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REVIEW

Epidemiology of invasive meningococcal disease in Greece, 2006–2016

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Abstract

The present study describes the epidemiology of invasive meningococcal disease (IMD) in Greece for the period 2006–2016. Combined data from notified and laboratory-confirmed IMD cases were obtained from the two involved National Centres (Epidemiology and Reference Laboratory). Laboratory identification and typing was carried out by both conventional (culture) and molecular methods (PCR, MLST, PorA, and FetA typing). A total of 796 IMD cases were notified; of those, 720 (91%) were laboratory confirmed. Overall, a decline on the annual incidence of confirmed cases was observed, ranging from 0.91 (2006) to 0.47 (2016) /100,000. A similar trend was observed in most age groups especially in children 0–4 years (7.7 to 2.9/100,000), with the exception of an increase in the incidence rate in adults > 20 years (0.21 to 0.32/100,000). The overall case fatality rate was 6.5% (52/796), annual range 2–13%. Among 658 strains which were typed by sero/genogroup, 80% were identified as MenB (annual range 65–92%); however, a decline was observed in MenB incidence from 5.3 (2006) to 2.7 (2016), among infants and toddlers, while MenW (1%), MenY (2%), and MenA (1%) remained low. During the 11 years, the annual incidence of IMD declined by 50%, especially in the 0–4-year age group, due mainly to MenB. Continuous surveillance of IMD is important for the development of future vaccination and public health policies.

Keywords Invasive meningococcal disease (IMD) · Epidemiology · Clonal complex · porA · fetA · Antimicrobial susceptibility

Introduction

Invasive meningococcal disease (IMD) is an acute, severe, and potentially life-threatening disease characterized by meningitis, sepsis or, less commonly, pneumonia, arthritis, pericarditis, and abdominal disorders [1] with an overall case fatality rate (CFR) of 10% [2].

The immunochemical structure of the *N. meningitidis* capsular polysaccharide defines 12 serogroups; only six of those (A, B, C, W, Y, and to lesser extent X) are responsible for most IMD [3].

Following the increase of MenC IMD incidence during mid-1990s, mostly from the circulation of a hypervirulent clonal complex 11 [4], 14 EU/EEA countries successfully introduced meningococcal C conjugate vaccine (MCC) into their routine national childhood immunization programs, resulting a dramatic decline of MenC cases. Furthermore, the quadrivalent protein–polysaccharide conjugate vaccine (MCV4) offering protection against serogroups A, C, W, and Y [5] was licensed in Europe since 2010, while recently (2013 and 2017), two novel protein-based vaccines against MenB have been launched in Europe [6]. Groups remaining at risk of IMD include infants and children < 5 years of age, adolescents, and young adults [7].

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The monovalent conjugate vaccine for MenC was introduced in Greece in January 2001 and included in the national immunization program (NIP) in 2005 for infants, resulting in a dramatic reduction of MenC cases. Estimated vaccination coverage was 51.4% in 2005 [8] and lately reached 89.6% (2014) in preschool children aged 2–3 years old [9]. Furthermore, in 2011, the MCV4 was included in the NIP as a booster dose in adolescents 11–18 years old. However, historical data showed that MenB has been consistently the main cause of disease in Greece. Two MenB vaccines (4CMenB, Bexsero™, GlaxoSmithKline and MenB-FHbp, Trumenba™, Pfizer) are available in Greece since 2014 and 2018, respectively, recommended only for high-risk groups, although pediatricians are offering vaccination privately.

Comprehensive high-quality surveillance is needed to monitor disease incidence trends, serogroup distribution, and strain characteristics in order to provide evidence for decision-making bodies. As the last epidemiological data on IMD in Greece were published for the time period 1993–2003 [10], the aim of the present study is the epidemiological analysis of IMD cases collected through the national surveillance system during an 11-year period (between 1 January 2006 and 31 December 2016), focusing on epidemiological indicators such as incidence, case fatality rate, age-specific trends, sero/genogroups, and molecular type distribution as well as antimicrobial susceptibility patterns.

