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Neisseria meningitidis: epidemiology and pertinence of the Portuguese vaccinal strategy

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NEISSERIA MENINGITIDIS: EPIDEMIOLOGY AND PERTINENCE OF THE PORTUGUESE VACCINAL STRATEGY

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ABSTRACT

Despite the constant scientifical developments and both diagnosis and therapeutics improvements, invasive meningococcal disease (IMD) remains an important cause for mortality and morbidity in the paediatric population, globally. IMD's morbidity is due to the potential serious sequalae such as: seizures, palsy, visual and/or hearing loss, psychiatric disorders, neurological deficits, and potential limb amputations. The disease mainly affects children aged from 3 to 12 months old, but it can also occur in adolescents (and, occasionally, in the elderly).

Most infections will not lead to disease. In fact, it is estimated that 10% of the world population is an asymptomatic carrier of *Neisseria meningitidis* (meningococcus), in the oropharynx. This is the microorganism responsible for IMD, and it is transmitted through the dissemination of respiratory droplets or via direct contact with a carrier.

Disease causing serogroups are identified and have an extremely variable epidemiology, both geographically and through time. Carrier state varies substantially through the age groups, being more prevalent in children younger than 1 year old, decreasing with age till adolescence where there is a new peek, being adolescents and young adults the main carriers.

Vaccination comes as an essential weapon in the combat against IMD development. Effective vaccination against the A, C, W and Y serogroups is available (through conjugated vaccines, which have demonstrated to be capable of affecting carriage). However, immunization against the B serogroup has not yet reached the same efficiency, as only protein-based vaccines are available, apparently unable to interfere with carriage.

Literature is scarce when it comes to *Neisseria meningitidis* epidemiological distribution in Portugal and the impacts of vaccination strategies adopted through times on the Portuguese population's carriage state.

The objective of this work is to, through a systematic literature review, understand the meningococcal epidemiological distribution, especially in Europe, and, through the example of other countries, understand the effects of vaccination on the disease's incidence, particularly on the reduction of carriers, to comprehend the pertinence of applying vaccination strategies targeting adolescents and young adults in Portugal.

The primary studies were obtained through automatic research on the platform PubMed, using the MeSH keywords: "*Neisseria meningitidis*", "meningococcal disease", "carrier state", "epidemiology", "vaccines", "Europe" and "Portugal", with the formula: ((((((neisseria meningitidis) AND (meningococcal disease)) AND (vaccines)) AND (epidemiology)) AND (europe)) OR (portugal)) AND (carrier state). Search was restricted to a time interval from 1st January 2000 through 31st

December 2019. The primary studies were then selected based on a set of eligibility criteria. Furthermore, other studies selected from reference and citation tracking were included, being subjected to same eligibility criteria.

RESUMO

Apesar da constante atualização científica e do aprimoramento, tanto do diagnóstico, como da terapêutica, a doença meningocócica invasiva continua a ser uma causa importante de mortalidade e morbilidade, na população pediátrica, globalmente. A morbilidade causada pela doença deve-se, sobretudo, às graves sequelas que podem ocorrer: convulsões, paralisia, perda visual e/ou auditiva, perturbações psiquiátricas, défices neurológicos e pode ser necessário recorrer a amputações de membros. A doença afeta primariamente crianças entre os 3 e os 12 meses de idade, mas ocorre também em adolescentes (e ocasionalmente em idosos).

A grande maioria das infeções não dá origem a doença. Aliás, estima-se que 10% da população mundial seja portador assintomático de *Neisseria meningitidis* (meningococo), na orofaringe. Este é o microrganismo responsável pela doença invasiva meningocócica e a sua transmissão ocorre através da disseminação de gotículas respiratórias ou via contacto direto com um portador.

Os serogrupos causadores de doença estão identificados e têm uma distribuição epidemiológica extremamente variável, tanto geograficamente, como ao longo do tempo. O estado de portador varia substancialmente ao longo dos grupos etários, sendo mais prevalente nas crianças com menos de 1 ano de idade, diminuindo com a progressão da idade até à adolescência em que há um novo pico, sendo os adolescentes e os jovens adultos os principais portadores do meningococo.

A vacinação surge como uma arma essencial no combate ao desenvolvimento da doença invasiva meningocócica. Está disponível vacinação eficaz contra os serogrupos A, C, W e Y, (através de vacinas conjugadas, que já se mostraram capazes de combater o estado de portador) mas as imunizações contra o meningococo do serogrupo B ainda não atingiram o mesmo nível de eficácia, uma vez que apenas estão disponíveis vacinas de base proteica, aparentemente sem capacidade de intervir ao nível do estado de portador.

A literatura é escassa no que toca à distribuição epidemiológica que a *Neisseria meningitidis* toma em Portugal e aos impactos das estratégias vacinais adotadas ao longo do tempo no estado de portador da população portuguesa.

O objetivo deste trabalho é, através de uma revisão sistemática da literatura, compreender a distribuição epidemiológica do meningococo, especialmente na Europa e, através do exemplo de outros países, compreender o efeito da vacinação sobre a incidência da doença, particularmente sobre a diminuição do número de portadores, de modo a aferir a pertinência de aplicar estratégias de vacinação focadas nos adolescentes ou jovens adultos, em Portugal.

Os estudos primários foram obtidos através de pesquisa automática na plataforma PubMed, usando as seguintes palavras-chave MeSH: "*Neisseria meningitidis*", "meningococcal disease", "carrier state", "epidemiology", "vaccines", "Europe" e "Portugal", com a fórmula: ((((((neisseria meningitidis) AND (meningococcal disease)) AND (vaccines)) AND (epidemiology)) AND (europe)) OR (portugal)) AND (carrier state). A pesquisa foi restringida ao intervalo de tempo desde 1 de janeiro de 2000 a 31 de dezembro de 2019. De seguida, os estudos primários foram submetidos a uma série de critérios de inclusão e de exclusão. Além disso, foram, ainda, incluídos trabalhos selecionados a partir das referências e das citações dos estudos primários, sendo estes submetidos, depois, aos mesmos critérios de inclusão e exclusão.

LIST OF ABBREVIATIONS

ACIP - Advisory Committee on Immunization Practices
CC – clonal complex
CSF – cerebrospinal fluid
DIC – disseminated intravascular coagulation
DGS – *direção geral da saúde* (general health administration)
ECDC - European Centre for Disease Prevention and Control
EMA – European Medicine Agency
ET – electrophoretic type
fHBP – H factor binding protein
IMD – Invasive Meningococcal Disease
IL-1β - interleukin-1-beta
INSA – *Instituto Nacional de Saúde Dr. Ricardo Jorge* (National Health Institute Doutor Ricardo Jorge)
LOS - lipo-oligosaccharide
MATS - meningococcal antigen typing system
MenA – meningococcal group A
MenACWY – meningococcal groups A, C, W and Y
MenB – meningococcal group B
MenC – meningococcal group C
MenW – meningococcal group W
MenY – meningococcal group Y
MLEE – multilocus electrophoresis
MLST – multilocus sequence typing
MSM – men who have sex with men
NadA – neisserial adhesion A
NG – nongroupable
NHBA – neisserial heparin-binding antigen
OMVs – outer membrane vesicles
PCR – polymerase chain reaction
PICU – paediatric intensive care unit
PO – per os (by mouth)
rplF – 50S ribosomal protein L6
rt-PCR – real time PCR
SASG - Slide Agglutination SeroGrouping
ST – sequence type
TNF-α - tumour necrosis factor-alpha
WGS – whole genome sequencing

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INTRODUCTION

Despite improvements in diagnosis and therapy, meningococcal disease remains a major cause of child morbidity and mortality, globally. [1, 2] It mostly affects children between 3 and 12 months of age, but also adolescents. [3] Invasive Meningococcal Disease, although a rare condition, is a potentially life-threatening disease, with a fatality rate of 5-10%, and an associated risk for severe clinical sequelae in survivors. [2, 4-6] *Neisseria meningitidis* is the microorganism responsible for IMD, and it is estimated that 10% [4, 7] of world population are asymptomatic carriers of the bacterium in the nasopharynx [4] and oropharynx [7].

Invasive meningococcal disease represents a major public health concern, because of its mortality rate, but especially due to clinical sequelae. [4] Especially when it comes to invasive group B meningococcal disease, for which vaccines are still not perfected. [5] It represents a particularly important burden, both in countries where is endemic but also where it occurs sporadically, being the most fatigated world region the African meningitis belt. [2]

In 2013 and 2014, Portugal reported an IMD incidence rate of 0,71 and 0,52 cases per 100000 population – values aligning with the incidence rate estimation for Europe in 2012 (0,68/100000), and that show a decrease in incidence, which has been noted since 2003. [8] This decrease is mainly attributed to MenC vaccination (started in 2006). [8] Ever since then, MenC has only been isolated in adults, presumably non-immunized. [9] MenB has been the leading serogroup causing IMD in Portugal, since 2003, and MenY has been the second leading serogroup, since 2011 (due to MenY hypervirulent strains – cc ST-23 – which emerged in Europe in the early 2000s, becoming endemic). [8, 9]

Incidence rates continued to decrease, reporting 0,67 and 0,41 cases per 100000 population in 2015 and 2016, respectively (still aligning with mean European values for the same time period – 0,61 and 0,63/100000 respectively). [9]

In Portugal and all around the world, IMD mainly affects infants younger than 1 year of age. [8-10] There is a heavy decrease in incidence in the 1-4 years old group, remaining low through the 10-14-year-olds age group, with a slight secondary peak in 15-19 years old adolescents. [8-10]

MenB disease affects mainly children below 1 year of age, peaking at 6 months of age, which is similar to data reported by other European countries. The Portuguese National Health Institute reports that "Invasive *Neisseria meningitidis* strains isolated in Portugal between 2013 and 2014 present a great diversity of sequence types (ST). Altogether,48,5% were characterized as

hypervirulent (…) The most frequent cc was ST-41/44, always associated with group B, and it represented about a quarter of all characterized strains." [8] In 2015 and 2016, incidence decreased almost 50%, comparing to 2007 and 2013, eventually due to the beginning of MenB vaccination in the country. [9] Even still, MenB remains the predominant serogroup causing IMD in Portugal. [9]

Mortality due to IMD between 2003 and 2016 rated 6,4-7,0%, which is bellow expected values; sequelae rates are unknown. [8, 9] The Portuguese National Health Institute reports 8 deaths from IMD during 2015-2016, 4 having occurred in children aged 1 to 5 years old (from MenB disease), 3 in adults aged 62 to 78 years old (from MenB, MenY and MenC disease), and 1 in a 15-year-old (from MenY disease). [9]

MATERIALS AND METHODS

The primary studies were obtained through automatic research on the platform PubMed, using the MeSH keywords: "*Neisseria meningitidis*", "meningococcal disease", "carrier state", "epidemiology", "vaccines", "Europe" and "Portugal". Search was restricted to a time interval from 1st January 2000 through 31st December 2019. The primary studies were then selected based on a set of eligibility criteria (fig. 1):

- Only articles in English and Portuguese were included
- Regarding article type, only classical articles, clinical studies, comparative studies, datasets, evaluation studies, guidelines, journal articles, meta-analysis, multicenter studies, observational studies, reviews, systematic reviews, and validation studies were included
- Articles on non-human species were excluded
- Articles on microorganisms other than *Neisseria meningitidis*, or on pathologies other than invasive meningococcal disease, were excluded
- Studies on antibiotic resistance were also excluded

33 studies resulted from the application of these inclusion and exclusion criteria, two of them were primarily focused on mathematical models, and were excluded. 4 other articles were retrieved from citation and reference tracking, resulting in a total of 36 studies.

Due to the lack of data concerning Portugal from these studies, three reports regarding meningococcal disease in the country (regarding the time intervals of 2000-2006, 2003-2014 and 2007-2016) from the National Health Institute were included.

For better understanding of the Portuguese course of action regarding IMD cases, it was also included a protocol from the Portuguese Paediatrics Society, regarding acute bacterial meningitis, and the National Immunization Program.

As a complement to the data retrieved from the selected studies, the ECDC Surveillance Atlas of Infectious Diseases was also consulted.

