Distribution of invasive meningococcal B disease in Italian pediatric population: Implications for vaccination timing[☆]

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ABSTRACT

Neisseria meningitidis group B (MenB) is a leading cause of meningitis and sepsis. A new vaccine has been recently licensed. The aim of the present study was to evaluate the epidemiology of MenB disease in pediatric age and define the optimal age for vaccination. All patients aged 0–18 years admitted with a diagnosis of meningitis or sepsis to the 83 participating Italian pediatric hospitals were included in the study. Blood and/or cerebrospinal fluid (CSF) samples were tested by Realtime-PCR and/or culture. One hundred and thirty-six cases (mean age 5.0 years, median 2.7) of MenB disease were found. Among these, 96/136 (70.6%) were between 0 and 5 years, 61/136 (44.9%) were between 0 and 2 years. Among the latter, 39/61 (63.9%) occurred during the first year of life with highest incidence between 4 and 8 months. A case-fatality rate of 13.2% was found, with 27.8% cases below 12 months. Sepsis lethality was 24.4%. RT-PCR was significantly more sensitive than culture: 82 patients were tested at the same time by both methods, either in blood or in CSF; MenB was found by RT-PCR in blood or CSF in 81/82 cases (98.8%), culture identified 27/82 (32.9%) infections (Cohen's Kappa 0.3; McNemar's: $p < 10^{-5}$). The study shows that the highest incidence of disease occurs in the first year of age, with a peak between 4 and 8 months of life; 30% of deaths occur before 12 months. The results suggest that the greatest prevention could be obtained starting MenB vaccination in the first months of life; a catch-up strategy up to the fifth year of life could be considered. Our results also confirm that Realtime PCR is significantly more sensitive than culture. In those countries where only isolate positive infections are counted as cases, the incidence of MenB infection results highly underestimated.

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1. Introduction

Neisseria meningitidis is one of the most frequent causes of bacterial meningitis and septicemia worldwide [1,2]. Meningococcal disease is associated with significant mortality (10–20%) and long-term morbidity [1], especially in pediatric age and 20% of affected people have permanent neurological disabilities [3–5]. Lethality of sepsis is over 20% in children [6,7]. Prevention is therefore a priority.

Thirteen different serotypes are known, but, as known, most invasive meningococcal disease is caused by one of six capsular groups A, B, C, W135, X and Y. Excellent conjugate vaccines have been licensed so far. In Italy, since the introduction of conjugate meningococcal C vaccine (MenC), a rapid and sustained reduction in the incidence of invasive MenC disease across all age groups occurred [8,9]. As a consequence, capsular group B (MenB) has become responsible for most cases [7,9]. A vaccine against group B has recently been licensed in Europe and other vaccines are under study; preliminary data regarding immunogenicity and safety are promising both in infants and adolescents or adults [10,11].

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With the aim to provide broader cross-protection, vaccines under development include highly conserved subcapsular proteins such as PorA, variants of factor H binding protein (fHbp), Neisserial Heparin binding Antigen (NHBA) and Neisserial adhesin A (NadA) [1]. In order to plan an effective vaccination schedule, it is important to know when the greatest burden of meningococcal B disease occurs and if vaccine prevention should be done during the first year of life or later. The aim of the present study is therefore to describe the epidemiology of invasive meningococcal B disease across pediatric age groups so to define the optimal age for vaccination.

2. Methods

2.1. Study design

This observational, retrospective, cohort study was designed to evaluate the distribution of meningococcal B invasive disease cases across age groups in children admitted with a clinical suspicion of community-acquired meningitis or sepsis to Pediatric Hospitals or Pediatric wards of general hospitals in Italy from December 2006 to December 2012. This study was a part of a prospective study aimed at obtaining epidemiological and clinical data of Italian children with invasive bacterial diseases [12]. Hospitals from all Italian regions were invited to participate (see Table A, provided as supplementary file, for the characteristic of the participating hospitals).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.09.055.

