

# Twenty years of surveillance of Invasive Meningococcal Diseases in Puglia, Italy

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## Abstract

**Introduction.** The study aims to investigate the change in the burden of disease and the microbiological characteristics of Invasive Meningococcal Disease (IMD) in Puglia in comparison with overall incidence from 1994 through 2014.

**Methods.** Data are gathered in the frame of the National Surveillance System coordinated by the National Reference Laboratory (NRL) of the Istituto Superiore di Sanità.

**Results.** In Puglia, from 1994 through 2014, the average incidence of IMD was 0.2 per 100 000 inhabitants, below the national average value (0.33). IMD cases tended to be older than the other cases reported in Italy (median age 19 vs 16). The case-fatality rate was 20.4% in Puglia vs 13.3% in Italy. Serogroups B and C were most frequently identified. Serogroups C and Y presented a fairly clonal pattern, whereas serogroup B was genetically rather heterogeneous.

**Conclusion.** Surveillance systems are critical in monitoring any change in the epidemiology of IMD.

## Key words

- invasive meningococcal disease
- surveillance
- Puglia
- Italy

## INTRODUCTION

Invasive Meningococcal Disease (IMD) due to the human pathogen *Neisseria meningitidis* is a severe disease. This infection has different clinical consequences: meningitis, septicemia, arthritis, pneumonia, and pericarditis; moreover, long-term sequelae including learning difficulties, hearing impairment, neurologic, behavioral and motor deficits. Cases occur worldwide; although the majority of cases are sporadic, small outbreaks may occur [1]. The majority of cases are reported in the African meningitis belt, the sub-Saharan region where recurrent epidemic are mainly due to *N. meningitidis* of serogroup A [2]. In Europe, IMD cases due to serogroup B, C, Y and more rarely W, have been reported (<http://ecdc.europa.eu/en/publications/Publications/AER-VPD-IBD-2014.pdf>).

In Italy, a third of bacterial meningitis and sepsis is caused by *N. meningitidis* ([www.iss.it/mabi/](http://www.iss.it/mabi/)), and the serogroup B remains the leading cause of IMD in the pediatric and adolescent age groups. In our Country, the National Surveillance System of Bacterial Meningitis was established in 1994; in 2007, the national sur-

veillance was extended to all invasive bacterial diseases, including those due to *N. meningitidis*.

Routine immunization for meningococcal serogroup C disease has recommended at the national level since its introduction in 2005. However, vaccination policies in Italy show territorial heterogeneity [3]. Vaccination meningococcal C coverage against *N. meningitidis* at the 24<sup>th</sup> month of age is very high but needs to be improved through specific strategies. New conjugate vaccines against serogroups A, C, Y, and W are required to offer greater protection; in fact, serogroup Y increased in recent years, and rare cases of W have been also reported ([www.iss.it/mabi/](http://www.iss.it/mabi/)). Moreover, the Bexsero vaccine (the novel multicomponent MenB vaccine based on immunogenic proteins), which is now available, has shown, in the pre-licensure study [4], a predicted strain coverage for Italy of 87% (IC 70-93).

Puglia is one of the largest and most populated regions of southern Italy (4085 millions of inhabitants; [www.istat.it/it/popolazione](http://www.istat.it/it/popolazione)). It was one of the first Region that introduced universal vaccination with the MCC conjugate vaccine. In particular, the vaccination is offered to

all children aged 15 months. Vaccination with the conjugate vaccine against ACYW is also offered to children around 12 years old, as well as to people considered at risk (i.e., a co-payment regiment is provided for travelers to endemic countries). The MenB vaccination is offered free of charge to the 2014 cohort of infants, with a vaccination schedule of three doses administered at 3-4 and 6 months of age, and a booster dose at 15<sup>th</sup> months of age (DRG n. 958 del 20/5/2014 [www.sanita.puglia.it/portal/pls/portal/docs/1/2097490.PDF](http://www.sanita.puglia.it/portal/pls/portal/docs/1/2097490.PDF)). The vaccine coverage of MCC in Puglia, for the birth cohorts 2004-2011, increased from 48% in 2006 to 83% in 2013 ([www.epicentro.iss.it/temi/vaccinazioni/pdf/Istruttoria%20MENINGOCOCCO%20B.pdf](http://www.epicentro.iss.it/temi/vaccinazioni/pdf/Istruttoria%20MENINGOCOCCO%20B.pdf); [www.sincon.it/dettaglio\\_media.php?id=76](http://www.sincon.it/dettaglio_media.php?id=76)). Lower coverage rates for MCC or quadrivalent ACYW were reported in 11-12 years-old children, ranging from 48% in 2006 (birth cohort of 1995) to less than 50% in 2013 (birth cohort of 2002), with a peak in the 1998 (70%) birth cohort. The Regional Surveillance System for IMD in Puglia has been implemented starting from 2013. Moreover, the Regional Reference Laboratory provides molecular test to ascertain the etiology of the disease, thus improving the surveillance system.