1. *NEISSERIA MENINGITIDIS* AND INVASIVE MENINGOCOCCAL DISEASE

Neisseria meningitidis (also referred to as meningococcus), the microorganism accountable for Invasive Meningococcal Disease, being the leading cause of meningitis and septicaemia globally [11], is an aerobic, Gram-negative, diplococcus bacterium. [2, 4, 12-14] The *N. meningitidis* is included in the *Neisseriaceae* family, and in the Proteobacteria phylum. [4] Humans are the only known reservoir of the meningococcus. [2, 11, 14, 15] The bacteria spread by inhalation of droplets of respiratory secretions or via direct contact with a carrier. [2, 4]

Most infections result in asymptomatic carriage, rather than invasive meningococcal disease. [2, 4, 5, 7, 16] It is estimated that 10% of the population asymptomatically carries the bacterium in the nasopharynx. [1, 2, 4, 7, 17-23] This is due to the fact that *N. meningitidis* has molecularly adapted in order to colonize and survive on the nasopharyngeal mucosa, developing a commensal relationship with the host. [1, 4, 14] Notwithstanding, albeit rare, the bacterium may cross the mucosal barrier, proliferate in the bloodstream and then, invade the meninges, leading to IMD. (fig.2) [2, 4, 13, 24] The mechanism underling the mucosal overpass is largely unknown. [4, 14, 15, 24]

Neisseria meningitidis evades the immune system by making use of different virulence factors, as the capsule and factor H binding protein (fHbp). [4, 13-15, 22] Ala'Aladeen *et al* (2000) have demonstrated the meningococcus' ability to perform capsule switching, in order to evade the immune system, as the capsule is highly immunogenic. [21] Gianchecchi *et al* (2017) describe the host's response as the activation of three main cascade reactions: the complement system, the fibrinolysis and coagulation pathway, and the inflammatory response. [4] Via the complement system, C3a and C5a promote inflammation. The fibrinolysis and coagulation pathway prompts to fibrin deposition and disseminated intravascular coagulation (DIC). [4] DIC depends on chemokines and cytokines, especially interleukin-1-beta (IL-1β) and tumour necrosis factor-alpha (TNF-α), [4, 10] which increase the permeability of the blood-brain barrier, and promote the neutrophil influx. [10]

The meningococcus, having invaded the meninges, leads to meningococcal meningitis in 80-85% [4] of cases, and sepsis in 50% [2, 4]. [16, 17, 21, 25] Within the first 12-15 hours following infection onset, the patient may present with selective meningitis manifestations (petechiae, purpuric rash (fig. 3), meningism, neck stiffness, photophobia, sepsis, and shock). [4] Within 15-24 hours, later signs (confusion or delirium, unconsciousness, septic shock, multisystem failure) and

eventually death may take place. [4] Sepsis may occur without the presence of meningeal signs, and it does so in 15-20% [4] of IMD. However, the patient may present with a clinical case resembling that of other common viral infections. [4] Sepsis manifests by exceedingly high levels of lipo-oligosaccharide (LOS) in cerebrospinal fluid (CSF) or blood, in addition to the onset of a purpuric or petechial rash and sudden fever. Furthermore, DIC leads to the formation of microthrombi and the release of circulating mediators, which damage endothelial cells (fig. 2), so the patient may present serious capillary leak syndrome. [4] Patient may also present Waterhouse-Friderichsen syndrome, characterized by adrenal haemorrhage and rash, that can lead to hypotension and multiple organ failure. [4, 14] Invasive meningococcal disease in older patients (mostly above 65 years old) may present as meningococcal pneumonia. [4, 6] The definitions of "confirmed" and "possible" cases have been agreed amongst the European countries, resulting in a set of criteria in 2012 (fig. 4). [26, 27]

It is estimated that the mortality rate associated with IMD is 5-10% in industrialized countries [2, 4, 14, 20, 22, 28], 50% in case of septic shock. [2, 14] Severe clinical sequelae may affect the patient in 12-24 hours from the first symptoms, in 12-20% [4, 14] of survivors. [13] There is a wide spectrum of possible severe sequelae, such as limb amputations, seizures, paralysis, visual and hearing loss, psychological disturbances, and neurological deficits. [4, 5, 13, 14]

An early intervention, with appropriate antibiotic treatment, upon recognition of IMD clinical signs and symptoms, is crucial for the survival and quality of life of the patient [4], and may help prevent secondary cases due to contact with the patients [4], but it has not been enough to overcome the burden this disease represents. [28]

Most IMD cases are due to 6 out of the 13 serogroups (WHO reports 12 [2]) described: A, B, C, W, Y, and, more rarely, X. [2-4, 7, 11-19, 21, 22, 24, 28] All disease associated serogroups express polysaccharide capsules, whereas carriage isolates are often noncapsulated (50% of cases [21]) – and, therefore, nongroupable (NG) - due to downregulation of genes responsible for capsule expression, inactivation of genes in the capsule gene cluster (*cps*) or the inexistence of *cps* in capsule null (*cnl*) meningococci. [15-18, 21] NG isolates have been labelled as non-pathogenic in the past, but, considering the variability in capsule expression, no such statement can be made. [21] Maiden *et al* (2008) state that "although the capsule is the major virulence determinant for meningococci, it cannot have evolved specifically to cause disease because meningococci gain no benefits from causing disease in terms of improved host-to-host transmission". [18] In fact, capsule switching appears to be more frequent among group C strains than group B ones, which may be due to the fact that MenC capsule is more immunogenic, and therefore subjected to more pressure. [21] Maiden *et al* (2008) alert that the capsule's role is not yet completely understood. [18]

The *cps* region in MenB, MenC, MenW and MenY differ in the *siaD* locus (place of 4 different allele classes: $siaD_B$, $siaD_C$, *siaDw*, and $siaD_Y$), each encoding serogroup-specific polysialyltransferases. [16] Claus *et al* (2005) have demonstrated that meningococci with the $siaD_C$ or with the $siaD_Y$, were less likely to express capsule than the others. [16]

1.1. RISK FACTORS FOR MENINGOCOCCAL DISEASE

Several factors predispose to IMD, being the absence of strain-specific bactericidal antibodies the most crucial one. [4, 29] Trotter *et al* (2003) affirm that "susceptibility to meningococcal disease is inversely related to the presence of serum bactericidal antibody". [29] New-borns and young infants are subjected to the early disappearance of maternal antibodies (considering that bactericidal antibodies are transmitted from mother to child [29]), alongside the high rate of nasopharyngeal colonization, making them prone to develop IMD. [4] Numerous types of immunodeficiencies (primary, like functional or anatomic asplenia; or secondary as inflicted by HIV infection, for example), can be hampering in the development of bactericidal activity, making the patient more susceptible to IMD onset. [4, 14] Moreover, host specific polymorphisms, as well as extremes of age (*i.e.* below 6 months of age and over 65 years old), have been correlated with higher risk for IMD. [4, 14, 21]

Data shows that invasive meningococcal disease targets specific age groups, with a high disease attack rate in young infants and another in teenager, the latter associated with high carriage rates. [1, 4, 15, 20] Age is an important risk factor for both meningococcal carriage and development of invasive meningococcal disease. [4, 20]

Several studies have shown that low socioeconomic status displays an association to the risk of developing invasive meningococcal disease. [4, 5]

Seasonality is especially relevant when it comes to capsular meningococci: there seems to be no impact of seasons on non-capsulated strains [30] – it could be due to the fact that the capsule protects the bacterium from drying during aerosol transmission [30].

Bacterial meningitis peaks in winter, in both hemispheres. [4] This trend is more evident at over 30ºN and below -20ºN latitudes (Europe, China, North America, and South Africa, South America, and Oceania), during the respective winter season. [1, 4] When it comes to the African meningitis belt (latitude between -5ºN and 20ºN), meningitis peaks during the dry winter season, characterized by the Harmattan winds. [4, 30] Interestingly, meningitis incidence is held back by

the African monsoon. [4] All this lines up with the hypothesis that meningococcal transmission is reduced by high levels of humidity [4]. This could be explained by the damage done to the pharyngeal mucosal barrier by mineral dust winds and dry air, allied to the effects of viral infections (promoted by cold climates), alters the mucosa's immunity power, favouring invasive bacterial infection (namely, by *N. meningitidis*, leading to IMD onset). [4]

Other high-risk patients are travellers to endemic areas (e.g. Sub Saharan Africa), and microbiologists and laboratory staff. [4]

Human proximity also poses a risk, namely for unimmunized household contacts of IMD by MenB patients, military recruits and college students residing in dormitories. [4] In fact, college students residing in dormitories have 3-fold higher risk compared to those residing off campus (who share the same IMD development risk as the general population). [4]

Miglietta *et al* (2018) studied the involvement of men who have sexual intercourse with men (MSM) and bisexuals in a MenC (cc11) outbreak in Tuscany, in 2015. [12] These individuals were especially affected by the outbreak, what could suggest meningococcal sexual transmission; however, the transmission could be due to the close contact. [12] Furthermore, as Miglietta *et al* (2018) state: "MSM/bisexual cases were more prone to using illicit drugs, sharing drinks, attending discos, and having sexual intercourses (a proxy for close contact) in the 10 days before symptom onset". [12] In 2016 a vaccination campaign targeting discos and LGBT associations took place in this Italian region and led to a decrease of MenC cases in 2017. [12]

1.2. CONTACT MANAGEMENT

Transmission of the meningococcus requires close prolonged contact, with secondary cases emerging within 1 week after the index case. [6] Close contacts of IMD patients are subjected to a 200-1200-fold enhanced risk for developing meningococcal disease. [4, 20]

Up until 2010, public health policies varied through different European countries, regarding the management of close contacts. [4] Therefore, the European Centre for Disease Prevention and Control (ECDC) took the 2007 European Meningococcal Disease Society's (EMGM) document as guidance. [4] This document resulted from a survey conducted by the EMGM, and it presents a weak recommendation for the chemoprophylaxis with antibiotic of close contacts (preschool, school or college colleagues, sharers of cigarettes, drinks, or other means of low-level salivary

contact) of a IMD case, and strong recommendation for chemoprophylaxis and adequate course of vaccination (if not previously immunized) for household contacts. [4, 31]

Most reported outbreaks and clusters in industrialized countries involve individuals aged 0-25 years, and take place in institutional settings, such as school, pre-school, universities, and the military. [6]

The identification and characterization of an outbreak is particularly important in terms of public health, because of the need to provide timely antibiotic chemoprophylaxis (for short-term protection) and vaccination (depending on the serogroup). [6] Cardeñosa *et al* (2001) conducted a population-based study on IMD cases and their contacts and found that 43% of household contacts carried a strain of the same serogroup as the index case. [20]

2. *N. MENINGITIDIS* DETECTION

Accurate diagnostic methods allow for the monitorization of the impact of vaccines on *N. meningitis* carriage, and IMD. [7, 17]

The bacterium is more commonly detected in the oropharynx than the nasopharynx. [32] Carriage is detected by sampling from the posterior pharyngeal wall with direct plating onto adequate growth media. [24]

It has been demonstrated that viable meningococci are present in carriers' saliva. [33] Because saliva testing would be more comfortable for both the subject and the investigators, Rodrigues *et al* (2019) compared saliva samples to oropharyngeal swabs in terms of meningococci detection, and showed that, even though saliva testing has lower sensitivity for detecting genogroupable strains, it is a much easier methodology and would allow for more frequent and numerous samples, which could increase detection sensitivity if it were used in addition to oropharyngeal swabs. [33]

The gold standard for the identification of *Neisseria meningitidis* resorts to direct culturing and molecular methods based on DNA isolation. [4]

The biomaterials used for the cultural examination (on blood agar plates or chocolate plates) should be from sterile sites, namely blood and CSF, but also synovial fluid, pleural fluid, and pericardial fluid. [4] Additionally, skin biopsy (of the petechial lesions) can also be used. [4] Furthermore, the Kovac's oxidase test and the carbohydrate utilization test can be used, to help characterize the colonies. [4]

Afterwards, the Slide Agglutination SeroGrouping (SASG) method allows for the identification of the serogroup. [4]

Considering that when dealing with meningococcal disease time is crucial, faster identification methods are favourable. [4] Therefore, diagnosis by molecular biology methods comes as a faster alternative to direct culturing. [4] Molecular methods are based on DNA extraction, and seek for the identification of the *ctr*, *sod sia*, *syn*, *sac*, and *xcb* genes, enabling the serogroup identification. [4] Furthermore, PCR offers higher sensibility (96% and it is not influenced by previous antibiotic therapy) than CSF or blood culture analyses. [4] Moreover, PCR conducted on skin biopsy has a sensitivity of 100%, higher than that of blood cultures (58.8%). [4] PCR offers higher reliability than agglutination antisera in capsular groups identifications. [32] Despite the

gains over traditional culture, rt-PCR reveals less sensitivity for the identification of MenB, comparing to WGS, possibly due to the lack of culture enrichment. [7]

