roy Soc Med 1916;10:44-60.

[28] Gates FL. A report on antimentitis vaccination and observation on agglutinins in the blood of chronic meningococcus carriers. J Exp Med 1918;28:449-74.

[29] Ferry NS. Active immunization with meningococcus toxin. Jama 1935;104:983-4.

[30] Kuhn D, Kisner P, Williams MP, et al. The control of meningococcal meningitis epidemics by active immunization with meningococcal soluble toxins: further studies. Jama 1938;110:484-7.

[31] Gotschlich EC, Goldschneider I, Artenstein MS. Human immunity to meningococcus. IV. Immunogenicity of group A and group C meningococcal polysaccharides in human volunteers. J Exp Med 1969;129:1367-84.

[32] Gasparini R, Amicizia D, Domnich A, et al. Neisseria meningitidis B vaccines: recent advances and possible immunization policies. Expert Rev Vaccines 2014;13:345-64.

[33] Tettelin H, Saunders NJ, Heidelberg J, et al. Complete genome sequence of Neisseria meningitidis serogroup B strain MC58. Science 2000;287:1809-15.

[34] Rappuoli R. Reverse vaccinology, a new genome-based approach to vaccine development. Vaccine 2001;19:2688-91.

[35] McKenzie LD, Bernfield L, Barniak V, et al. Vaccine potential of the Neisseria meningitidis 2086 lipoprotein. Infect Immun 2004;72:2088-100.

[36] Murphy E, Andrew L, Lee KL, et al. Sequence diversity of the factor H binding protein vaccine candidate in epidemiologically relevant strains of serogroup B Neisseria meningitidis. J Infect Dis 2009;200:579-89.

[37] Pajon R, Beerink PT, Harrison LH, et al. Frequency of factor H-binding protein modular groups and susceptibility to cross-reactive bactericidal activity in invasive meningococcal isolates. Vaccine 2010;28:2122-9.

[38] Mascioni A, Bentley BE, Camarda R, et al. Structural Basis for the Immunogenic Properties of the Meningococcal Vaccine Candidate LP2086. J Biol Chem 2009;284:8738-46.

[39] Martinon-Torres F, Gimenez-Sanchez F, Bernaloa-Iurbe E, et al. A randomized, phase 1/2 trial of the safety, tolerability, and immunogenicity of bivalent rLP2086 meningococcal B vaccine in healthy infants. Vaccine 2014;32:5206-11.

[40] Kaaijk P, van Straaten I, van de Waterbeemd B, et al. Preclinical safety and immunogenicity evaluation of a nonavalent PorA native outer membrane vesicle vaccine against serogroup B meningococcal disease. Vaccine 2013;31:1065-71.

[41] Lee M, Bortec C, Montana M, et al. Meningococcal vaccines: Current state and future outlook. Pathol Biol 2015;63:144-51.

[42] FDA. US Food and Drug Administration. Tramunba. Available at: http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm421020.htm Accessed on 1 July 2015.

[43] Miller E, Salisbury D, Ramsay M. Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. Vaccine 2001;20:S58-67.

[44] Fine PEM, Mulholland k. Community immunity. In Plotkin S, Orenstein W. Offit P. Vaccines Fifth Edition. Elsevier 2008, pp: 1573-92.

[45] Trotter CL, Gay NJ, Edmunds WJ. Dynamic models of meningococcal carriage, disease, and the impact of serogroup C conjugate vaccination. Am J Epidemiol 2005;162:89-100.

[46] Pan-Canadian Public Health Network. The Recommended Use of the Multicomponent Meningococcal B (4CMenB) Vaccine in Canada: Common Guidance Statement of the Multicomponent Meningococcal B vaccine. Available at: http://www.phac-aspc.gc.ca/naci-ccni/mening-4cmenb-exec-resum-eng.php Accessed On 1 July 2015.

[47] Anderson RM, Nokes DJ. Mathematical models of transmission and control. In Holland WW, Detels R, Knox G. Oxford Textbook of Public Health. Oxford Medical Publications, Second Edition, Oxford University Press, New York 1999.

[48] Zoppia G, Trucchi C. Prevention of invasive diseases: strategies to increase vaccination coverage in children and adolescents. J Prev Med Hyg 2012;53:125-9.

[49] Trotter CL, Maiden MC. Meningococcal vaccines and herd immunity: lessons learned from serogroup C conjugate vaccination programs. Expert Rev Vaccines 2009;8:851-61.

[50] Maiden MC, Ibzar-Payon AB, Urwin R, et al. Impact of Meningococcal Serogroup C Conjugate Vaccines on Carriage and Herd Immunity. J Infect Dis 2008;197:737-43.
[51] WHO. Vaccine Introduction Guidelines Adding a vaccine to a national immunization programme: decision and implementation. http://www.who.int/immunization/hpv/plan/vaccine_introduction_guidelines_who_2005.pdf Accessed on 28 June 2015.

[52] Gasparini R, Amicizia D, Lai PL, et al. Health Technology Assessment and vaccinations in Italy. GRITTA 2014;1:16-24.