Methods

Data collection

Surveillance of IMD is mandatory. IMD cases are notified to the National Public Health Organization (NPHO) by physicians nationwide as well as the Greek Public Health directorates. Data collected include demographic (in accordance with the Hellenic Statistical Authority ELSTAT) and epidemiological characteristics, vaccination status, clinical manifestations, potential antibiotic treatment prior to CSF collection, and disease outcome. Laboratory testing is performed by the Hellenic National Meningitis Reference Laboratory (HNMRL, Dept of Public Health Policy, School of Public Health, University of West Attica). Isolates and biological fluids (CSF, blood) received by the HNMRL are obtained as part of the routine activity and analyzed anonymously.

Case definition

The IMD case definition used complied with EU definitions where a case can be categorized as possible, probable, or confirmed based on specific clinical, laboratory, and epidemiological criteria (Commission Implementing Decision 2012/506/EU of 8 August 2012 of the EU-2012).

Laboratory methods

Meningococcal isolates were cultured on Chocolate Columbia Agar (OXOID Ltd., Basingstoke, UK) and incubated at 37 °C and 5–10% CO_2 for 24 h. Serogroups were identified by a slide agglutination test (Remel Europe Ltd. UK) according to the manufacturer's instructions. For culture negative suspected biological samples, a multiplex PCR assay was carried out for identification and typing of *N. meningitidis* [11]. Genogroup was determined by multiplex PCR targeting specific capsule group genes, as described previously [12].

Minimum inhibitory concentration (MIC) for seven antimicrobial agents (penicillin, cefotaxime, ceftriaxone, rifampicin, chloramphenicol, cefaclor and ciprofloxacin) was assessed by the E-test (AB Biodisc, Solna, Sweden or LIOFILCHEM S.R.L Italy since 2009) and performed according to EUCAST guidelines.

Molecular characterization (MLST, PorA, FetA)

Isolates and biological fluids belonging to a sero/genogroup were characterized by 'finetyping' (MLST and PorA and FetA typing), as described previously [13], using the PubMLST.org/neisseria database (http://pubmlst.org/neisseria/). Sequence types (ST) were defined and grouped into Clonal Complexes (ccs). PorA genotyping for variable regions 1 and 2 (VR1 and VR2) was performed as described previously [14] and compared with the variable sequences in the *Neisseria* PorA database (http://pubmlst.org/neisseria/PorA/). Similarly, the FetA variable region was also obtained for all typable isolates, as previously described [15] and compared with variable sequences in the *Neisseria* FetA database (http://pubmlst.org/neisseria/FetA/).

Data analysis

Data analysis included sero/genogroup, clinical presentation (meningitis, septicaemia, both), and outcome and was stratified by five age groups (0–4, 5–9, 10–14, 15–19, and > 20 years during the study period (2006–2016). Descriptive analysis was performed using STATA version 12.1. Graphs and tables were processed with STATA and Microsoft Excel.

Results

Study population and incidence

Overall, 796 cases of IMD were notified during the study period; 720 (90%) were laboratory confirmed, 5% (37/796) were defined as probable and 5% (39/796) as possible cases.

Of the 720 laboratory-confirmed cases, 278 cases were positive for both culture and PCR, 71 cases were confirmed by culture only, while 350 cases were positive solely by PCR Fifteen (15) cases were confirmed by microscopy/antigen detection, while for 6 cases, the confirmation technique was not reported. Meningitis was reported in 52% ($n = 413$) cases, septicaemia in 23% ($n = 181$), while both meningitis and septicaemia were reported in 25% of cases ($n = 202$).