In the past, the foundation of *N. meningitidis* genotyping, was the study of constitutional genes related to metabolic functions. [4] The study by multilocus enzyme electrophoresis (MLEE) can distinguish electrophoretic types (ETs), while multilocus sequence typing (MLST) recognizes sequence types (STs) – the standard for determining genetic lineage. [4, 13, 16] A new technique, applied to the 50S ribosomal protein L6 (rplF) gene can be used for research and diagnosis, while being cheaper and faster. [4] STs found equivalent are grouped as clonal complexes (CCs), notwithstanding a high antigen diversity within the same CC (being genome mutations or recombination during carriage one of the reasons for such). [4, 13, 16] The meningococcus' plasticity comes as a tool for evading the immune system of the host. [4]

Over the last decade whole genome sequencing (WGS) has been becoming more common and allow to sort meningococcal populations into lineages with an epidemiologic interest, using a subset of meningococcal core genes. [13]

Ravenhorst *et al* (2017) concluded that WGS of cultured isolates has a higher sensitivity for serogroup identification, than Ouchterlony and rt-PCR, having compared these techniques. According to the authors, WGS of cultured isolates provides a "precise picture of both genogroup and isolate identity". [7] In the present day, WGS analysis are done routinely, allowing for the identification of CC, ST and differentiating highly similar clones. [17]

3. MENINGOCOCCAL CARRIAGE

Neisseria meningitidis colonizes the human upper respiratory tract, [2, 4, 7, 19, 21, 22] and this colonization can last for days or months (> 6 months [21]). [4, 23] When carrier rate surpasses 20% in a community, there is danger for an epidemic, usually by the most prevalent serogroup. [1] Even after an individual eradicates one episode of meningococcal carriage, they remain susceptible to further colonization. [21]

The meningococcus is transmitted through respiratory droplets or direct contact with infected subjects (fig. 2). [2, 4] The most common form of transmission occurs with asymptomatic carriers [2, 4, 7, 23], especially if not immunized. [4, 23]

The main carriers are children younger than 1 year old, adolescents (15-19 years old) and young adults (20-24 years old). [2, 4, 6, 7, 13, 18, 19, 21-23, 25, 34] According to Pavlopoulou *et al* (2004), meningococcal carriage "varies from <2% in young children, to 5-15% in teenagers and young adults, to *c.* 50% in people who live un severely overcrowded conditions such as institutions and military camps". [1] Multi-strain carriage has been described. [4, 35]

The repetitive carriage of the meningococcus and of different *Neisseria* seams to provide cross-protection against IMD (but there is no consensus among authors) [1, 4, 5], *i.e.* exposure to *Neisseria lactamica* (a non-pathogenic bacterium, genetically close to *Neisseria meningitidis*), for example, may promote anti-meningococcal antibodies. [29]

There may be a correlation concerning the temporal extension of *N. meningitidis* carriage and the development of a long-term commensalism between the host and the bacterium, that only rarely will evolve into disease. [4] In fact, most strains of the meningococcus do not cause IMD, and are restricted to asymptomatic carriage. [4, 19] The strains isolated from individuals with IMD are extremely different genetically and antigenically from the majority, that does not cause disease, and belong to a limited number of CCs, known as hyperinvasive lineages. [4, 13, 15, 16, 18, 21, 23] It is believed that long-term meningococcal carriage provides for the formation of bactericidal antibodies (lost in childhood, alongside other maternal antibodies). [29] Serogroup specific antibodies can be identified within 2 weeks of colonization. [1] However, the levels are extremely low, especially compared to those obtained via vaccination. [29]

Gasparini *et al* (2014) conducted a longitudinal carrier study in Italian students aged 14 to 22 years old and found that all strains isolated from the same individual were either identical or had accumulated small changes over time. [15] However, some authors state it is possible that high levels of carriage can predispose to genetic recombination. [4, 35] According to Gianchecci *et al* (2017), disease-associated strains can acquire genetic information from carriage-associated strains,

and it can cause new pathogenic strains. [4] No core genomic differences were associated with disease onset, so far. [4]

The majority of data from carriage studies concerns Europe, existing fewer information on North America, Africa, the Middle East, and Africa. [4] Studies consistently show that meningococcal carriage prevalence peaks in young infants, adolescents, and young adults (peaking at around 19 years old in developed countries [30]), and that the epidemiology of the different serogroups varies through time and geographically. [2, 4, 7, 16, 19, 28, 30] MenB is reportedly the dominant capsular group carried, notwithstanding the high frequency of MenC, MenW and MenY. [4]

3.1. RISK FACTORS FOR MENINGOCOCCAL CARRIAGE

Cleary *et al* (2015) describe age as "a key predictor of meningococcal carriage prevalence". [5] Carriage prevalence suffers an increasement from 5% in young children to a peak at 19 years old of 24%, then decreasing to 8% at 50 years old. [5] Age is an important risk factor for both meningococcal carriage and development of invasive meningococcal disease, but several other risk factors for meningococcal carriage have been identified. [4, 5, 14, 30, 34, 36]

Conversely, MacLennan *et al* (2006) state that, when it comes to adolescents, social behaviour and not age is the responsible for the high carriage rates. [20] Active and passive smoking, intimate kissing (mouth to mouth contact has also been established as a risk factor for meningococcal carriage [33, 34]) and pub/club attendance (which might be associated with smoking, considering that at the time of MacLennan's study it was allowed in closed public spaces, and the fact that, with loud music, people stand closer together to be heard which promotes transmission) are associated with higher meningococcal carriage. [11, 14, 20]

Studies point to a higher prevalence of meningococcal carriage in males. [1, 4, 14, 30] Although MacLennan *et al* (2006) state that behaviour is the "driving force behind the increased risk of meningococcal carriage in teenagers", rather than sex. [20]

As pointed above, several studies have shown that low socioeconomic status displays an association to the risk of developing invasive meningococcal disease, but there is not much research of the relationship between deprivation and meningococcal carriage. [5] The first study to examine this link was by MacLennan *et al* (2006), and there was not found any evidence of an association. [20] However, Cleary *et al* (2015), through a cross-sectional study, who sought to identify risk

factors for meningococcal carriage, upon the study of an apparent cluster of invasive meningococcal disease in West Cumbria, United Kingdom, have shown a relationship between poverty and meningococcal carriage; although, as the investigators warn, deprivation could simply represent a greater prevalence of other more direct risk factors (as active and passive smoking, which increases susceptibility to bacterial infection and is an established risk factor for meningococcal disease [1, 4, 5, 11, 14, 22, 30, 36]). [5] The authors alert for the fact that, considering deprivation as an important risk factor, the evaluation of effectiveness and cost-effectiveness introduction of MenB vaccines is of upmost importance. [5]

Carriage seems to be increased in rural areas, when comparing to urban areas. [4, 30]

Despite the lack of consensus among authors, some studies appoint concurrent bacterial or viral respiratory infections [such as influenza virus or respiratory syncytial virus] as having a positive relation to carriage acquisition. [4, 14, 22, 23, 30]

The number and the closeness of social interactions appears as another important risk factor [4, 25], and it appears to be the reason for the higher carriage rates among adolescents and young adults. [17, 34, 37] Crowding in general, but namely household crowding [30], are risk factors for meningococcal carriage. [4, 22, 23, 25, 37] In closed environments, carriage rates can reach 40-80%.[2] Among teenagers, house crowding may be less relevant, considering this age group tends to spend less time at home. [20] Military recruits (possibly due to associating in small platoons [21]), adolescents, and college students are at high risk. [4, 17, 20-22, 25]

Military personals originate from different geographical areas and must live in crowded conditions. [2] Jounio *et al* (2011) studied carriage rates in vaccinated (MenACWY polysaccharide vaccine) military recruits and found a significant increase during the military service. [23]

College students represent a group of individuals with high mobility [25], and are associated with risk behaviours such as smoking and alcohol drinking. [4] Increase in meningococcal carriage has been associated with university dormitories or social spaces on campuses. [11, 25]

Ala'Aldeen *et al* (2000) state that, as university students originate from different parts of the country (and, sometimes, abroad), the carriage investigated in the first days of the academic year, will be a representation of the most prevalent strains in the country. [21] This could lead to a bigger dissemination of hyper-virulent strains. [37] Therefore, the investigators screened first-time university students over 4 consecutive days, in the first week of school, and demonstrated an increase in carriage rates over this period of time, especially among students who shared catering facilities. [21] Then, with the objective of studying effects of long-term carriage, the researchers analysed the students 3 more times over the course of the academic year. [21]

4. MENINGOCOCCAL EPIDEMIOLOGIC SURVEILLANCE

The epidemiology of *Neisseria meningitidis* varies geographically and through time, being extremely dynamic. [2, 4, 6, 14-17, 19, 30, 38] In fact, IMD incidence varies throughout the world, depending on population immunity and environmental factors, affecting mainly young infants and, at a smaller scale, teenagers. [1, 3, 4, 6, 15, 21, 30] Different countries report discrepant IMD incidence, in part, due to differences in the surveillance systems. [4, 38] The understanding of meningococcal epidemiology is crucial when it comes to targeting individuals who are responsible for the most transmission. [30] Moreover, it is pivotal for the adequate development of effective vaccination programs. [4, 22, 30] Monitoring by continuous surveillance of the ever-changing epidemiology of the meningococcus is crucial for the management of IMD overall. [4, 6, 7, 15, 19, 30]

Until World War II, when it disappeared for unknown reasons, MenA had been causing outbreaks in industrialized countries. [4, 14] In the African meningitis belt, the world region with the highest burden of IMD [30], MenA was the most common serogroup during 2007-2009, having been replaced by MenW from 2010-2011. [4] MenX, rare in other parts of the world, is responsible for a substantial quota of IMD cases in sub-Saharan Africa. [2]

Unlike what happens in Europe, the distribution of carriage by age has not been thoroughly studied in the African meningitis belt. [30] In the African meningitis belt, meningococcal carriage peaks at a younger age (5-19 years old), comparing to what is recorded on Europe; however, this is a region with more variability, comparing to industrialized countries. [30] Furthermore, studies show that children in this region of 10-14 years old have the highest frequency of contacts. [30]

The mathematic meningococcal transmission model developed by Cooper *et al* (2019) suggest that meningococcal carriage increases rapidly in childhood, peaking at 10 years of age, and gradually declining after this point. [30]

MenA has been, however, reported in Russia and Greece in the last decade [24] as well as in Austria, Czechia, Denmark, Estonia, France, Germany, Italy, Latvia, Luxembourg, Poland, Portugal, Romania, and Sweden, but at residual values. MenA was the most prevalent serogroup in Lithuania in 2000 (fig. 5). [38]

MenA is also the predominant serogroup in Asia. [14]

MenB and MenC represent a particular danger because they can lead to multiple organ failure in up to 30% [4] of IMD cases. IMD is endemic in Europe, where MenB is the predominant serogroup [7, 11, 14] (ST-41, ST-32, ST-269, but also ST-11 and ST-8), followed by MenC (ST-11 and ST-8). [24] These are the serogroups responsible for most cases (sporadic and isolated outbreaks) in developed countries overall. [2, 4, 13] Regardless of variations in the epidemiology of *Neisseria Meningitidis* through time and geographically (especially notorious in Europe in the Northern region), MenB remains the dominant serogroup in general, in Europe (fig. 5) [38] and in Australia. [14] MenB and MenC are also the predominant serogroups in Latin America. [14]

Even though historically uncommon causes of IMD [6, 7], MenY and MenW have been becoming more prevalent in several countries (as in the Northern region of Europe [38]). [2, 4, 7, 19] These strains affect more commonly individuals over 65 years of age. [6, 14]

In the USA, MenY is the most prevalent capsular group [2, 4, 19], being also common in Europe, namely in Scandinavia and Slovenia. [2, 4, 38] Ravenhorst *et al* (2017) claim that MenY's incidence has been increasing in the Netherlands, being, as of 2017, the second most common serogroup of IMD cases in adolescents and young adults. [7] It was surpassed by MenB the following year. [38]

Lawler *et al* (2019) studied a cluster of two IMD cases of MenW in an elderly care home, in the United Kingdom, in 2015. Epidemiological information was reviewed, and phenotyping of case specimens conducted public health action, which included immunization and carriage assessment by throat swabs, followed by whole genome sequencing. [6] These patients presented with bacterial pneumonia (which is usually due to *Streptococcus pneumonia*, leading to an underdiagnosis of meningococcal pneumonia [6]), due to a "rare but expanding hypervirulent clone". [6]