The study aims to investigate the change in the burden of disease and the microbiological characteristics of IMD cases in Puglia in comparison with overall incidence from 1994 through 2014.

## MATERIALS AND METHODS

### Surveillance system

In Italy, all cases of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* are notified in the frame of the National Surveillance System of Bacterial Invasive Diseases at the ISS. Confirmed cases are reported to regional and national authorities through the Local Health Units. The 2008 EU case definition (revised in 2012), based on clinical and laboratory criteria, is used for surveillance purposes.

For each IMD case, epidemiological information and samples are sent to ISS [5]. Data are managed using a dedicated database. Microbiological analysis, including serogroup confirmation and typing data, are performed routinely on all reported cases and the strains are stored at -80 °C. Molecular testing is also performed by the Regional Reference Centre. Surveillance reports are published periodically ([www.iss.it/mabi/](http://www.iss.it/mabi/)).

### Microbiological analyses

Serogroup was confirmed by slide agglutination with commercial antisera (Remel Europe, Ltd, UK) or by multiplex PCR [6]. Susceptibility to penicillin G, rifampicin, ciprofloxacin and ceftriaxone was determined by E-test Method (bioMérieux SA, France) on Mueller-Hinton agar (Oxoid) supplemented with 5% of sheep blood. The breakpoints were those recommended by the European Committee on Antimicrobial Susceptibility Testing – EUCAST version 5.0, January 1, 2015 ([www.eucast.org/](http://www.eucast.org/)).

### Molecular typing

Chromosomal DNA was extracted by using the QIAamp DNA minikit (Qiagen, Hilden, Germany), ac-

ording to the manufacturer's instructions. Multilocus sequence typing (MLST), PorA and FetA typing were defined as described in <http://neisseria.org/>. The fine-type was as follows: capsular group: *porA* (P1). VR1, VR2: *fetA* VR: ST (cc). MLST data were analysed by eBURST, version 3, (<http://eburst.mlst.net>), [7]. eBurst analysis was set up referring to the most stringent setting of six identical out of the seven housekeeping gene alleles.

### Statistical analysis

The data were analysed using EpiInfo (version 3.4.5. July 30, 2013). Odds ratios (OR), 95% confidence interval were obtained to measure the strength of the association of variables of interest. Statistical differences were tested using standard tests.

## RESULTS

Over twenty years, from 1994 to 2014, 174 IMD cases, out of 4263 nationally reported ([www.iss.mabi](http://www.iss.mabi)) occurred in Puglia. The average incidence in the period was 0.2 cases per 100 000 inhabitants, lower than the national incidence rate (0.33 per 100 000 inhabitants). However, the regional incidence trend was consistent with the national one, with two peaks in 2000 and 2005, respectively, (Figure 1). Differently from what reported for the rest of Italy, an increase of cases was detected in Puglia in 2013, with an incidence of 0.46 cases per 100 000 inhabitants, higher than the overall incidence (0.27 per 100 000 inhabitants), (Figure 1) maybe to the implementation of the regional surveillance system. All the 19 cases occurred in 2013 were sporadic, except for 3 cases of unknown serogroup, with a geo-temporal clustering pattern, occurring from 20<sup>th</sup> to 27<sup>th</sup> of February 2013 in a small geographic area (around 20 km) of the Region.

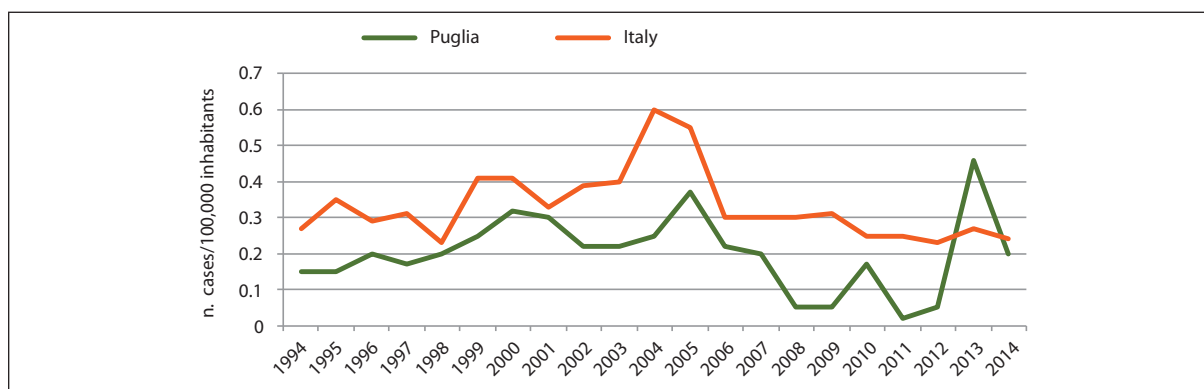
Consistently with the seasonal pattern observed in Italy in the period, also in Puglia most cases (44.8%) occurred during the January-March trimester.

