Persistent occurrence of serogroup Y/sequence type (ST)-23 complex invasive meningococcal disease among patients aged five to 14 years, Italy, 2007 to 2013

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In Italy, the incidence of invasive meningococcal disease (IMD) has remained stable since 2007 (around 0.3 cases/100,000 inhabitants). However, as reported for other European countries, an increase of serogroup Y *Neisseria meningitidis* has been observed. In this study we report IMD cases from 2007 to 2013 in Italy and investigate the clinical and epidemiological features of cases affected by serogroup Y. Molecular characteristics of serogroup Y strains are also described. During the study period, the proportion of IMD cases due to serogroup Y increased, ranging from 2% in 2007 to 17% in 2013 (odds ratio (OR): 8.8), whereby the five to 14 years age group was mostly affected ($p < 0.001$). Overall 81 serogroup Y IMD cases were identified, with a median age of 18 years, ranging from three months to 84 years. Of the 81 respective patient samples, 56 were further subject to molecular typing. The sequence type (ST)-23 complex (clonal complex (cc)23) was predominant among serogroup Y meningococci (54/56 samples), and included nine different STs. Presumably, ST-23 was the founding genotype, with all the other STs presenting as single-locus variants. All cc23 isolates analysed harboured mutations in the *lpxL1* gene; however, no associations among *lpxL1* mutations, ST and age group were identified. Overall, these findings generate scientific evidence for the use of the quadrivalent meningococcal conjugate vaccine in the five to 14 years age group.

Introduction

Since the 1990s, some significant changes in serogroup Y *Neisseria meningitidis* (MenY) epidemiology have been reported worldwide. During the beginning of this period, an increase of MenY cases was observed in the United States (US) [1], as well as in Latin American countries [2]. In Colombia the proportion of MenY cases peaked at 50% in 2006 [3]. MenY incidence increased also in Europe [4,5]. In France, MenY accounted for only 5.5% of all cases of invasive meningococcal disease (IMD) in 2010 but for 10% in 2013 [6]. In Norway and in Finland, MenY represented respectively 31% and 38% of all cases reported in 2010 [7,8]. MenY emergence was observed also in Sweden, with an increase of the incidence from <0.05 cases per 100,000 inhabitants in 2000 to 0.23 in 2010 [9].

In Italy, although the incidence of IMD remained stable since 2007 (around 0.3 cases/100,000 inhabitants; data from National Surveillance System <http://www.iss.it/mabi/>), some changes were noted in the frequency distribution of specific meningococcal serogroups. In our country, similarly to other European countries, serogroup B and C are responsible for the majority of IMD cases, however, an increase in the proportion of MenY has been observed, from 4% before 2005 to 7% in 2006 [10]. Some changes in the distribution of serogroups may be due to the introduction of the meningococcal serogroup C conjugate (MCC) vaccination (between 2005 and 2007), which has been included in the 2012 to 2014 national immunisation plan (NIP), in accordance with regional policies; the

FIGURE 1

Annual proportion of invasive meningococcal disease (IMD) cases due to serogroups B, C, Y, W, Italy, 2007–2013

The serogroups B, C, W and Y are represented by different colours as specified in the legend.

vaccine is recommended to all children between 13 and 15 months of age, and to 11 to 18 year-old individuals, if not previously vaccinated, and to those belonging to risk categories [11].

The aims of present study were: (i) to describe the trend of MenY IMD cases from 2007 to 2013 in Italy and (ii) to investigate the clinical and epidemiological features and the molecular characteristics of MenY cases.

Methods

Bacterial isolates

In Italy, notification of all cases of IMD is mandatory. Clinical and epidemiological information and meningococcal isolates are collected in the frame of the National Surveillance System coordinated by the National Reference Laboratory (NRL) of the Istituto Superiore di Sanità.

Every year, the NRL receives an average of 75% of the meningococci isolated by local hospital laboratories throughout the country. Epidemiological and microbiological data for each IMD case are managed using a dedicated database. Local laboratories send the isolates to the NRL, where they are stored at -80 °C before complete microbiological characterisation.

Microbiological analyses

Serogroup is confirmed by slide agglutination with commercial antisera (Remel Europe, Ltd, United Kingdom) or by multiplex polymerase chain reaction (PCR) [12]. Susceptibility to ceftriaxone, ciprofloxacin, penicillin G and rifampicin is determined by E-test method (bioMérieux SA - France) on Mueller-Hinton agar (Oxoid) supplemented with 5% of sheep blood. The breakpoints are those recommended by the European Committee

on Antimicrobial Susceptibility Testing – EUCAST version 5.0, 1 January 2015 (<http://www.eucast.org/>).

Molecular typing

Chromosomal DNA is extracted by using the QIAamp DNA minikit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Multilocus sequence typing (MLST), porin A (PorA) and ferric enterobactin transport protein A (FetA) typing are defined as described in <http://neisseria.org/>. The finetype is identified as follows: capsular group: *porA* (P1). Variable region (VR)₁, VR₂: *fetA* VR: sequence type (ST) (clonal complex (cc)). The *lpxL1* gene amplification and sequencing were performed as indicated by Ladhani et al. [13].

eBurst

MLST data were analysed by eBURST, version 3, (<http://eburst.mlst.net>) [14]. eBurst analysis was set up referring to the most stringent setting of identity of alleles in six of the seven housekeeping genes.

Statistical analysis

The data were analysed using EpiInfo (version 3.4.5, July 30, 2013). Odds ratios (OR), 95% confidence intervals (CI) and p values, were obtained to measure the strength of the association between serogroup Y and other variables. Statistical differences were tested using standard tests (i.e. chi-squared and chi-squared for trend); the level of statistical significance is set at $p < 0.05$.