For the past twenty years, several countries have reported an increase in the incidence of MenW disease from the ST-11 lineage. [17] This lineage is associated with atypical clinical presentations, such as gastrointestinal symptoms [17], pneumonia, septic arthritis, endocarditis and epiglottitis/supraglottitis. [4, 6]. The dissemination of the hypervirulent strain MenW:cc11 (ET-15) to Europe (having become endemic) is attributed to the Hajj pilgrimage. [4, 13, 17] This strain disseminated into Africa, later to South America and in 2009 started affecting the United Kingdom. [6, 13, 17, 25] This lineage, W: P1.5,2: F1-1: ST-11 (CC11), is known as the South American/United Kingdom strain. [13, 17] A clone of this strain originated an outbreak in 2013 in the United Kingdom and is now endemic in several European countries, such as the United Kingdom, France, Sweden, and the Netherlands. [13] This highlights the danger for strain importation within Europe. [24]

The cc11 lineage is highly clonal, which makes it hard to differentiate among clonal variants, so much so that MLST identifies all cc11 strains as sequence 11 (most are homogeneous for the PorA outer membrane protein, and type as P1.5,2 using monoclonal antisera). [13] Even antigen fine-typing cannot, as Mulhall *et al* (2019) state, "establish the true phylogenetic relationships among contemporary circulating clones" or "historically significant strain types". [13]

Hypervirulent ST-complexes appear to be rare, and differ in their ability to establish a commensal relationship with the host: ST-11 are poor colonizers, for instance, while ST-23 are well-adapted to commensalism. [35]

In the United Kingdom, where most literature on meningococcal disease originates (being, by far, the European country more fatigated by IMD [38] (fig. 6)), IMD incidence has declined from 3.8/100000 population to 1.5/100000 population, in 2001/2002. [6] From 2009 to 2015/2016, the incidence of MenW IMD has been increasing. [6]

During the 1990s, MenC disease showed an increasement in the United Kingdom, because of the spread of a serogroup C variant of the ST-11 complex (first identified in Canada, known to cause disease worldwide [16, 39]). [18] MenC strains c11 are associated with high mortality rates, and are responsible for outbreaks in several countries, like Germany, France, and Italy. [39] There appears to be an association between the $siaD_C$ expression and ST-11 complex, which, considering the virulence of the capsule, can account for ST-11 meningococci's invasive character. [16]

With the introduction of MenC vaccination, MenB became the most common cause of meningococcal disease. [18] Jeppesen *et al* (2015) conducted the largest carriage study through the second decade of life and found that the most commonly carried serogroups in the United Kingdom were MenB and MenY, which had been rising since from 10 years prior. [11]

In the United Kingdom, MenY disease peaks in persons over 65 years of age. [11]

In the Republic of Ireland, MenC conjugate vaccine was introduced in 2000, as response to MenC increasing incidence, which resulted in a significant MenC disease decrease. [13]

There has been a recent increase in MenC disease, therefore Mulhall *et al* (2019) set to understand if it was due to the emergence of a new clone, or the re-emergence of clones prevalent at the time of MenC vaccination introduction. To do so, the investigators subjected MenC:cc11 isolates from the 1997/98 to 2016/17 epidemiologic years to WGS and found that most MenC disease was being caused by a distinct strain from the MenC:cc11 ET-15 clone of the late 1990s (still in circulation, but only causing sporadic cases). [13]

Aiming to understand the currently circulating MenW strains, Mulhall *et al* (2019) compared all MenW isolates from 2010/11 to 2016/17 to the original Hajj clone, to the 2009 United Kingdom lineage, and the novel 2013 United Kingdom lineage. The researchers did not identify any

Hajj clone, only the United Kingdom lineages – now established in several other European countries, and endemic in the Republic of Ireland. These findings led to a deliberation on a vaccine policy change, in order to protect adolescents. [13]

5. MENINGOCOCCAL VACCINATION

Meningococcal vaccination promotes the development of serum bacterial antibodies and induces mucosal immunity, with effects on carriage and transmission. [16] Ever since meningococcal vaccines have been introduced in the market (early 1970s), the burden of IMD has been lowered. [4] The first formulations were based on plain capsular polysaccharides, and even though they were able to reduce the burden of IMD, they showed low immunogenicity in children, and no effect on nasopharyngeal carriage. [4, 16, 20]

Then, vaccines based on polysaccharides, but now conjugated with protein carriers, were developed, showing satisfactory results in various studies and programs. [4, 18, 29, 40] Cooper *et al* (2019) state that "vaccinating carriers is the only way to generate herd protection". [30]

The first glycoconjugate vaccines were used in Europe during the 1990s, in the context of an international epidemic of MenC:cc11 meningococci. [13]

5.1. THE PARTICULARITY OF MenB VACCINATION

There are conjugated vaccines against the A, C, W and Y serogroups. However, only protein-based vaccines are available against MenB, one of the major causes of IMD. [11, 17, 22, 41]

Because of the molecular mimicry between the polysaccharide capsule and the human neural cell adhesion molecule, MenB vaccines have been proving a challenge to produce, [4, 11, 41] as, even when conjugated with a carrier protein, MenB capsular polysaccharide's low immunogenicity represents a risk for autoimmunity development. [41]

Originally, MenB vaccines were based on outer membrane vesicles (OMVs), directed towards localized outbreaks. [4, 16] These vaccines were based on the PorA potein. [41] However, being strain-specific, these vaccines could not provide cross-protection. [4, 41]

The need to seek a new approach led to reverse vaccinology usage. [41] Using WGS, three well conserved surface proteins were discovered, with the potential to induce cross-protection: factor H-binding protein (fHBP), neisserial adhesion A (NadA), and neisserial heparin-binding antigen (NHBA). [41] With this discovery, two "broad spectrum" meningococcal vaccines, as Gianchecchi *et al* (2017) refer to it, were produced – multicomponent meningococcal B vaccine (4CMenB – Bexsero®, by GSK Vaccines) and the bivalent factor H binding protein vaccine (2MB /

rLP2086 – Trumenba®, by Pfzier). [4, 7, 37] 4CMenB has four components – fHBP 1.1, NadA, NHBA subvariant 2, PorA P1.7-2.4 based on the NZ98/254 isolate - [3, 15, 41], and rLP2086 has two – fHBP 1.55 and fHBP 3.45. [41]

rLP2086 was strictly approved for individuals aged 10-25 years, in the USA, while 4CMenB was approved to use in individuals above 2 months old, with no age upper limit, in Europe. [4, 11] In 2013, the European Medicine Agency licensed 4CMenB for individuals above 2 months of age, in a 2-3 doses immunization schedule, plus a booster dose. [4] There seems to be no difference in terms of immunogenicity or safety, comparing the 2 and the 3 doses regimen, in lactates younger than 6 months. [4] rLP2086 has been licensed in Europe for use in individuals aged from 10 years. [37]

5.2. EFFECTS OF VACCINATION

Claus *et al* (2005) state that "the resultant population effects [of vaccination] can be either advantageous, if transmission of the disease-associated meningococcus is interrupted, or disadvantageous, if they promote the emergence of vaccine escape variants". [16]

In the African meningitis belt, MenA is the most prevalent serogroup. [4] Almost 2 years after the beginning of the start of mass vaccination program in Burkina Faso in 2010, there was a considerable reduction in the number of MenA-related meningitis and a decline on nasopharyngeal colonization. [4] This was supported by the results obtained in Chad (94% decline in meningitis cases and elimination of MenA carriage), and in Niger (isolates tested in 2011 did not show any evidence of MenA presence). [4] It has been demonstrated that MenA vaccination contributes to herd immunity. [7, 17] Cooper *et al* (2019) suggest that the vaccination of children around 10 years old in Africa could be effective in reducing *N. meningitidis* transmission. [30]

During the first year of a MenC vaccination campaign in 1999 in the United Kingdom, the incidence of IMD in individuals younger than 18 years old reduced 80%; and 2 years after the beginning of the program, nasopharynx colonization declined 75%. [4] MenC vaccination contributes to herd protection, as it reduces colonization and transmission of MenC, after the immunization of children and adolescents. [1, 2, 7, 17, 18, 22, 32] However, Ravenhorts *et al* (2017) warn that, in the United Kingdom, before the introduction of vaccination, low rates of MenC carriage were detected, despite the high MenC disease rates [25], and therefore the investigators hypothesise that MenC carriage might be difficult to detect. [7]

Fernández *et al* (2002) studied the impact of meningococcal vaccination for the combination of MenA and MenC on carriage of *N. meningitidis* C, in Galicia, Spain, where meningococcal disease was an endemic disease. The investigators showed that vaccination resulted in a marked decrease of meningococcal disease cases (1 year after vaccination enabled a decline from 10.42 to 4.3 cases per 100 000 population) and a decline in prevalence of carriers 10-19 years old (with a slight rise in the 5-9 years old age group). [40] Moreover, MenB (not targeted by the immunization program) did not replace MenC in the asymptomatic nasopharyngeal carriers. [40]

Since 2005, in several countries, quadrivalent meningococcal vaccines against the A, C, W and Y serogroups, conjugated with protein carriers, has been being introduced. [4] These formulations promote protective antibodies in individuals of all ages, and to induce immunological memory, alongside a positive effect on carriage. [4, 17, 25] However, it is important to note that the effects of MenACWY on carriage may be due to the decline in MenY and MenC carriage (the most common serogroups, prior to the vaccine introduction). [17] In fact, during the 2000s, MenY became more prevalent in Finland, despite vaccination against MenACWY. [23] Moreover, MenACWY vaccination might not have a significant impact on MenW carriage, as suggested by Oldfield *et al* (2018). [17]

The effects of MenB vaccination are hard to appreciate because clinical outcomes are not robust enough, considering the low IMD incidence and the fact that MenB vaccination is very recent. [3, 11, 41] Therefore, several authors defend that other approaches must be adopted, like performing genetic analysis of carriage isolates in hopes to find the antigens included in vaccination, which would prove the vaccine updated and well targeted. [3, 41] The titer of serum bactericidal assay with human complement (hSBA) correlates to the level of protection against IMD (≥ 1:4 translates in protection against meningococcal disease), translating in the amount of complement-mediated killing of bacteria by functional antibodies in sera from vaccines. [3, 15] However, it would be impractical to apply this method to a large number of samples. [3]

MATS is a modified ELISA method with the capability to quantify expression and the level of matching with the corresponding antigen in the vaccine (fHBP, NHBA, and NadA) on bacterial lysates, and it could be useful tracking spatial and temporal changes in MenB epidemiology and their repercussions on 4cMenB coverage; it is a fast and resource-saving alternative (comparing to other methods). [3, 15]

4CMenB has proved to reduce meningococcal carriage for any serogroup after 2 immunization doses. [4] The foundation in recombinant proteins for the development of MenB vaccines allowed for the prevention of outbreaks. [4] Moreover, with “broad-spectrum” vaccines, the delay between the characterization of a new outbreak and the availability of the vaccine is reduced. [4]

In Italy, the introduction of MenC conjugate vaccines (fig. 7) (in 2005 for 2-year-olds and 11-12-year-olds [15, 19, 39]) changed the epidemiology of the meningococcus [14], and as of 2017 approximately 70% of IMD cases were due to MenB. [4] The National Immunization Prevention Plan did not, according to Gianchecci *et al* (2017), reach the goal of 95% coverage in any Italian region, going from 42.7% in Campania to 88.3% in Emilia Romagna. [4]

Germinario *et al* (2010), through a cross-sectional study involving college students in Italy, demonstrated the positive effects of vaccination (having not detected any MenC carrier, the predominant serogroup before vaccination introduction). [19]

In the United Kingdom, MenC and MenB are included in the children vaccination program, since 1999 and 2015, respectively (fig. 8). [6, 37] MenC vaccine was offered to 13-14-year-olds and new university entrants (below 26 years of age), until 2015. [6] Close contacts of MenA, MenC, MenW or MenY IMD are also offered vaccination, unless previously vaccinated (within 12 months prior). [6] In spite of the 71% MenACWY vaccine coverage, data reveals an increase in MenW carriage. [17, 25]

In 2009/2010, MenY carriage was highly prevalent among university students, in the United Kingdom. [25] Concomitantly, IMD cases by MenY were also elevated, and have plateaued since then. [25]