2.2. Case definition

Bacterial meningitis was suspected in the presence of at least two of the following clinical signs: bulging fontanelle, drowsiness or irritability, opisthotonus, neck stiffness, vomit or seizures [13] A bacterial meningitis case was defined when clinical signs were associated to the positivity of RT-PCR (Realtime Polymerase Chain Reaction) and/or blood or CSF (Cerebral Spinal Fluid) culture for a bacterium. Meningococcal meningitis was defined by the presence of clinical suspicion together with chemical CSF tests and the positivity of culture or RT-PCR on CSF for *N. meningitidis*. Meningococcal meningitis was defined associated to sepsis when RT-PCR was positive for *N. meningitidis* in blood, too. Sepsis was clinically suspected in the presence of previously described signs [14,15] and confirmed by culture or RT-PCR for *N. meningitidis*.

2.3. Patients

All patients aged 0–18 years admitted with a diagnosis of meningitis or sepsis to the participating centers during the study period were included in the study. Data regarding age, sex, clinical presentation, blood tests, radiologic exams and vaccination status were collected. Biological samples were obtained as part of routine exams for etiologic definition. The study, partially funded by the Italian Center for Disease Control (CCM), was approved by the local institutional review board.

2.4. Clinical samples

Samples of blood and/or CSF, according to the clinical presentation, were obtained from all children included in the study as soon as possible after hospital admission and were used for molecular testing by RT-PCR and/or culture. All samples for cultural tests were immediately sent to the local laboratory using the procedures established by each hospital for culture tests. All samples for molecular tests were sent to the central Laboratory (Immunology Laboratory, Anna Meyer Children Hospital, Florence, Italy) using

a free-post carrier, delivered within the following day and tested within 2 h after delivery. All the samples for molecular tests were accompanied by a form collecting demographic and laboratory data and the main clinical findings of the patient.

For culture purposes, 4–6 ml of blood samples (up to 3 sets) were used. All cases in which RT-PCR or culture demonstrated the presence of *N. meningitidis* were serogrouped using molecular techniques; in the central Laboratory 200 μl of whole blood were used for both diagnosis and serogrouping by RT-PCR.

2.5. DNA extraction

Bacterial genomic DNA was extracted from 200 μl of biological samples using the QIAmp Dneasy Blood & Tissue kit (Qiagen), according to the manufacturer's instructions.

2.6. RT-PCR

RT-PCR amplification was performed in 25 μl reaction volumes containing 2× TaqMan Universal Master Mix (Applied Biosystem, Foster City, CA, USA); primers were used at a concentration of 400 nM; FAM labeled probes at a concentration of 200 nM. Six μl of DNA extract was used for each reaction. All reactions were performed in triplicate. A negative control (no-template) and a positive control were included in every run. DNA was amplified in an ABI 7500 sequence detection system (Applied Biosystem, Foster City, CA, USA) using, for all the primers couples, the same cycling parameters as follows: 50° for 2 min for UNG digestion 95 °C for 10 min followed by 45 cycles of a two-stage temperature profile of 95 °C for 15 s and 60 °C for 1 min. If no increase in fluorescent signal was observed after 40 cycles, the sample was assumed to be negative.

2.7. Meningococcal serogrouping

All samples which were positive in Realtime-PCR for *ctra* gene were included in serogrouping analysis. The following serogroups were tested: A, B, C, W135, Y using primers and probes as described in Table 1.

2.8. Statistical analysis

Data was processed with the SPSSX 11.0 statistical package; p values < 0.05 were considered statistically significant. Results were expressed as mean levels and standard deviations (SD) or as median and interquartile range as appropriate. χ^2 was used to assess group differences in categorical variables. Odd ratio (OR) and 95% confidence limits (95% CL), when possible, were calculated. For continuous variables, the t-test was used with Logarithmic transformation of non-normal distributed variables.

3. Results

3.1. Case distribution according to age

In the study period, 136 cases of invasive meningococcal B disease were reported. The mean age was 5.0 years, median 2.7 years, interquartile range 10.2 months–6.4 years.