[53] Ministero della Salute. Piano Nazionale per la Prevenzione Vaccinale 2012-2014. http://www.salute.gov.it/imgs/C_17_pubblicazioni_1721_allegato.pdf. Accessed on 1 July 2015.

[54] Christensen H, Hickman M, Edmunds WJ, et al. Introducing vaccination against serogroup B meningococcal disease: an economic and mathematical modelling study of potential impact. Vaccine 2013;31:2638-46.

[55] Christensen H, Trotter CL, Hickman M, et al. Re-evaluating cost effectiveness of universal meningitis vaccination (Bexsero) in England: modelling study. BMJ 2014;349:g5725.

[56] Capri S, Veneziano MA, de Waure C. Valutazione economica di Bexsero®. QUPH 2013;13:68-79.

[57] Tirani M, Meregaglia M, Melegaro A. Health and economic outcomes of introducing the new MenB vaccine (Bexsero) into the Italian routine infant immunisation programme. PLoS One 2015;10:e0123383.

[58] Regione Piemonte. ASL TO4. Vaccinazione pediatrica. Document available at: http://www.astloto4.piemonte.it/document.asp?codice=11392004&codType=2. Accessed on 2 June 2015.

[59] Regione Emilia Romagna. ASL TO4. Vaccinazione pediatrica. Document available at: http://www.astloto4.piemonte.it/document.asp?codice=11392004&codType=2. Accessed on 2 June 2015.

[60] Regione Basilicata. Dipartimento Politiche per la Persona. Deliberazione n. 167 dell’11 febbraio 2014. Approvazione del documento tecnico-scientifico dal titolo “Programma di campagna vaccinale per la prevenzione primaria della malattia invasiva da Meningococco di gruppo B”. Available at: http://operservice.regione.basilicata.it/opendata/home.jsp?tile=DELIBERE_delibere.jsp&year=2014&p=page=38. Accessed on June 29, 2015.

[61] Regione Puglia. Deliberazione n. 956 del 20-05-2014. Commissione Regionale Vaccini. Modifica Calendario Regionale per la vita 2012 – DGR 241/2013. Approvazione nuovo Calendario Vaccinale per la vita 2014. Available at: http://www.regione.puglia.it/index.php?page=delibere&opz=view&kid=12256. Accessed on June 29, 2015.

[62] Regione Veneto. deliberazione della giunta regionale n. 1564 del 26 agosto 2014. Approvazione Nuovo “Calendario Vaccinale” della Regione del Veneto. Parziale modifica della D.G.R. n. 411 del 26.02.2008, approvazione documento “Offerta vaccinazioni soggetti a rischio”, approvazione “Programma di formazione per gli operatori sanitari”, approvazione documento “Piano di comunicazione a sostegno delle malattie infettive prevenibili con vaccino”. Available at: http://bur.regione.veneto.it/BurrS-ervices/pubblica/DettagliodGdr.aspx?id=281075. Accessed on June 29, 2015.

[63] Regione Friuli Venezia Giulia. Allegato alla Delibera n. 2535 del 18 dicembre 2014. Offerta Vaccinale regionale: infanzia e adolescenza. Available at: http://ntom.regione.fvg.it/stor age/2014_2535/Allegato%201%20Delibera%202535-2014.pdf. Accessed on June 29, 2015.

[64] Regione Toscana. Calendario vaccinale della Regione Toscana e direttive in materia di vaccinazioni. Aggiornamento al 2014. Available at: http://www301.regione.toscana.it/bancadati/atti/Contenuto.xml?id=5090161&nomeFile=Delibera_n._823_del_06-10-2014. Accessed on June 29, 2015.

[65] Regione Sicilia. Decreto assessorale 38/2015. Modifica e Integrazione del Calendario Vaccinale per la vita. Adottato con DA n° 0820/2012. Available at: http://pti.regione.sicilia.it/portal/page/portal/PiR_PORTELE/PiR_LaStrutturaRegionale/PiR_AssessoratoSalute/PiR_Decreti/PiR_Decreti2015/PiR_Decretiassessoriali/2015/12/2001%202015%20SERV%20(38).pdf. Accessed on June 29, 2015.

[66] Regione Liguria. Deliberazione della giunta regionale 22.12.2014 n. 1701 Piano Regionale Prevenzione Vaccinale, aggiornamento anno 2015. Bollettino Ufficiale Della Regione Liguria. Anno XLVI N. 3 del 21 gennaio 2015: 99-113. Available at: http://www.bur.liguriainrete.it/ArchivioFile/B_202815032000.pdf. Accessed on June 29, 2015.

[67] Provincia Autonoma di Bolzano. Dipartimento di Prevenzione. La vaccinazione protegge. http://www.provincia.bz.it/sanita/download/la_vaccinazione_protegge_2014(1).pdf. Accessed on 2 June 2015.

[68] Stefanelli P, Fazio C, Neri A, et al. Changing epidemiology of Infant Meningococcal Disease after the introduction of meningococcal serogroup C vaccine in Italy, 2006-2014. Vaccine 2015;33:3678-81.