Since the national MenW outbreak of 2015, MenC vaccine was replaced by the MenACWY conjugate vaccine (fig. 8) and broadened the offer to 14-18-year-olds, with a catch-up campaign associated. [6, 17, 25] Using WGS, Oldfield *et al* (2018) studied the impact of this vaccination campaign on carriage of meningococci in immunized university students. [17] The investigators noted “no significant change in carriage of MenY organisms”, but “a rapid and significant rise in carriage of MenW strains with PorB serotypes and *porA* and *fHbp* STs that matched alleles harboured by endemic United Kingdom MenW:cc11 invasive isolates”. [17] Oldfield *et al* (2018) noted that, even though both the original and the 2013-strain MenW:cc11 strains were isolated in students, only the later became more prevalent. [17] The authors point as possible explanations differences in strains transmissibility and/or differences when it comes to the host susceptibility to carriage. [17]

Oldfield *et al* (2017) conducted a cross-sectional meningococcal carriage study in first-year university students, in the United Kingdom, where vaccine coverage for MenACWY was 71%. [25] The investigators noted an increase in MenW carriage (in spite of vaccination coverage), and alert for the need to further monitor this issue, because it may be promoting the increase of MenW disease in the United Kingdom. [25]

MenB vaccination has led to a ~50% reduction in MenB disease in the United Kingdom. [37] However, without showing any effect in carriage, Clark *et al* (2019) do not consider MenB vaccination in adolescents to be cost-effective in the United Kingdom. [37]

As of 2004, MenB was the predominant serogroup in Greece, followed by MenC. [1] MenC vaccination was introduced in Greece in January 2001 (fig. 9), and included in the national immunization program, targeting older children and teenagers in 2005. [22] In April 2011, MenACWY was also included in the national immunization program, as a booster dose for 11-16-year-olds. [22] Vaccination coverage was found to be as high as 83,4% in teenagers, in 2009. [22] Tryfinopoulou *et al* (2016) demonstrated a significant decrease in carriage among military recruits and university students, following vaccination, except when it comes to MenB. [22] MenB disease has been increasing excessively since 2000, in Greece, and, with the decrease of MenC cases, became the predominant serogroup. [22] The researchers also found a significant increase in MenY carriage among university students, aligning with an increase of MenY cases in various European countries since 2010. [22] Additionally, the identification of MenX isolates is noteworthy, as of until 2013 there were no described cases of MenX disease in Greece. [22]

In the Netherlands, in 2002, all children aged 1-18 years were vaccinated with a single dose of MenC conjugate vaccine (fig. 10), with routine vaccination at 14 months old being subsequently introduced. [7] As of 2017, MenC vaccination coverage surpassed 90%. [7] In the Netherlands, MenB remained as the predominant serogroup until 2017, when it was surpassed by MenW, whose incidence had been increasing since 2014. [38]

5.3. WHO TO VACCINATE

Vaccination is the most effective preventive measure against IMD. [3, 4] Vaccines not only induce immunological memory among immunized persons, but also reduce carriage and transmission among the unvaccinated population. [18]

There is no recommendation for the vaccination of healthy individuals above 21 years of age who do not belong to a risk population. [4] The Advisory Committee on Immunization Practices (ACIP) advices for the immunization of adolescents and risk populations, as soon as possible. [4]

Ravenhorst *et al* (2017), advocate for the vaccination before the age of 15, for individual immunization and, potentially, herd protection. [7]

Considering the investigators found a gradual rise in carriage in later adolescence, Jeppesen *et al* (2015), affirm that "any immunisation campaign designed to reduce pharyngeal carriage of MenB (or MenY), and thereby induce herd immunity, should aim to immunise prior to this rise, i.e. in the early teenage years or before, in order to prevent acquisitions and interrupt transmission". [11]

MenACWY vaccination seems to be an effective strategy in suppressing meningococcal carriage in the military. [2] It should be combined with 4CMenB, as it does not eliminate MenB carriage. [2]

Different countries, based on geographical epidemiologic discrepancies, have different vaccination policies. For example, since the MenA epidemic in the 1970s, there has not been any IMD outbreak in Finland; therefore, vaccination is only recommended in high-risk groups, like military recruits. [23]

6. THE PORTUGUESE REALITY

IMD has been monitored in Portugal since 1927, being its notification compulsory. In 2000, the Portuguese general health administration (DGS), alongside the National Health Institute Doutor Ricardo Jorge (INSA), following the 1999 market introduction of a conjugated vaccine against MenC in the United Kingdom, created the Meningococcal Disease Integrated Vigilance Program, in an effort to evaluate the impacts of vaccination against IMD during the 2000-2006 time period, in Portugal. [8, 10]

MenC vaccination started being commercialized in Portugal, in 2001, and it had been prescribed ever since. [10, 42] In January 2006, Portugal introduced the MenC conjugate vaccine (2+1) for infants and children at 1 year of age, in the national immunization plan (table I), with a catch-up campaign which achieved 80-91% coverage among the 1989-1994 cohorts. [43] At the time, there were different vaccination schemes (table I), considering the age at start of vaccination and the number of administered doses before and after the age of 12 months, due to the catch-up campaign. The recommended vaccination schedule contained 3 doses, administrated each at 3, 5 and 12 months of age. [10] In 2012, the vaccination scheme was altered to a single dose at 12 months of age, with no vaccination of adolescents. [42, 43] After the 2006 inclusion of MenC vaccination in the national immunization program, the first IMD case by MenC in a child below 12 months of age, took place only in 2014 (and, at least until 2016 – the most recently published data -, this remained the only reported death by MenC disease in Portugal [9]). [8, 9]

MenB vaccination was introduced commercially in Portugal in April 2014, having been included in the national immunization program in 2020 (table II). [8] Both Bexsero® (table III) and Trumemba® (table IV) vaccines are available, being Bexsero® the one included in the national immunization program. [42]

MenACWY is also available commercially (both Nimenrix® [table V] and Menveo® [table VI]), in Portugal, but it is not included in the national immunization program, being recommended in particular cases of immune system impairment (table II). [44]

Different approaches have been adopted towards IMD cases quantification throughout the years (due to the use of different sources through the years as well as changes in the definition of what constituted a case – updated in 2012 in consonance with the European Comission [8] (fig. 4), and reviewed in 2016 (fig. 11) [27]), resulting in some unavoidable under or over-quantification in some years. [8, 10] IMD incidence rates during the 1990's oscillated between 1,82 and 3,23 cases/100 000 population [10], but from 2000 on there has been a decrease. [10] Between 2000

and 2002, incidence rates varied from 2,98 to 3,81 cases per 100 000 population, but from 2003 forward the impact of the commercialization of MenC vaccination showed profit as incidences varied from 1,25 to 1,99 cases per 100 000 population [10], with accentuated decreases especially in the years 2005-2006 (22,4%), 2007-2008 (32,4%) and 2013-2014 (25,4%). [8] In 2016 (the most recent year with published data), incidence rate was 0,41 cases per 100 000 population – showing a very significant decrease since the introduction of vaccination against IMD. [9]

Until the 2000-2006 time period, MenC was the main serogroup responsible for IMD in Portugal, followed by MenB. [10] Since the 2006 inclusion of MenC vaccination in the national immunization program, MenC disease has become residual in the country, with MenB becoming the most frequent serogroup since 2003. [8] It is still unknown why, but MenB incidence has decreased since the MenC vaccination introduction. [8] There are still no data on the impact of MenB vaccination on disease rates. [8, 9]

Most IMD cases are confirmed by cultural exams, by laboratories from the national hospital network. Negative cases are confirmed by INSA by PCR techniques (as off until 2010) – it is important to monitor these cases, as it predicts how early antimicrobial therapy was initialized. [10] With hospital laboratories being equipped to perform PCR analysis (2010), the percentage of confirmed IMD cases has showed an increase; however, the proportion of confirmed cases without strain characterization has been decreasing, but still remaining high, which could be due to the decreasing need to send samples over to INSA for analysis, and therefore having less sampled DNA characterized. [8] In 2015-2016 (the latest data), all cases that resulted in death by IMD had serogroup identification, with a great part having been analysed at INSA. [9]

INSA conducts a molecular characterization of all meningococcal strains received, and from October 2002 to December 2006, serogroup C showed the smallest genetic diversity (high prevalence of cc11 [8-10]), whereas serogroup B accounted for 42 different phenotypes identified (being ST-41/44 the most frequent cc, since 2003 [8]). [10] During this time period, 7 strains were identified to originate from capsular switching processes, none of the patients implied had been previously vaccinated against MenC. [10]

As off 2016, MenB continued to be the most prevalent serogroup, with ST-41/44 remaining the most frequently identified cc, followed by ST-213. [9] Since 2011, MenY became the second most prevalent serogroup in Portugal, showing low genetic diversity (cc23 accounted for 59,4% of MenY disease). [9] Differently from what was observed in other countries, where MenY was more frequently associated with disease in adults, in Portugal, this serogroup was responsible, mainly, for disease amongst individuals younger than 10 years of age, between 2007 and 2016. [9]

Regarding risk factors associated with disease onset, it has been shown that, as in other countries, IMD presents with a seasonal pattern in Portugal, peaking between the 45th week of a year and the 16th week of the following year. [10] Also, DGS and INSA report a slightly higher incidence in males over females of IMD, with 54,5% (2000-2006) of cases occurring on males. [10] Other factors, such as socioeconomical status, for example, have not been studied.

Death rates have been decreasing, since 2000. [10] Between 2000 and 2006, the mean death rate of IMD was 7,7%, with MenB being responsible for the most deaths in the <5 and 5-14 years-old groups, and MenC in individuals older than 15 years old. [10] From 2006 onwards, MenB has become the most deadly serogroup in Portugal (as well as the most prevalent). [8, 9] From 2003 to 2016, death rate decreased further (now 7,0%), remaining bellow the expected values (10%). [9] Death rates appear do be higher, higher the age of the patient (fig. 12). [9]

Sequelae rates are unknown. [8-10]

There are but two meningococcal carriage studies conducted in Portugal. [33, 43]

Rodrigues *et al* (2015) performed the first meningococcal carriage study in Portugal on 601 university students from Coimbra university, having obtain a 13.3% carriage rate. [43] The predominant serogroup was MenB, followed by MenY. [43] MenC and MenW isolates were subjected to WGS and found to be distinct from the cc11 strains. [43] The investigators suggest that it may be beneficial to, as occurred in the United Kingdom, apply a vaccination program targeting teenagers, in Portugal. [43]

4 years later, Rodrigues *et al* (2019) reassessed the meningococcal carriage in Coimbra University students and found an overall carriage rate of 12,5% (being MenB the predominant serogroup, and the main cause of meningococcal disease in Portugal). [33] MenW isolates were subjected to WGS and all belonged to the ST-11 complex (two were from the South African lineage and one was from the “original” United Kingdom strain). [33] There was only one subject who carried more than one serogroup (MenW and MenY, namely). [33]

In spite of the increase in MenW carriage reported in several European countries, the researchers have not noticed any increase in MenW carriage in Portugal. However, the MenW isolates belonged to the hyperinvasive lineage that has been responsible for the increase of MenW disease rates – ST-11. [33]

CONCLUSION

In spite of the incessant scientifical developments and both diagnosis and therapeutics advancements, IMD remains an important cause for mortality and morbidity in the paediatric population, all over the world. [1, 2] Most infections by *Neisseria Meningitidis* result in asymptomatic carriage rather than disease, being ~10% of the world population asymptomatic carriers. [2, 4, 7] Nevertheless, in both scenarios, meningococcal carriage is fundamental for the development of IMD, as it is key for disease transmission, and infection onset. [4, 7, 20] Moreover, carriage correlates with the emergence of new pathogenic variants, considering that with a higher level of carriage, comes a higher potential of genetic recombination. [4, 7] As Cooper *et al* (2019) put it, "meningococcal carriage dynamics drive patterns of invasive disease". [30]

The majority of *Neisseria meningitidis* transmission happens among carriers. [30] Carriage rate is higher among adolescents and young adults, being these the main spreaders of the meningococcus. [2, 4, 6, 7, 13, 18, 19, 21-25] Infants, also important carriers, lack the complete development of the immune system and lose maternal antibodies early on, becoming particularly vulnerable. [4] Therefore, carriage studies are key for understanding the ever-changing meningococcal epidemiology and direct vaccination strategies. [2, 7, 11, 15, 17, 19, 21, 23, 25, 28, 32-35, 43]