Among these, 96/136 (70.6%) patients were between 0 and 5 years, 61/136 (45.2%) patients were between 0 and 2 years. Among cases under 2 years of age, 39/61 (63.9%) occurred during the first year of life. Distribution of cases according to age is shown in Fig. 1. Within the first year of age the highest incidence was observed between the 4th and the 8th month of age, where 20/39 (51.3%) cases occurred. Case distribution according to months of age is shown in Fig. 2.

Table 1
Primers and probes used for diagnosis and serotyping of meningococcal infection.

| Target	Gene	Forward primer	Reverse primer	Probe
N. meningitidis	ctra	gctgcggtaggtggttcaa	gctgcggtaggtggttcaa	FAM‿cattgccacgtgtcagctgcacat
Serogroup A	sacB	cccccagcatggctagattt	agggcactttgtggcataattt	FAM‿accctaaaattcaatgggtatatcacga
Serogroup B	siaD B	ttggacttggttaagctgacctaa	gttgacaacatctccattttatcttacc	FAM‿ttagatatgacaaataaattgttacgtgggg
Serogroup C	siaD C	agggaaccgcaacctatgc	cacaaaacgttgctcaaattttg	FAM‿ccactcttagaatcatttacatacaaaccc
Serogroup W135/Y	siaD W135/Y	agggaaccgcaacctatgc	cacaaaacgttgctcaaattttg	FAM‿ accctaaaattcaatgggtatatcacga
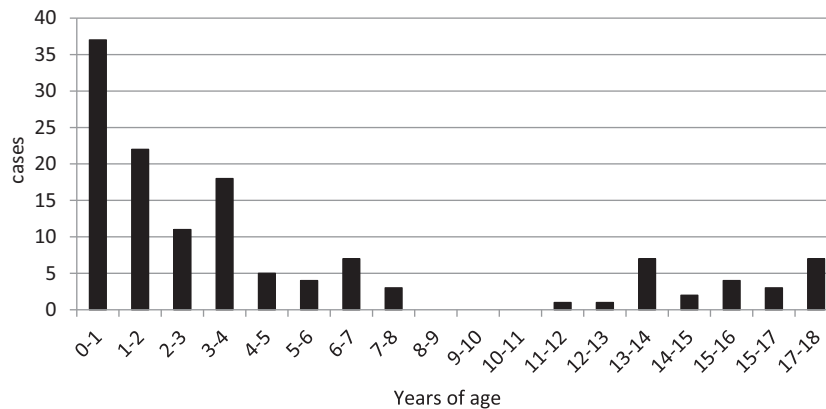
Fig. 1. Distribution, according to age, of cases of invasive meningococcal infection (meningitis or sepsis).

3.2. Culture and RT-PCR sensitivity in blood or CSF

Fifty-two blood samples were tested both by culture and RT-PCR. MenB was found in 43/52 (82.7%); the 9 (17.3%) patients who were negative for both tests in blood were positive by RT-PCR in CSF. MenB was identified by RT-PCR alone in 32/43 (74.4%) patients and by both RT-PCR and culture in 11/43 (25.6%) patients (McNemar's $p < 10^{-3}$); no sample was identified by culture alone.

Fifty-nine CSF samples were tested both by culture and RT-PCR. MenB was found in 57/59 (96.6%); the 2 (3.4%) patients who were negative for both tests in CSF were positive by RT-PCR in blood. MenB was identified by RT-PCR alone in 35/57 (61.4%) patients; by culture alone in 1/57 (1.8%) and by both RT-PCR and culture in 21/57 (36.8%) patients (McNemar's $p < 10^{-3}$).

Overall, 82 patients were tested at the same time by both molecular and cultural tests either in blood or in CSF or in both and a Neisseria meningitidis infection was found by RT-PCR in blood or CSF in 81/82 cases (98.8%). In the same patients culture could identify 27/82 (32.9%) infections. RT-PCR was significantly more sensitive than culture in achieving laboratory diagnosis of meningococcal infection (Cohen's Kappa: 0.3; McNemar $p < 10^{-5}$).