The annual incidence of confirmed IMD cases (per 100,000 population) decreased from 0.91 ($n = 100$) in 2006 to 0.47 in 2016 ($n = 51$), with a mean annual incidence of 0.52 (95% CI 0.37–0.73) (Fig. 1). The highest incidence was observed in the age group of 0–4-year-old (range 2.4–7.7) followed by the age groups of 5–9 years (0.6–4.5), 15–19 years (0.6–1.8), and 10–14 years (0.4–1.5).

Consequently, the incidence declined in the age groups of 0–4 years (62%; 7.7 to 2.9), 5–9 years (84%; 4.5 to 0.7), 10–14 years (64%; 1.1 to 0.4), and 15–19 years (57%; 1.4 to 0.6) for the years 2006 and 2016, respectively. While relatively low, there was a non-significant increase in incidence in age groups ≥ 20 years (0.2 to 0.3) (2006–2016) (Fig. 1). Considering infants < 1 year of age, incidence declined by 73% from 13.8 (2006) to 3.8 (2016).

IMD-confirmed cases by sero/genogroup

Sero/genogroup was identified in 657 of 720 (91%) of confirmed cases. MenB was the most predominant (80.4%; 528/657, annual range 65–92%) followed by MenC (4.7%; 31/657, annual range 0–13%), MenY (2.4%; 16/657, annual range 0–8%), MenW (1.2%; 8/657, annual range 0–4%), and MenA (1.2%; 8/657, annual range 0–5%), while, sero/genogroup was not identified in 10% of the cases (65/657), all of which confirmed by PCR.

Overall, MenB incidence decreased from 0.65 (2006) to 0.35 (2016) (mean 0.44) per 100,000 (Fig. 2), which was observed in all age groups except for age group ≥ 20 years. Specifically, a reduction of 49% in MenB incidence was observed for children 0–4 years of age (5.3 to 2.7); a similar reduction (50%) was observed in infants < 1 year (7.3 to 3.7) for the years 2006 and 2016, respectively. A higher MenB incidence reduction of 89% was observed in children 5–9 years of age (incidence rate 3.5 (2006) to 0.4 (2016)), while a lower reduction (43%) was observed in the 10–14-year age group (incidence rate 0.7 (2006) to 0.4 (2016)) (Fig. 3).

Analysis by geographical region incidence revealed that the highest average MenB incidence was observed in the Thessaly region (Central Greece) (0.66/100,000) followed by the Greater Athens area (0.54/100,000).

The incidence rates for MenA, C, W, and Y remained low throughout the study period (Fig. 2).

Case fatality ratio

During 2006–2016, 52 fatal cases were reported, case fatality ratio (CFR) of 6.5%. The CFR for confirmed cases was 5.4% (39/720) ranging from 0% (2011) to 10.7% (2012). The highest CFR was observed among the 15–19 years of age (8.3%) while the lowest was observed in the age group of 10–14 years (2.1%). Among the identified sero/genogroups, case fatality was 6% (33/528) for MenB, 6% (2/31) for MenC, and 6% (1/16) for MenY, while for the rest of the cases (16/52), post mortem material was not available for further sero/genogroup identification.

Molecular characteristics

MLST analysis identified 14 clonal complexes (cc) among the 332 tested isolates, while no clonal complex was identified in 11 isolates. Among the 295 Men B isolates tested, the most common clonal complexes were 269 cc

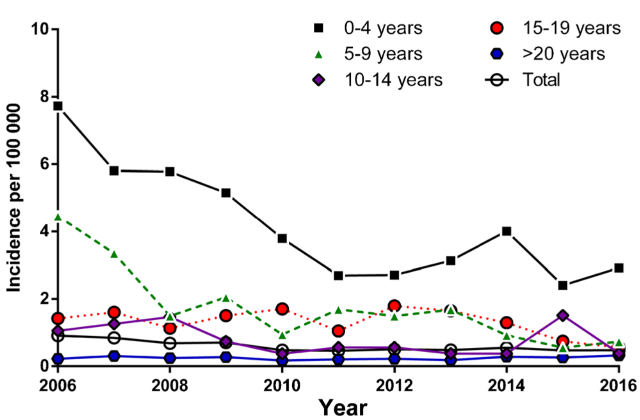

Fig. 1 Annual incidence of IMD-confirmed cases by year and age group in Greece, 2006–2016