Carriage studies are scarce in Portugal. [33, 43] Rodrigues *et al* (2015) performed the first meningococcal carriage study in Portugal which demonstrated that the predominant serogroup was MenB, followed by MenY. [43] 4 years later, Rodrigues *et al* (2019) reassessed the meningococcal carriage in an equivalent population and MenB remained the predominant serogroup. [33] Despite the increase in MenW carriage reported in several European countries, the researchers have not noticed any increase in MenW carriage in Portugal, but the MenW isolates found belonged to the hyperinvasive lineage that has been responsible for the increase of MenW disease rates – ST-11. [33] More studies are needed to understand the carriage patterns in the country. [8, 9, 33, 43]

As data shows in most European countries, MenB is the lead cause of IMD in Portugal, now. [8, 9, 38] It also appears to be the most carried serogroup in Portugal. [33, 42]

At the moment, the Portuguese National Immunization Program includes vaccination against MenC and MenB (while MenACWY is also available), in children, as the program does not

contemplate the vaccination of adolescents against IMD as standard practice. [42, 44] MenC vaccination, older in the country, has achieved remarkable results in decreasing the incidence of MenC (and, surprisingly, MenB as well) disease. [8, 9] This vaccine has also been responsible for the decrease in MenC carriage in other countries. [18, 29, 40] MenB vaccines are relatively newer and there is less data on their effects. Regardless, it appears to be effective when it comes to reducing the burden of MenB disease but has not showed any effect on carriage, in other countries. [37]

Vaccination is of upmost importance in inducing immunological memory and reducing carriage levels, contributing to herd immunity. [18] Ravenhorst *et al* (2017) and Jeppensen *et al* (2015) highlight the importance of vaccinating not only infants, but also adolescents, in hopes of decreasing carriage levels. [7, 11] Rodrigues *et al* (2019) suggest that a vaccination program targeting teenagers, in Portugal, could be beneficial. [43]

Vaccination has shown remarkable results in Portugal. More studies are need in order to conclude whether or not the vaccination of adolescents would be cost-beneficial.

APPENDIX

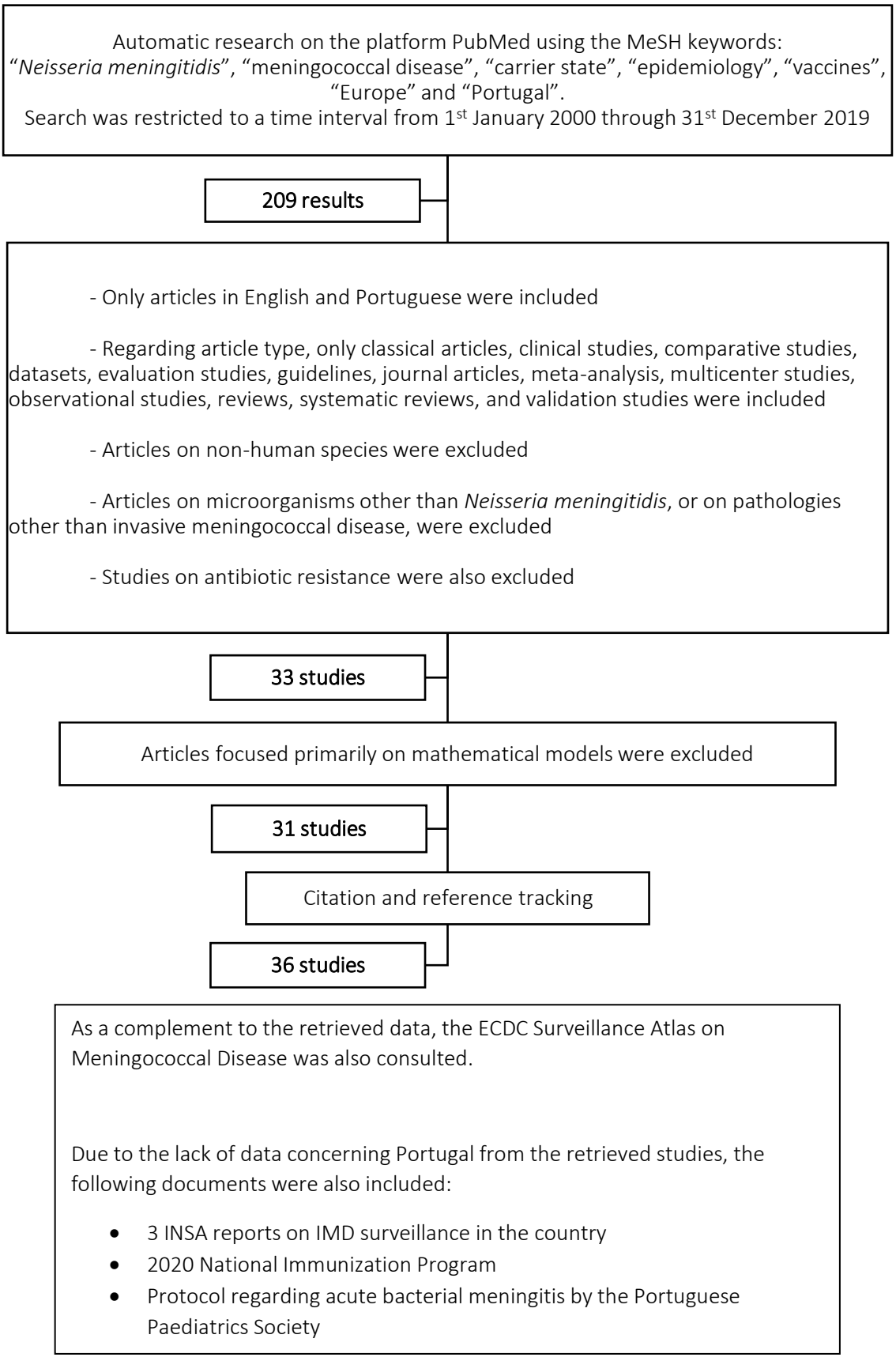

Figure 1 - Work methodology

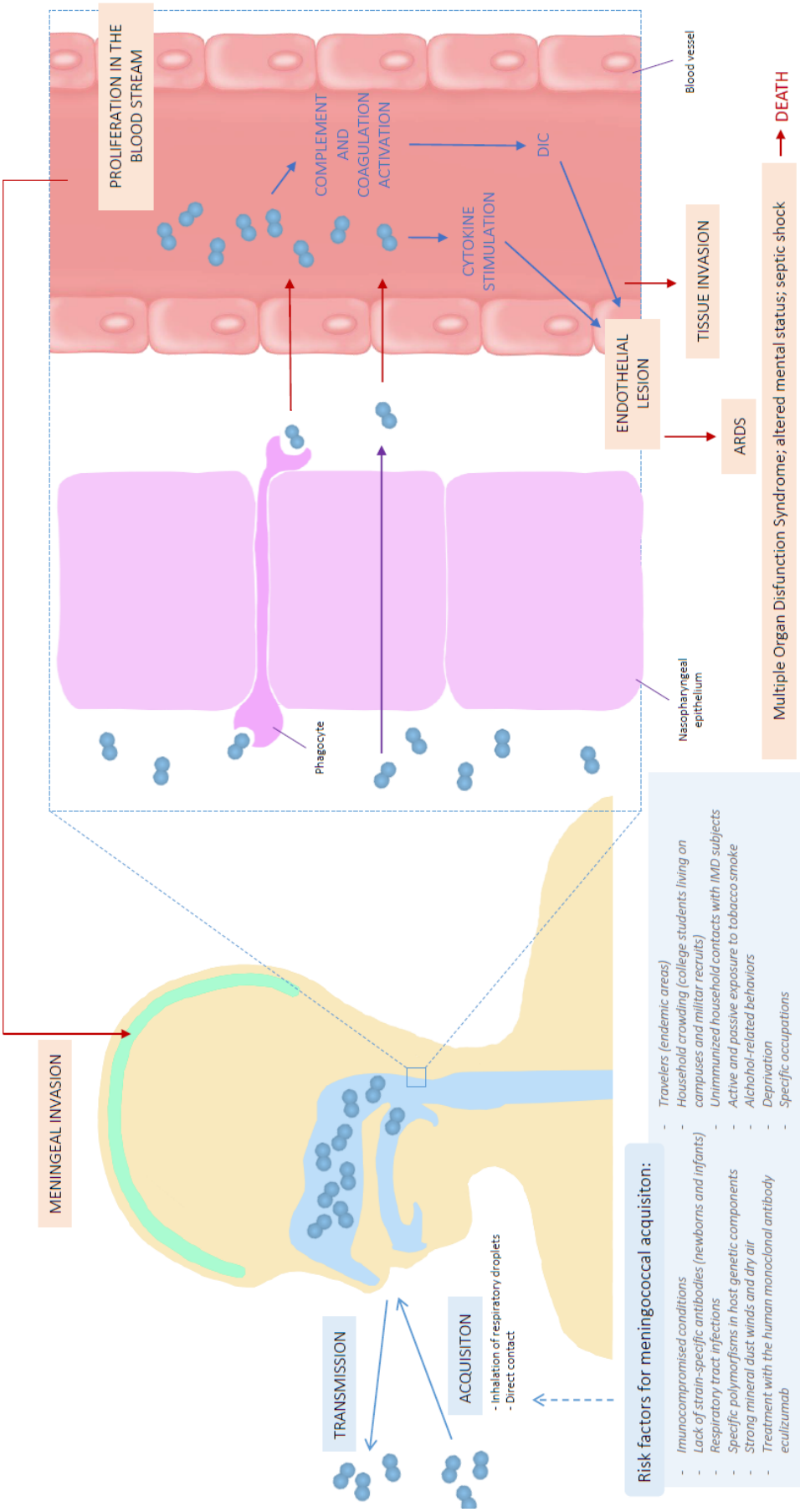

Figure 2 - Menincogoccal pathogenisis [17, 45]

Figure 3 - Infant with severe IMD admitted to PICU

Clinical criteria (at least 1 of the following)		
•Fever •Meningeal signs •Petechiae •Septic shock •Septic arthritis		
Laboratorial criteria (at least 1 of the following)		
•Isolation of *Neisseria Meningitidis* from an usually sterile locale, including skin lesions •Meningococcal DNA detection from an usually sterile locale, including skin lesions •Meningococcal antigen detection in CSF •Detection of Gram negative diplococci in CSF		
Epidemiological criteria		
•Epidemiological relatioship by person to person contagiation		
Case classification	A) Possible case	Fulfills clinical criteria
	B) Probable case	Fulfills clinical criteria and presents epidemiological relationship
	C) Confirmed case	Fulfills laboratorial criteria

Figure 4 - Case definition for the notification of IMD [46]

Figure 5 - Most prevalent serogroup in each European country (2000 on the left, 2017 on the right)
Only countries with available data for both the year 2000 and the year 2017 were included