Sensitivity according to clinical presentation was evaluated. In 44 patients who were admitted to hospital with the diagnosis of sepsis with or without meningitis, RT-PCR was performed in the blood of 29/44 and in CSF of 15/44 and was positive in 29/29 (100%) blood and in 13/15 (86.7%) CSF. Culture was performed in the blood of 24/44 and in the CSF of 10/44 and was positive in 6/24 (25.0%) blood and in 2/10 (20.0%) CSF. As for meningitis, in 90 patients with the diagnosis of meningitis with no sign of sepsis, RT-PCR was performed in 39 blood samples and in 61 CSF samples and was positive in 29/39 (74.4%) blood samples and 60/61 (98.4%) CSF samples. Culture was performed in 31 blood samples and 50 CSF samples and was positive in 5/31 (16.1%) blood samples and 21/50 (42.0%) CSF samples. As expected, CSF is the most suitable sample for diagnosis of meningococcal meningitis and blood is the most suitable sample in meningococcal sepsis. RT-PCR has always a greater sensitivity (2–8 times higher) when compared to culture, ranging from 2.3 times in the CSF of patients with meningitis, to 8.7 times in CSF of patients with sepsis.
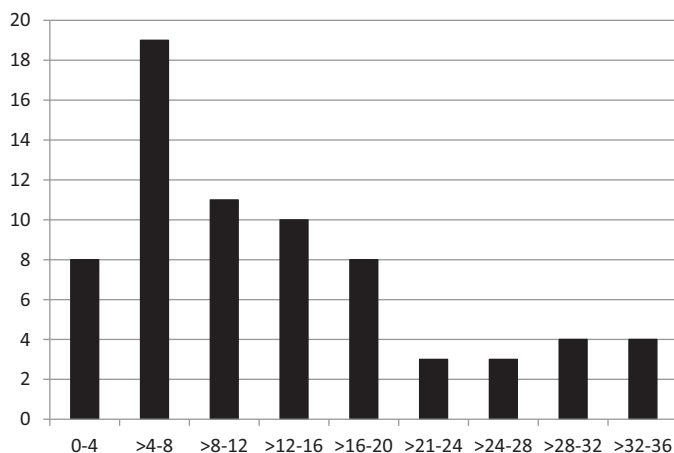
3.3. Case-fatality rate, follow-up and outcome

Over the study period there were 18 deaths, constituting an overall case fatality ratio (CFR) of 13.2%. Five out of 18 (27.8%) deaths occurred in the first year of age, 9 out of 18 (50.0%) occurred between the second and the fifth year of age; 3 cases occurred in adolescents (13–17 years of age). One case occurred at 6.2 years. CFR was 24.4% (11/45 cases) in children admitted with a diagnosis of sepsis, and 7.7% (7/91 cases) in children admitted for meningitis and in whom sepsis was not mentioned at admission. Twelve patients (8.9%) had complications during the acute phase of disease (cutaneous or subcutaneous necrosis, acute renal failure, seizures). During the follow-up, severe sequelae such as abnormalities in Nuclear Magnetic Resonance of brain (gliosis, idrocephalus) associated with neurologic symptoms, mental retardation, amputation of both hand and foot fingers have been reported in 4 patients (3.0%).

Fig. 2. Distribution of meningococcal B cases within the first 36 months of age.

4. Discussion

The results, obtained in a large pediatric population of Italian patients, demonstrate that invasive meningococcal infection has the highest incidence in the first 5 years of life where over 70% cases occur and in particular in the first year of age, where over 20% of all cases found in pediatric age are found. The incidence peak, similarly to what reported in other countries [16], is between the 4th and the 8th month of life.

In parallel with the introduction of routine MenC vaccination in different Italian regions, the incidence of meningococcal infection due to serogroup C has progressively decreased in infants and adolescents [8,9,13,17]. However, invasive meningococcal disease is still the first cause of meningitis and is second only to pneumococcal infection for cases of sepsis. The most common cause of invasive meningococcal disease, accounting for over 80% of cases found in patients younger than 24 years of age [9,17] is now MenB.