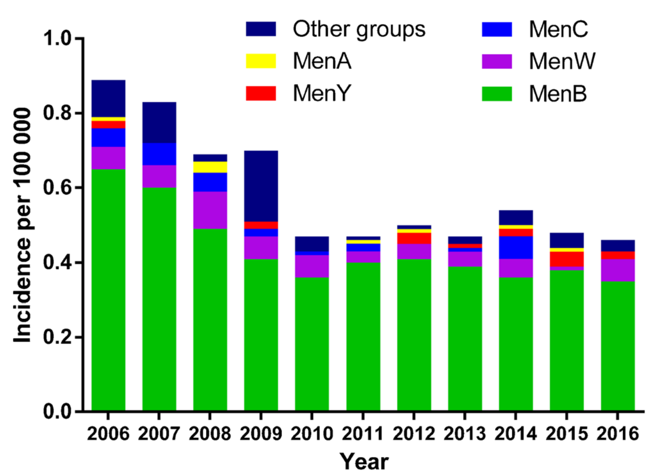

Fig. 2 Annual incidence of IMD-confirmed cases by epidemiological year and sero/genogroup distribution in Greece, 2006–2016

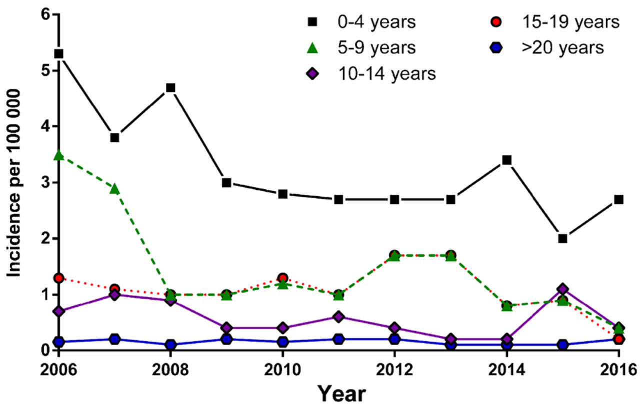

Fig. 3 Annual incidence of IMD-confirmed cases related to MenB by epidemiological year and age group in Greece, 2006–2016

(34.9%; 103/295), 32 cc (24.4%; 72/295), 162 cc and 41/44 cc (12.9%; 38/295 each, respectively), and 162 cc (11.2%; 33/295) all accounting for 83.4% while in 11 cases (3.7%), no clonal complex was identified.

Among the 12 MenC isolates tested, the most common clonal complexes identified were 11 cc (66.7%; 8/12), 41/44 cc, and 32 cc (8.3%; 1/12 each, respectively) while in two isolates (16.7%), no clonal complex was identified. Among the five MenW isolates, 22 cc (80%) was identified in four isolates, while in all MenY isolates ($n = 6$), 23 cc was identified. For the 2 MenA cases, 23 cc and 32 cc were identified, while in the single MenX case ST, 1135 was identified not assigned to any cc.

PorA variable region genotyping in 537 positive available samples tested revealed 29 combinations for the two variable regions VR1 and VR2 (Table 1). PorA variant P1.7-2,4, present in the 4CMenB vaccine, was found in 40 MenB isolates (7.6%), mostly associated to 41/44 cc.

FetA gene analysis revealed 24 different variable regions; variants F5-1 (30.5%), F3-3 (24%), and F1-5 (11.8%) most prevalent mostly associated to MenB.