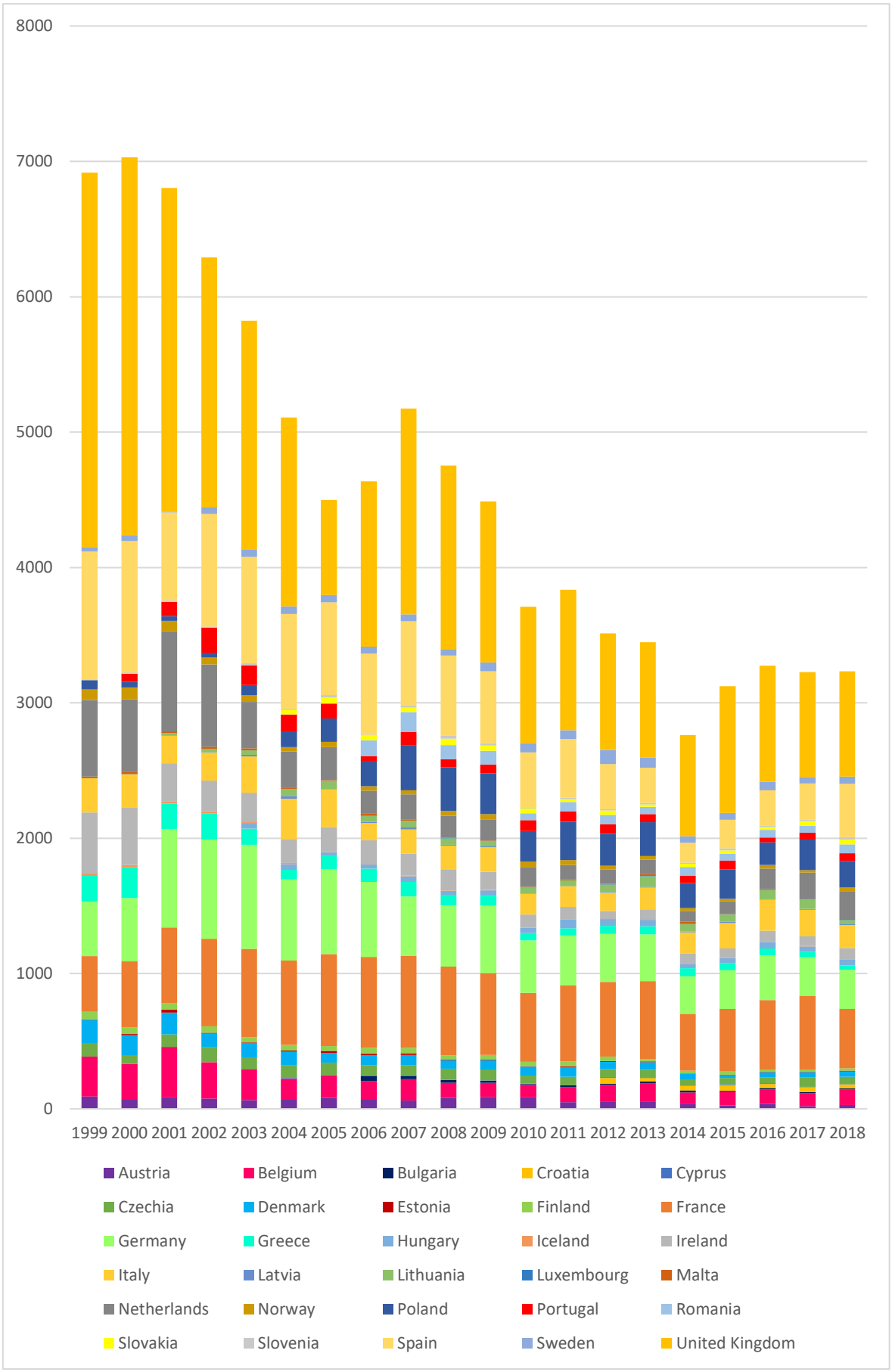

Figure 6 - Number of IMD cases per each European country, from 1999 to 2018 [38]

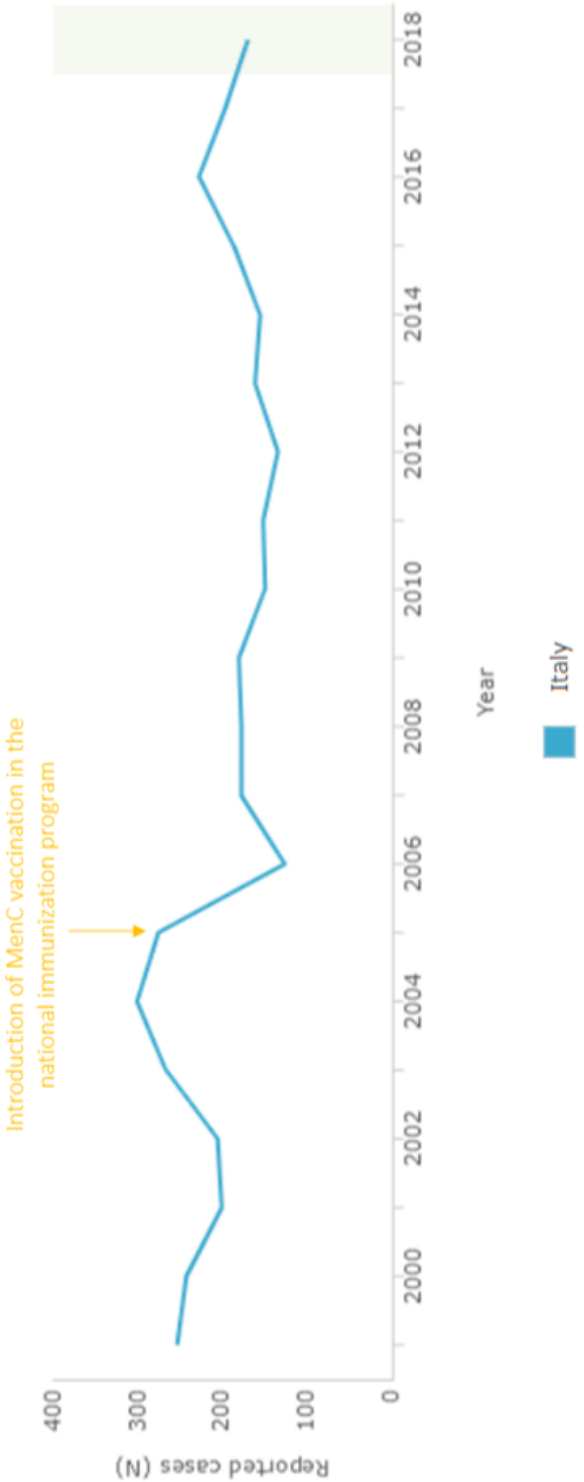

Figure 7 - Number of reported cases in Italy from 1999 to 2018. Vaccine introduction date is marked. Adapted from [38]

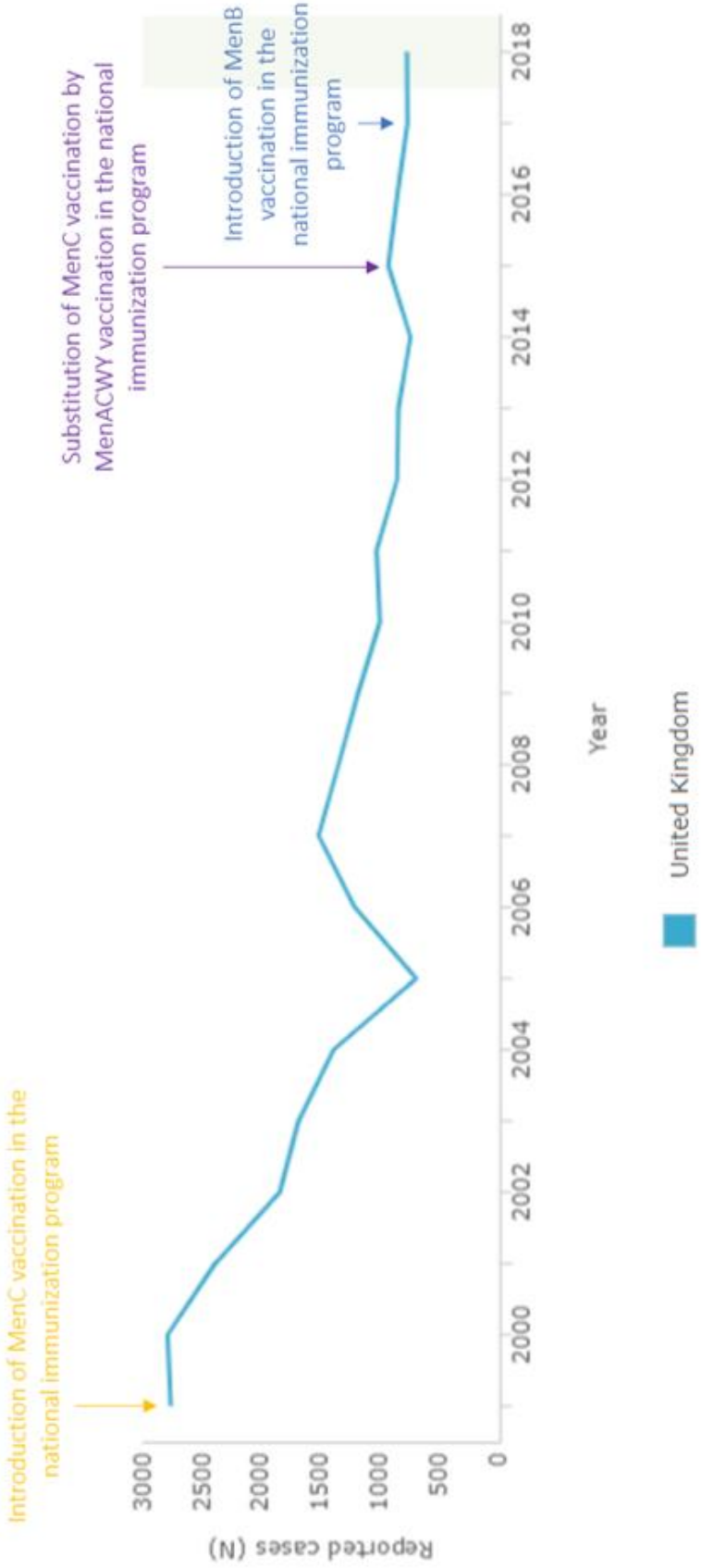

Figure 8 - Number of reported cases in the United Kingdom from 1999 to 2018. Vaccine introduction dates are marked. Adapted from[38]

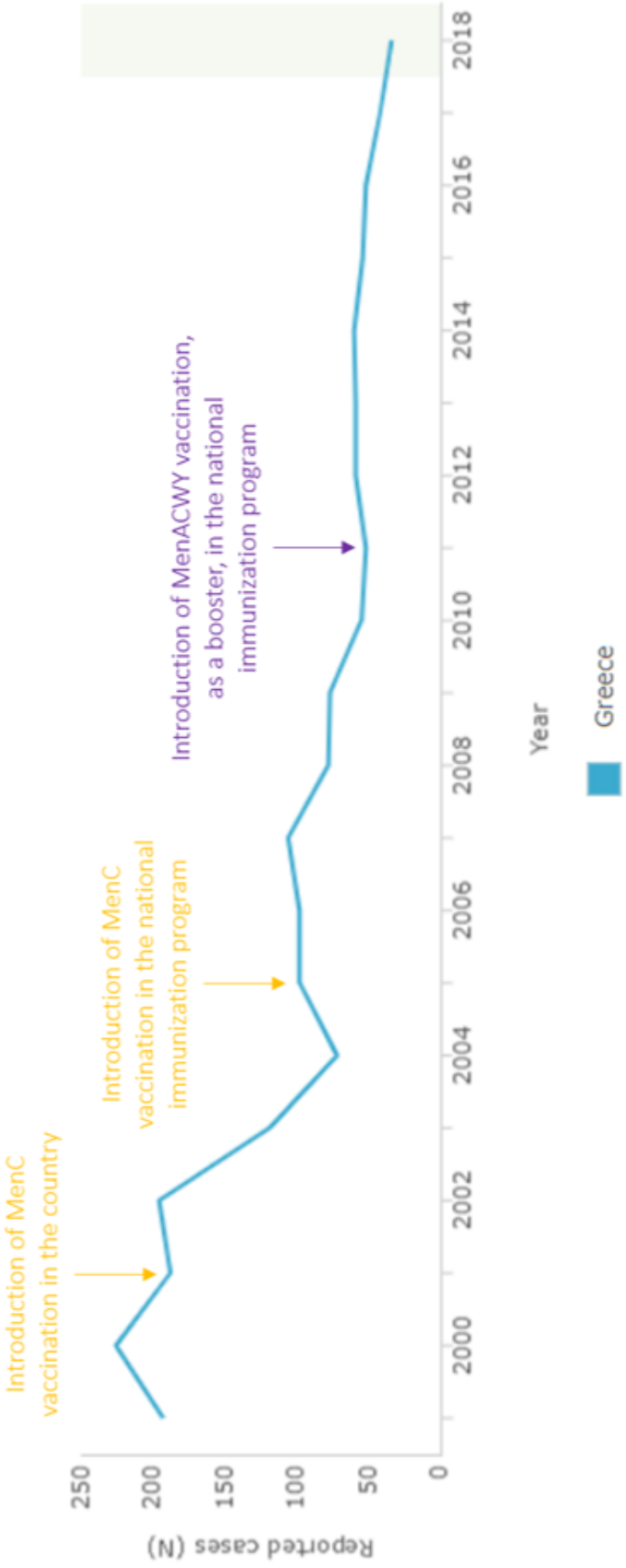

Figure 9 - Number of reported cases in Greece from 1999 to 2018. Vaccine introduction dates are marked. Adapted from [38]