Culture has been, so far, the most used technique for meningococcal surveillance; however, bacterial culture leads to an important underestimation of disease burden. Confirming previous results, [16,18,19] once again Realtime PCR results significantly more sensitive than culture in identifying meningococcal infection, independent of the biological sample used and the clinical presentation. In fact, in our data obtained in patient tested at the same time with both methods, sensitivity of culture was less than one third that of Realtime PCR. This data is particularly important and suggests that in those countries (as in Italy) [9] where only isolate positive samples are counted as meningococcal cases, the incidence results largely underestimated. Furthermore, it is well known that culture-based methods have even lower sensitivity compared to molecular methods when the patient has been treated with antibiotics [13].

Realtime-PCR has the advantage of providing a diagnosis in the presence of culture-negative samples [12,13,20,21]; and can also determine the capsular group and even the complete sequence of bacterial genes when needed. Therefore, some countries have included PCR-based approaches in surveillance procedures, while performing cultural tests too. In the United Kingdom, 58% of laboratory-confirmed meningococcal cases were identified by PCR alone [22]; that percentage is even higher in countries with lower health resources, where sample transport and storage negatively influence the results; among them Brazil, where the use of PCR has almost doubled the figures obtained by culture tests [19].

RT-PCR has the additional advantage of providing results in less than 2 h [12] so allowing to start prophylaxis of contacts very soon and only when needed.

Case fatality ratio has been recently described to be about 5% for MenB in patients of any age [16]. Our data, obtained in a pediatric population, show a higher fatality rate of 13.2% with almost 30% cases in the first year of age and over 75% in the first 5 years of age. The CFR is even higher for patients presenting with sepsis, where it reaches 24.4%.

As reported in other western countries [16,23,24] the number of cases found in our study rapidly increased in the first months of life, with a peak between the 4th and 8th month of age. Therefore, in order to obtain the highest effectiveness, the vaccine should be offered to all infants in the first months of life.

It has been recently demonstrated that the recently licensed 4CMenB is highly immunogenic in infants after 3 doses given at 2, 3, 4 or 2, 4, 6 months of life [10].

However as demonstrated for other vaccines (either made of polysaccharides conjugated to proteins or of proteins) in order to establish good immune memory and long term protection a dose in the second year of age is always recommended [25].

It cannot be excluded that a single dose given after the first year of age could protect also infants through a mechanism of herd protection, but this hypothesis has not been demonstrated, so far. Reduction in carriage is considered an important determinant of the MenC vaccination success [25] and was obtained vaccinating at the same time both infants and adolescent and young adults; classes, the latter, in which the carriage state is more frequent. The effect of MenB vaccines on carriage is still under study, but, if undergoing studies will demonstrate carriage can be eliminated by vaccination, inclusion of adolescents in vaccination programs would have also an advantage on protection of infants.

Other approaches for protections of infants in the first year of life could evaluate a cocoon strategy, in which mothers are vaccinated during pregnancy in order to offer the newborns a passive antibody protection. No data are available on this procedure which has not been proven very effective with other vaccines [26] for the presence of frequent non-household sources of infections.

The present work provided country specific data which can be an important key point, as suggested by international recommendations [1] to make sustainable decisions, given the great regional variability in MenB incidence and serogroup distribution. Since the available vaccine is made of a mix of 4 subcapsular protein of MenB, which can be absent in different MenB isolates, it will be mandatory to go on with epidemiological studies to evaluate whether, under the immune pressure induced by vaccination, new mutants which do not express the 4 proteins target of the vaccine will escape the immune system [27].

Large epidemiological studies will continue to be needed to monitor and evaluate the introduction of this new vaccine, and to measure the impact of vaccination on achieving the goal of eliminating meningococcal disease and RT-PCR should be included in all surveillance programs in order to obtain more precise evaluation of incidence, case fatality rate and serogroup distribution.