Antimicrobial susceptibility

E-test was performed for 244 of 349 available isolates (105 isolates failed due to lack of appropriate growth). Most isolates (71.7%; 175/244) were highly susceptible to penicillin (MIC < 0.094 mg/L) while 18.9% (46/244) showed intermediate susceptibility (MIC 0.094–0.25 mg/L). Resistance (MIC > 0.25 mg/L) was recorded in 9.4% (23/244) of the isolates, observed mainly during the years 2013–2016 (74%; 17/23), and was associated to MenB (91.3%; 21/23) and MenC (8.7%; 2/23).

Most isolates (99.2%; 242/244) were susceptible to cefotaxime (MIC ≤ 0.125 mg/L) and all to ceftriaxone (MIC ≤ 0.125 mg/L). Low resistance rates (1.6%; 4/244) were observed to rifampicin (MIC > 0.25 mg/L), chloramphenicol (0.8%; 2/244, MIC > 4.0 mg/L), and ciprofloxacin (1.2%; 3/244, MIC > 0.03 mg/L).

Discussion

IMD surveillance in Greece through mandatory notification is nationwide and comprehensive. During the study period (2006–2016), 90% of notified cases were laboratory confirmed. IMD showed a similar downward trend compared to other European countries; the incidence rate decreased from 1.0 in 2006 to 0.5 in 2016 per 100,000 population [7]. Moreover, the overall annual notification rate (0.66/100,000) was lower than the average European incidence rate (0.77/100,000) for the same period [16].

In our study, incidence is highest in infants and young children with a second peak in adolescents/young adults, similar to that observed to the rest of EU/EEA countries, Australia and the USA [17]. Nonetheless, the present study showed a striking decrease of 73% in the incidence of confirmed cases in infants to 3.8 per 100,000, in line with other European countries according to incidence rates declined from 17.36 (2006) to 8.5 (2016) per 100,000 (16) for the same age group. A similar decrease was observed in the age group of 1–4 years (from 5.76 in 2006 to 2.69 in 2016) (16).

MenB remains the most common sero/genogroup in Greece since 1997 [10] accounting for 80% (annual range 65–92%) of the IMD cases; lower than observed in UK, Netherlands, Belgium, Ireland, and Spain [16], while the mean MenB incidence of 0.44/100,000 for the study period was slightly lower compared to EU/EEA countries (0.50/100,000) (16). Almost 30% of MenB cases in Greece occurred in children younger than 5 years of age, as compared to an estimate of 50% for Europe between 2008 and 2011 [18].

The incidence in MenC cases remained low throughout the study period and was among the lowest compared to other European countries [16], mainly connected to the initiation of MenC vaccination in the early 2000s.

Similarly, the incidence of MenW (0.06/100,000) was low, in contrast to UK (0.15/100,000) and Netherlands (0.37/100,000) in 2015–2016 [19] due to the rapid expansion of the hypervirulent MenW 11 cc clone which emerged in UK in 2013 accounting for 24% of IMD cases [20], while a similar increase was observed in the following years in The Netherlands, Norway, Spain, Sweden, Switzerland, and Germany [21]. Furthermore, the incidence of MenY in Greece remained low similar to the Eastern and South-eastern European countries, and in contrast to Northern European Countries (UK-Scotland, Switzerland, and Scandinavian countries) [22].

The CFR for confirmed cases was considerably lower (5.4%) than the mean CFR (8.6%) found in the EU/EEA

Table 1 Most common PorA VR1 and VR2 combinations among the sero/genogroups in Greece (2006–2016)

Sero/genogroup	PorA VR1/VR2 combination	number of samples	(%)
MenB	1.19–1,15–11	117	(25.1%)
	1.7, 16	84	18.0%
	1.22, 14	55	(11.8%)
	1.7–2, 4	40	(8.6%)
	All combinations	466	(100%))
MenC	1.5–1,10–8	9	(33.3%)
	All combinations	27	(100%)
MenW	1.18-,3	2	(28.6)
	All combinations	7	(100%)
MenY	1.5–1,10–1	10	(71.4%)
	All combinations	14	(100%)
Other sero/genogroups[a]	All combinations	23	(100%)
Total number of tested samples		537	

Identification was carried out in both meningococcal isolates and clinical material identified as *N. meningitidis*

[a] PCR-confirmed cases for other serogroups or for which genogroup was not identified

countries between 2006 and 2016. The CFR in Europe was stable for the last decade, although with significant variation among countries, the highest CFR being reported in Hungary (16.3% (2007) and 17.9% (2011)), Poland (15.2% in 2007), and Slovakia (40% in 2011) [16]. In a recent UK study, the CFR was slightly higher (6.9%) than in Greece [23] most probably due to the increase of MenW cases since 2013 [20].