In Puglia, patients tended to be older (median age 19 years vs 16 years in the rest of Italy), ranging from 1 month to 87 years with respect to cases reported in the rest of Italy. During the twenty years study period, the 22% of cases occurred in the age group from 5 to 14 years. The percentages in the other age groups (< 1, 1-4, 15-24, 25-44, 45-64, and > 64) were 7.7%, 12%, 15.5%, 16.1%, 16.7% and 10%, respectively.

However, since 2013, half of the IMD cases occurred among patients aged  $\geq$  45 years old (52%). Moreover, in the entire period, the IMD incidence among infants less than 1 year of age was higher than the overall population (1.95 per 100 000 inhabitants). The incidences in the other age groups (1-4, 5-14, 15-24, 25-44, 45-64, and > 64) were 0.7, 0.46, 0.28, 0.12, 0.12 and 0.1 per 100 000 inhabitants, respectively.

Not statistically significant difference was found between females (84) and males (90).

As in the rest of Italy, meningitis was the main clinical picture (62% of cases), followed by sepsis and meningitis plus sepsis. Of note, in the years 2013-2014, the frequency of meningitis in Puglia was significantly higher than in Italy (85% vs 43%,  $p < 0.05$ ).



**Figure 1**  
Trend of Invasive Meningococcal Disease incidence from 1994 through 2014 in Italy and in Puglia.

The outcome of the disease, available for 54 out of 174 cases, was fatal for 11 patients (OR1.67 in comparison to the overall Italian data). From 1994 through 2014, the case-fatality rate in Puglia was 20.4% vs 13.3% reported in Italy.

The serogroup was defined for 40 out of 174 invasive meningococcal cases. The serogroup distribution was as follows: 22 (55%) serogroup B, 13 (32.5%) serogroup C, 4 (10%) serogroup Y, and 1 (2.5%) serogroup W. The proportion of serogroup B and C was consistent with the national data, where these serogroups represented 59% and 33% of the cases, respectively. Three out of the 4 serogroup Y meningococci were isolated in 2013, in 2 teen-agers and 1 adult; no epidemiological link was found among them.

All the analysed meningococci were susceptible to rifampicin, ciprofloxacin, and ceftriaxone. Moreover, 47% showed a decreased susceptibility to penicillin G ( $MIC_{50}$  and  $MIC_{90}$  were 0.064 and 0.125mg/L, respectively).

### Molecular analyses

Molecular analysis, started in 2010, was performed on 16 out of 19 isolates/clinical samples received by the NRL (Table 1). Among serogroup B, ST-41/44 complex (cc41/44) was identified as the major clonal complex (71.4%). The remaining isolates belonged to ST-461 complex (cc461) and to a new sequence type ST-11119 (clonal complex unknown), corresponding to a new MLST profile occurred in 2014.

Four different sequence types (STs) were found in the cc41/44: ST-1403 (2 isolates), ST-3615, ST-41 and ST-414. The sequence types ST-41 and ST-414 were single-locus variants (SLV) at the eBurst analysis. Six different finetypes was identified of which B: P1.7-2,4: F1-5: ST-1403 (cc41/44) was found among two unrelated isolates.

The ST-11 complex (cc11) characterized serogroup C meningococci. Four out of them showed the same finetype C: P1.5-1,10-8: F3-6: ST-11 (cc11); two of these strains were isolated in January 2014 in the same city (S. Giovanni Rotondo).

Three serogroup Y meningococci, isolated in 2013, belonged to ST-23 complex (cc23), as already reported for the majority of serogroup Y isolated in the country, [8].

### CONCLUSIONS

The national surveillance system for invasive meningococcal disease provides a better understanding of the dimensions of the problem and of the impact of immunization strategies on the incidence of IMD.

In Puglia, the increasing incidence of IMD reported since 2013 can be in part due to the improvement of the efficiency of the surveillance system (*i.e.*, case detection) due to the use of more sensitive diagnostic tests.

Prior to 2005, serogroup C meningococci were responsible for most of the IMD; since that year, the epidemiology of IMD in Italy has changed, showing a decrease of serogroup C and a relative increase of serogroup B. The decline appears to be associated with the introduction and widespread use of the conjugate serogroup C vaccine in the country. The development of new vaccines against invasive meningococcal disease provides, in fact, important opportunities for reducing the burden of disease. Therefore, it will be extremely interesting to monitor the dynamic of invasive meningococcal disease over time, in order to evaluate the impact of the immunization programs.