Appendix. Italian group for the study of Invasive Bacterial Disease

The member of the Italian group are: Agostiniani R, Pistoia; Allievi P., Garbagnate Milanese; Allù G., Ragusa; Amigoni A., Ravenna; Bartolini E., Firenze; Bernardi F., Bologna; Bernardini R., Empoli; Biban P., Verona; Bigi M., Rimini; Bossi G., Pavia; Bottone U., Massa; Cardinale A., Montevarchi; Cardona A., Foligno; Castronari R., Perugia; Celandroni A., Pontedera; Chiossi M., Cuneo; Colleselli P., Vicenza; Correra A., Napoli; Cortimiglia M., Firenze; D'Ascola G, Arezzo; De Benedictis F.M., Ancona; de Martino M., Firenze; Dini E., Firenze; Dollfus L., Terni; Domenici R., Lucca; Flacco V., Lanciano; Verrotti A., Perugia; Gaetti MT, Jesi; Gagliardi L.,Viareggio; Galli L., Firenze; Giglio P., Gubbio; Guala A., Verbania; Lanari M., Imola; Lasagni D., Firenze; Lizzoli C., Magenta; Lombardi E., Firenze; Magnini M., Esine; Mattei R., Lucca; Memmini G., Carrara; Mesirca P., Montebelluna; Gragnani S., Livorno; Migliozzi L., Senigallia; Nunziata F., Solofra; Pecile P., Udine; Pepe G., Gallipoli; Perferi G., Firenze; Peris A., Firenze; Perri P.F., Macerata; Pescollderungg L., Bolzano; Pezzati M., Firenze; Poggi G.M., Firenze; Poggiolesi C., Firenze; Rapisardi G., Firenze; Ratta L., Rimini; Ricci S., Firenze; Ridi F., Firenze; Riva A., Brescia; Rizzo L., Portoferraio; Roman B., Vimercate; Romano F., Firenze; Toffolo A., Oderzo; Strano M., Borgo San Lorenzo; Trapani S., Firenze; Valleriani C., Firenze; Vasarri P., Prato;

Vascotto M., Siena; Vergine G., Rimini; Verini M., Chieti; Zorzi C., Camposanpiero.

References

[1] The Global Meningococcal Initiative. Recommendations for reducing the global burden of meningococcal disease. Vaccine 2011;29: 3363–71.

[2] Sáfadi MA, González-Ayala S, Jäkel A, Wieffer H, Moreno C, Vyse A. The epidemiology of meningococcal disease in Latin America 1945–2010: an unpredictable and changing landscape. Epidemiol Infect 2012 Aug;9:1–12.

[3] Bedford H, de Louvois J, Halket S, Peckham C, Hurley R, Harvey D. Meningitis in infancy in England and Wales: follow up at age 5 years. Br Med J 2001;323(7312):533–6.

[4] Halket S, de Louvois J, Holt DE, Harvey D. Long term follow up after meningitis in infancy: behaviour of teenagers. Arch Dis Child 2003;88(5): 395–8.

[5] de Louvois J, Halket S, Harvey D. Effect of meningitis in infancy on school-leaving examination results. Arch Dis Child 2007;92(11):959–62.

[6] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29(7):1303–10.

[7] Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, Angus DC. The epidemiology of severe sepsis in children in the United States. Am J Respir Crit Care Med 2003;167(5):695–701.

[8] Bechini A, Levi M, Boccalini S, Tiscione E, Balocchini E, Canessa C, et al. Impact on disease incidence of a routine universal and catch-up vaccination strategy against *Neisseria meningitidis* C in Tuscany, Italy. Vaccine 2012;30(45):6396–401.

[9] http://www.simi.iss.it/files/Report_MBI.pdf [accessed 27.03.13].

[10] Gossger N, Snape MD, Yu LM, Finn A, Bona G, Esposito S, et al. European MenB Vaccine Study Group. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. J Am Med Assoc 2012;307(6):573–82.