The low CFR observed in our study may be attributed to several reasons. The awareness of IMD in Greece is high both at the physician and at the public level, thus leading to immediate reference by physicians and access by the public to tertiary hospitals and advanced care. The existing active surveillance of IMD cases enables the prompt detection of contacts and implementation of prevention and control measures including initiation of chemoprophylaxis where indicated. In addition, the inclusion of MCV4 vaccine in the national immunization schedule for routine vaccination of both children and adolescents would have an impact, and perhaps, any private prescriptions for MenB in children.

Hyperinvasive CCs are clonal complexes with particular epidemiological importance as there is an association between particular ccs and certain serogroups differing between countries. In our study, 269 cc (35%) and 32 cc (24%) were the most predominant clonal complexes associated with MenB, similar to Spain [24]. Although 162 cc was predominant in Greece during the time period 1999–2010 and its proportion was higher compared to other European countries [25], analysis revealed that a reduction was observed since 2011 with a simultaneous increase of the 269 cc. Low proportions of 41/44 cc cc (12%) were recorded in contrast to many European countries (England and Wales, Northern Ireland, Czech Republic, Poland, France, Germany, Norway, and Italy) where this particular cc is most prevalent [16].

Among the MenC cases, the hypervirulent 11 cc was predominant which was responsible for the outbreaks in the mid 1990s in Greece [26], similar to other EU countries such as Croatia [27], Czech Republic [28], and France [29]. Furthermore, 22 cc and 23 cc related to MenW and MenY, respectively, were found among the Greek isolates, while none of the MenW isolates was related to 11 cc.

Identification of PorA variants remains important as the protein is a major immunogenic component of several meningococcal vaccines under development. The most common combinations for PorA were P1. 19-1, 15-11, and P1.7,16 for the variable regions VR 1 and 2, respectively, while the PorA variant P1.7-2,4 present in the 4CMenB vaccine was present in 8% of the MenB positive samples and mainly associated to 41/44 cc.

Most isolates were susceptible to the antimicrobial agents tested. Resistance to ciprofloxacin, chloramphenicol, or rifampicin was rare, similar to previous studies [30]. The majority of the isolates were highly susceptible to penicillin.

In conclusion, IMD incidence rate in Greece was similar to other European Countries; yet, the mortality in Greece was lower. The striking decline in incidence of 50% over the 11-year period was reflected in younger groups, although MenB remained predominant. As meningococcal epidemiology is unpredictable and incompletely understood and IMD incidence is highly variable around the world, continued surveillance for monitoring emerging clones is recommended to assess epidemiological trends and plan vaccine policies.

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Authors' contribution Anastasia Flountzi (AF) analysis and interpretation of data, drafting of manuscript. Theano Georgakopoulou (TG) acquisition of data, analysis and interpretation of data, critical revision. Sooria Balasegaram (SB) analysis and interpretation of data, drafting of manuscript. Konstantinos Kesanopoulos (KK) acquisition of data. Athanasia Xirogianni (AX) acquisition of data. Anastasia Papandreou (AP) acquisition of data. Georgina Tzanakaki (GT) study conception and design, acquisition of data, critical revision.

Compliance with ethical standards

Ethics statements Ethic approval was not required since depersonalized data from notifiable disease registry and laboratory findings were used.

Conflict of interest The authors declare that they have no conflict of interest.

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