The Puglia Region, one of the largest and most populated regions in the south of Italy, was one of the first to introduce the universal vaccination with the conjugate MCC vaccine. However, no major differences between regional and national data were found, with regard to the incidence of the disease and the main circulating serogroups (B and C). Some minor differences concerned the median age of infected patients, which was higher in Puglia (19 vs 16 year old in the rest of the country), and the case-fatality rate, which was about 1.5 time higher than the rest of Italy. We cannot exclude the higher case-fatality be due to more efficient data collection in the region, allowing for a more accurate count of real number of deaths.

The higher median age of the patients with IMD observed in Puglia could be partially explained by age-dependent nasopharyngeal carriage and/or to an indirect effect due to MCC vaccination [9]. However, the determinants of such difference remain undefined. The decreasing susceptibility to penicillin together with the decrease of the incidence rate is a phenomenon already described [9, 10]. The genetic characteristics of the circulating isolates in Puglia show the presence of

**Table 1**  
Microbiological and epidemiological data of 16 invasive meningococcal diseases cases, 2010-2014

Finetype [ <i>porA</i> (P1). VR1,VR2: <i>fetA</i> VR: ST (cc)]	Year	Month	City	Clinical picture	Age (years)
B: P1.7-35,4: F1-5: ST-3615 (cc41/44)	2010	January	S. Giovanni Rotondo	meningitis	7
B: P1.7-2,4: F1-5: ST-41 (cc41/44)	2010	May	S. Giovanni Rotondo	sepsis	6
B: P1.18,25: F1-84: ST-414 (cc41/44)	2010	December	Foggia	meningitis	8
B: P1.7-2,4: F1-5: ST-1403 (cc41/44)	2012	August	Foggia	meningitis + sepsis	17
B: P1.19-2,13-1: F3-9: ST-461 (cc41/44)	2013	June	Bari	meningitis	29
B: P1.7-2,13-2: F1-5: ST-11119 (UNK)	2014	February	Bari	meningitis	3
B: P1.7-2,4: F1-5: ST-1403 (cc41/44)	2014	August	S. Giovanni Rotondo	meningitis	60
C: P1.5-1,10-8: F3-6: ST-11 (cc11)	2013	October	Bari	meningitis	13
C: P1.5-1,10-8: F3-6: ST-11 (cc11)	2014	January	S. Giovanni Rotondo	sepsis	0
C: P1.5-1,10-8: F3-6: ST-11 (cc11)	2014	January	S. Giovanni Rotondo	meningitis	51
C: P1.5-2,nd: F1-6: ST-nd (cc11)	2014	April	Taranto	sepsis	63
C: P1.5-1,10-8: F3-6: ST-11 (cc11)	2014	May	Bari	meningitis	18
Y: P1.5-2,10-21: F2-13: ST-23 (cc23)	2013	March	Brindisi	meningitis	53
Y: P1.5-2,10-2: F2-13: ST-9253 (cc23)	2013	April	Foggia	meningitis + sepsis	18
Y: P1.5-2,10-2: F2-13: ST-023 (cc23)	2013	June	S. Giovanni Rotondo	meningitis	12
W: P1.18-1,3: F4-1: ST-3189 (cc22)	2011	March	Foggia	meningitis	14

UNK: not known  
nd: not determined

the major clonal complexes identified in the rest of the country, except for one case with a new Sequence Type. The serogroups C and Y presented a fairly clonal pattern, whereas serogroup B isolates were rather genetically heterogeneous.

Before concluding, some limits of the analysis need to be mentioned. Firstly, IMD cases are likely to be underestimated, since only laboratory confirmed cases are reported. Secondly, because of the lack of molecular data prior to the 2010, the circulation of the main hypervirulent meningococcal clonal complexes in Puglia and in the rest of the country cannot be compared.

In conclusion, a surveillance system including molecular diagnosis and typing of the circulating meningococci is essential to monitor any changes in the epidemiological pattern of invasive meningococcal disease due to immunization strategies adopted locally and nationally. Our data support the need for an harmonized vaccination schedule for MenB and Men ACYW conjugate vaccine through the country.

#### Author's contribution statement

SP designed the purpose of this article and drafted the manuscript; FC and NA contributed in the microbiological analyses; DTA, LM isolated the meningococci and collect the data; DRAL, LD carried out the laboratory analyses on strains isolated in Puglia; MD analyzed the data on vaccine coverage in Puglia; CM reviewed critically the manuscript.

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#### Conflict of interest statement

There are no potential conflict of interest or any financial or personal relationships with other people of organization that could inappropriately bias conduct and findings of this study.

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