[11] Santolaya ME, O'Ryan ML, Valenzuela MT, Prado V, Vergara R, Muñoz A, et al. V72P10 Meningococcal B Adolescent Vaccine Study group. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. Lancet 2012;379(9816):617–24.

[12] Resti M, Moriondo M, Cortimiglia M, Indolfi G, Canessa C, Becciolini L, et al. Italian Group for the Study of Invasive Pneumococcal Disease, Community-acquired bacteremic pneumococcal pneumonia in children: diagnosis and serotyping by real-time polymerase chain reaction using blood samples. Clin Infect Dis 2010;51(9):1042–9.

[13] Resti M, Micheli A, Moriondo M, Becciolini L, Cortimiglia M, Canessa C, 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(6):1266–73.

[14] Azzari C, Moriondo M, Indolfi G, Massai C, Becciolini L, de Martino M, et al. Molecular detection and serotyping on clinical samples improve diagnostic sensitivity and reveal increased incidence of invasive disease by *Streptococcus pneumoniae* in Italian children. J Med Microbiol 2008;57(Pt 10):1205–12.

[15] Russell JA. Management of sepsis. N Engl J Med 2006;355:1699–713.

[16] Ladhani SN, Flood JS, Ramsay ME, Campbell H, Gray SJ, Kaczmarski EB, et al. Invasive meningococcal disease in England and Wales: implications for the introduction of new vaccines. Vaccine 2012;30:3710–6.

[17] http://www.simi.iss.it/files/Report_MBI.pdf [accessed 27.03.13].

[18] Gray S, Campbell H, Marsh J, Carr A, Newbold L, Mallard R, et al. The epidemiology and surveillance of meningococcal disease in England and Wales. In: Poster P041. 17th International pathogenic Neisseria conference (IPNC). 2010. Available at: http://neisseria.org/ipnc/2010/IPNC 2010 abstracts.pdf [accessed 27.03.13].

[19] Sacchi CT, Fukasawa LO, Goncalves MG, Salgado MM, Shutt KA, Carvalhanas TR, et al. Incorporation of RT-PCR into routine public health surveillance for meningococcal meningitis in São Paulo State, Brazil. In: Poster P042. 17th International pathogenic Neisseria conference (IPNC). 2010. Available at: http://neisseria.org/ipnc/2010/IPNC 2010 abstracts.pdf [accessed 27.03.13].

[20] Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol 2001;39:1553–8.

[21] Gurley ES, Hossain MJ, Montgomery SP, Petersen LR, Sejvar JJ, Mayer LW, et al. Etiologies of bacterial meningitis in Bangladesh: results from a hospital-based study. Am J Trop Med Hyg 2009;81:475–83.

[22] Gray S, Campbell H, Marsh J, Carr A, Newbold L, Mallard R, et al. The epidemiology and surveillance of meningococcal disease in England and Wales. In: Poster P041. 17th International pathogenic conference (IPNC). 2010. Available at: http://neisseria.org/ipnc/2010/IPNC_2010_abstracts.pdf [accessed 27.03.13].

[23] Levy C, Taha MK, Weil OC, Quinet B, Lecuyer A, Alonso JM, et al. Association of meningococcal phenotypes and genotypes with clinical characteristics and mortality of meningitis in children. Pediatr Infect Dis J 2010;29: 618–23.

[24] Hershey JH, Hitchcock W. Epidemiology and meningococcal serogroup distribution in the United States. Clin Pediatr (Phila) 2010;49: 519–24.

[25] Trotter CL, Edmunds WJ. Modelling cost effectiveness of meningococcal serogroup C conjugate vaccination campaign in England and Wales. Br Med J 2002;324(7341):809.

[26] Wiley KE, Zuo Y, Macartney KK, McIntyre PB. Sources of pertussis infection in young infants: a review of key evidence informing targeting of the cocoon strategy. Vaccine 2013;31(4):618–25.

[27] Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, et al. Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. Lancet Infect Dis 2013 May;13(5):416–25.