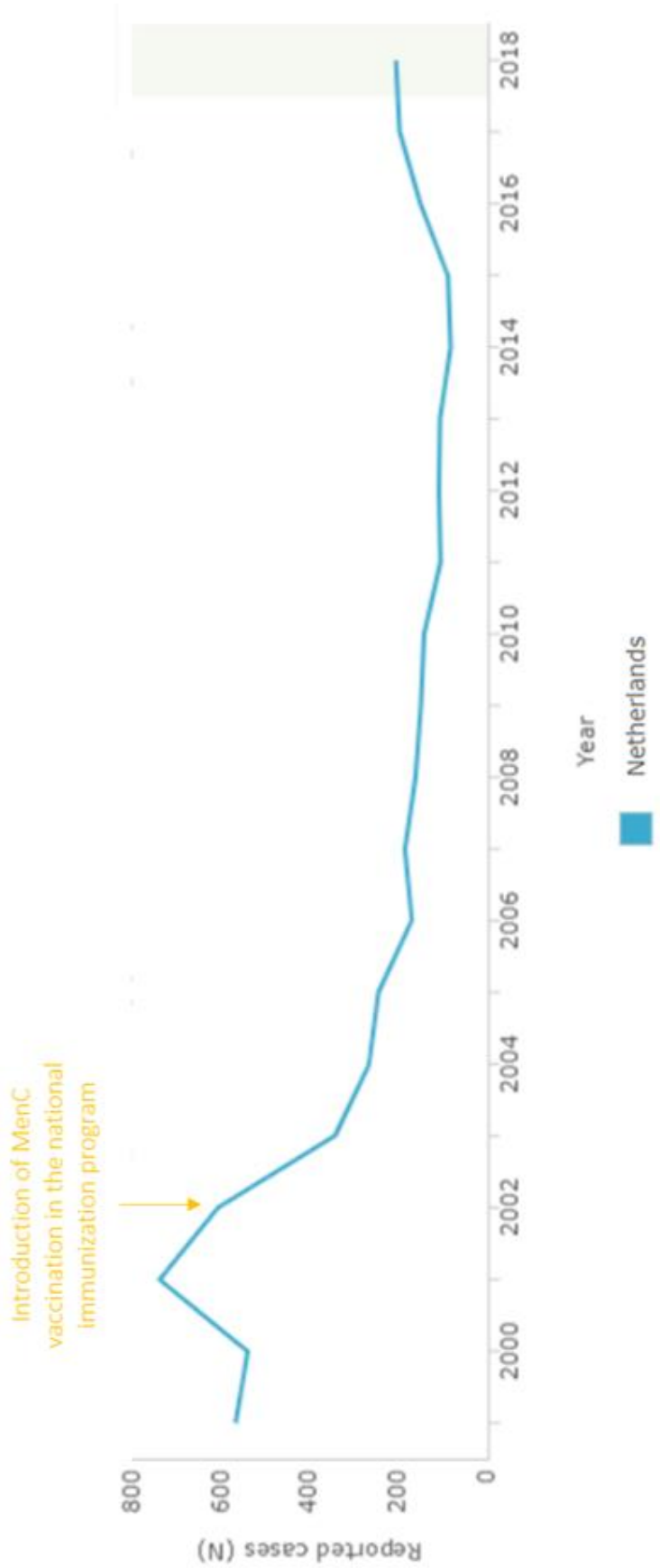

Figure 10 - Number of reported cases in the Netherlands from 1999 to 2018. Vaccine introduction date is marked. Adapted from [38]

Clinical criteria (at least 1 of the following)

- Meningeal signs and symptoms
- Petechiae
- Septic shock
- Septic arthritis

Laboratorial criteria (at least 1 of the following)

- Isolation of *Neisseria Meningitidis* from an usually sterile locale, including skin lesions
- Meningococcal DNA detection from an usually sterile locale, including skin lesions
- Meningococcal antigen detection in CSF
- Detection of Gram negative diplococci in CSF

Epidemiological criteria

- Epidemiological relatioship by person to person contagiation

Case classification	A) Possible case	Fulfills clinical criteria
	B) Probable case	Fulfills clinical criteria and presents epidemiological relationship
	C) Confirmed case	Fulfills laboratorial criteria

Figure 11 - Case definition for the notification of IMD [8]

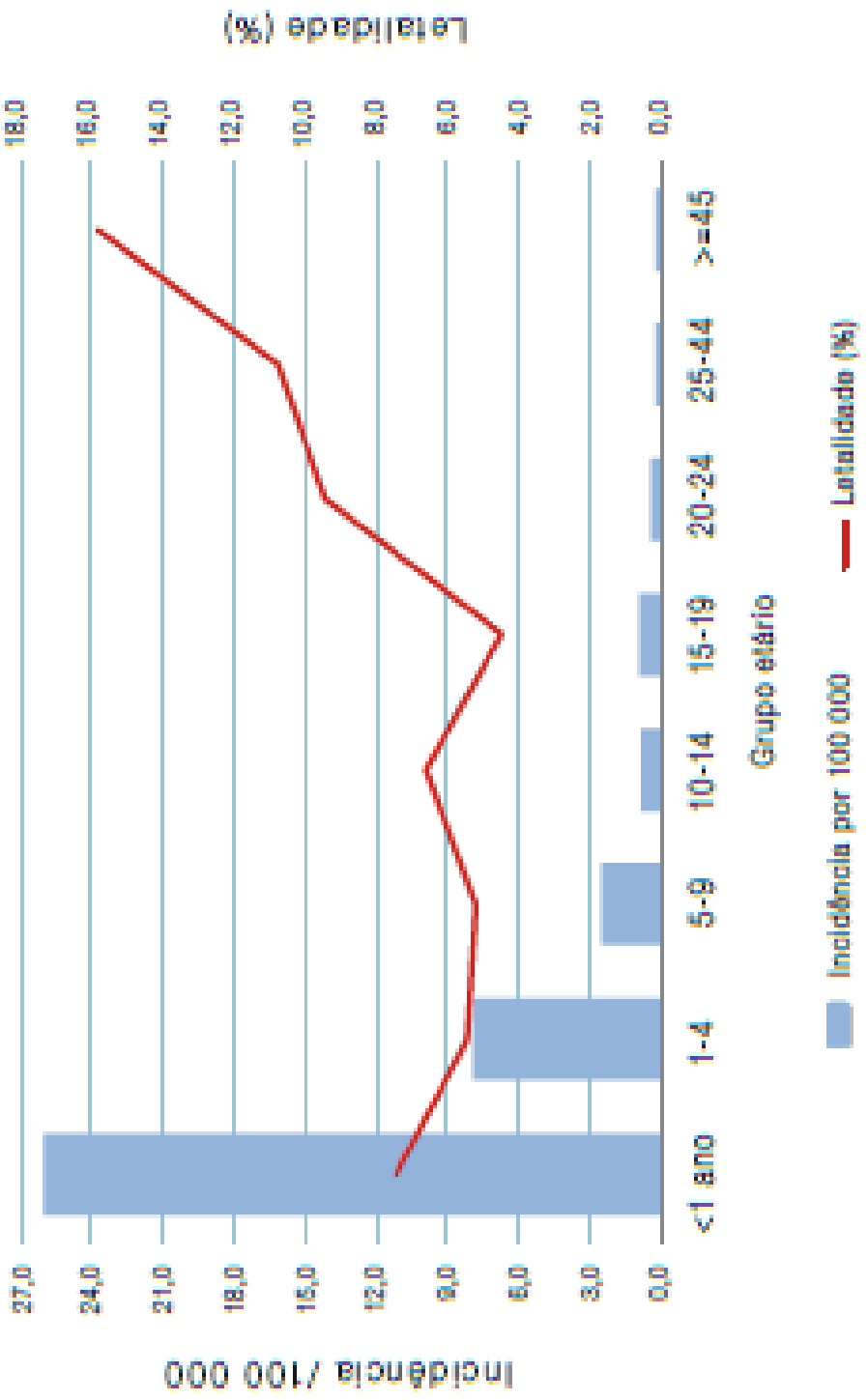

Figure 12 - IMD incidence per age group and respective death rates from 2003 to 2016, in Portugal [9]

Table I - Recommended MenC vaccination schemes as of 2006 [10]

AGE AT START OF VACCINATION	NUMBER OF DOSES ADMINISTRATED	
	< 12 months	≥ 12 months
6 WEEKS TO 3 MONTHS	2 doses	1 dose at 15 months
3 MONTHS	2 doses (at 3 and 5 months)	
2-9 MONTHS	2 doses	
10-11 MONTHS	1 dose	1 dose
≥ 12 MONTHS		1 dose, at first opportunity

Table II - National immunization program, regarding meningococcal vaccination (adapted from [44])

	MenC	MenB	MenACWY
VACCINE TYPE	Conjugated vaccine	Protein-based vaccine (containing the following antigens: NHBA, fHbp, NadA, OMVs from the NZ98/254 (PorA P1.4) strain)	Conjugated vaccine
INDICATIONS	Included in the national immunization program (as monovalent) *≥ 6 weeks and < 18 years of age* May be administered at ≥ 18 years of age if immune system impairment [(1)]	Included in the national immunization program ≥ 8 weeks and < 5 years of age May be administered at ≥ 5 and ≤ 50 years of age if immune system impairment [(1)]	Not included in the national immunization program ≥ 6 weeks of age, if immune system impairment [(1)]
PRECAUTIONS *(other than hypersensibility reactions, severe acute disease, and coagulation impairment)*	-	Prematures of ≤ 28 gestational weeks should undergo hospital surveillance of their first vaccines, with cardiorespiratory monitorization for at least 6-8 hours	-
ADVERSE REACTIONS	Local inflammatory signs (mostly in the first 24h) Systemic most frequent reactions: crying, irritability, somnolence or other sleep alterations, anorexia, nausea, diarrhoea, abdominal pain, and vomiting. ≥ 2 years old, also: headache, myalgia, and malaise *Severe adverse reactions are mostly rare or exceedingly rare*	Local inflammatory signs Systemic most frequent reactions: fever and irritability, diarrhoea, vomiting, anorexia, somnolence, abnormal crying, and rash. In adolescents and adults also: malaise, nausea, myalgia, arthralgia, and headache *Severe adverse reactions are mostly rare or exceedingly rare*	Local inflammatory signs Systemic most frequent reactions: irritability, somnolence, malaise, fatigue, fever, shivering, anorexia, nausea, diarrhoea, vomiting, headache, myalgia, and arthralgia Severe adverse reactions are mostly rare or exceedingly rare

[(1)] Individuals of < 18 years old with functional or anatomic asplenia, hyposplenism, congenital complement deficit, or who are receiving treatment with Eculizumab

Being *Neisseria Meningitidis* a capsulated bacterium, future receivers of stem cells transplants should be vaccinated against MenB and MenACWY, regardless of age; those who have already received a transplant, should be vaccinated agains MenB if < 50 years old and MenACWY if ≥ 12 months old.
Persons with anatomic or functional asplenia and complement deficit should be vaccinated against MenB (if < 50 years old) and MenACWY.
Persons with coagulation pathologies should undergo MenB, MenACWY and MenC vaccination intramuscularly, if prescribed by the assistant physician.

Table III – Bexsero® recommended vaccination schemes [42]

Age Group	**Primary Immunization**	**Interval between primary immunization doses**	**Booster dose**
2-5 months of age	3 doses	> 1 month	A dose at 12-15 months of age, at least > 6 after primary immunization
6-11 months of age	2 doses	> 2 months	A dose in the 2nd year of life, at least > 2 months after primary immunization
12-23 months of age			A dose at least 12-23 months after primary immunization
2-10 years of age *≥ 11 years of age*		> 1 month	Consider a booster dose for individuals at risk for exposure to IMD

Table IV – Trumenba® recommended vaccination schemes [42]

Age Group	**Primary Immunization**	**Interval between primary immunization doses**	**Booster dose**
Non-vaccinated adolescents (> 10 years old)	2 doses	6 months	Consider a booster dose for individuals at risk for exposure to IMD
	3 doses	2 doses at least 1 month apart, followed by a 3rd dose at least 4 months after the 2nd dose	

Table V – Nimenrix® recommended vaccination schemes [42]

Age group	**Primary Immunization**	**Interval between primary immunization doses**	**Booster dose**
> 6 weeks and 6 months old	2 doses	2 months	1 dose at 12 months old, > 2 months apart from the last dose administered
≥ 6 months and < 12 months old	1 dose	-	
≥ 12 months	1 dose	-	Consider a booster dose for individuals at risk for exposure to IMD

Table VI – Menveo® recommended vaccination schemes [42]

Age group	**Primary Immunization**	**Interval between primary immunization doses**	**Booster dose**
> 2 years old	1 dose	-	Consider a booster dose for individuals at risk for exposure to IMD, if 2-5 years old, a second dose 2 months apart from the first

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