Results

From 2007 to 2013, a total of 1,157 IMD cases were detected in Italy, with an average annual incidence of 0.27 cases per 100,000 inhabitants. The annual proportions of IMD cases attributable to the principal serogroups (B, C, Y, W) by year are shown in Figure 1. The serogroup was obtained for 902 cases (78%), including 514 (57%) for serogroup B, 253 (28%) for serogroup C, 81 (9%) for serogroup Y, 23 (3%) for serogroup W, 10 (1%) for serogroup A, and 21 (2%) for other serogroups. The proportion of MenY IMD cases increased over the years, ranging from 2% in 2007 (3/134 cases) to 17% (20/119 cases) in 2013 (the OR for 2013 compared with 2007 was 8.8, $p < 0.001$).

The median age of the patients infected by MenY was 18 years, ranging from three months to 84 years; patients infected with serogroup Y appeared to be older than patients infected by other serogroups (18 years vs 16). Overall, 35% (28/81) of MenY cases occurred in the age group comprising five to 14 year-olds, and this age group was the most affected since 2008.

The distribution of cases attributable to MenY and to other serogroups by single variable of interest is shown in the Table. In the study period, among all patients in the age group five to 14 years, almost 20% (28/141) were infected with MenY; patients in this age group were more likely to be infected with MenY compared

FIGURE 2

Distribution of sequence types (STs) belonging to clonal complex (cc) 23 (n=54)^a among a subset of samples from serogroup Y *Neisseria meningitidis* cases, which were typed, Italy, 2007–2013

^a During the study period, a total of 56 samples from serogroup Y invasive meningococcal disease cases were analysed. Of these, samples from 54 cases belonged to cc23 and are depicted in the figure according to ST.

with all the other age groups (OR:3.3; 95% CI:1.94–5.59), (data not shown).

Differences with regard to the risk of being infected with MenY according to sex were not statistically significant.

As expected, meningitis and septicaemia represented the main clinical pictures among IMD cases. There was no significant difference in MenY infection among cases with different clinical presentation. However, among patients with MenY, an increase of septicaemia, from 19% (3/16 cases) in 2011 to 42% (8/19 cases) in 2013 was observed. The respective proportion of serogroup Y in the south and the islands was higher than in northern and central Italy, with an OR of 2.18 (Table). The outcome, available for 52 of 81 cases, was fatal for three patients: two women (67 and 45 years-old) and a six year-old child with sepsis, corresponding to a case fatality ratio of six per cent.

A total of 59 samples from the 81 serogroup Y IMD cases, were received by the NRL, allowing further typing. Moreover bacterial isolates derived from 50 patients respectively, were also obtained, and could be used for antibiotic susceptibility testing. All MenY isolates retrieved from cases were susceptible to ceftriaxone, ciprofloxacin and rifampicin. Moreover, 21 of 50 isolates showed a decreased susceptibility to penicillin G (minimum inhibitory concentration (MIC)₅₀ and MIC₉₀ were 0.047 and 0.125mg/L, respectively).

Molecular analyses

Molecular analyses were performed on 56 of 59 MenY samples received by the NRL. MLST identified the ST-23/clonal A3 complex (cc23) as the major cc (54/56 samples). The remaining two belonged to ST-167 complex (cc167), one was ST-767 and one was ST-884. Nine different STs were found in the cc23: ST-23 (30 samples), ST-9253 (8 samples), ST-3171 (7 samples), ST-2692 (3 samples), ST-1655 (2 samples), ST-2533 (1 sample), ST-9326 (1 sample), ST-10348 (1 sample) and ST-10098 (1 sample) corresponding to a new MLST profile defined for the first time in this study (Figure 2). ST-23 was detected during the whole period, with 10 samples in the age group comprising five to 14 year-olds. ST-1655, ST-10098 and ST-10348 appeared in Italy for the first time in 2012.

eBURST analysis clustered all STs belonging to the cc23 in a same group. The ST-23 was determined to be the founding genotype, with all others STs as single-locus variants (SLV).

PorA typing

Among the 56 samples which were typed, *porA* VR1 identified two different types: P1.5–2 in 45 samples and P1.5–1 in 11 samples (20%). *porA* VR2 identified eight different types, including P1.10–2 (35 samples), P1.10–1 (8 samples), P1.2–2 (8 samples), and P1.10–4, P1.10–8, P1.10–28, P1.10–92, P1.13–2 as singletons. The 5–2, 10–2 was the *porA* VR1, VR2 combination more frequently detected (35 samples).

FetA

F2–13 was present in 28 samples, F4–1 in 13 and the F5–8 in eight. Moreover, seven *fetA* types (F1–3, F1–12, F1–15, F1–23, F1–80, F2–9, F5–8) were identified as single samples.

Finetypes

A total of 25 different finetypes was identified. The two main were Y: P1.5–2,10–2: F2–13: ST-23 (cc23) (16 samples) and Y: P1.5–2,10–2: F2–13: ST-9253 (cc23) (7 samples). The former one appeared in 2009 and increased through 2013; differently, the latter finetype decreased from 2008 through 2012.

lpxL1

All 56 serogroup Y samples were analysed for the *lpxL1* gene however the gene amplification using the existing primers failed for eight samples. These belonged either to the finetypes Y: P1.5–1,2–2: F5–8: ST-23 (cc23) or Y: P1.5–1,2–2: F5–8: ST-3171 (cc23).

All cc23 samples analysed (n=48) harbour a mutation in the *lpxL1* gene. In particular, 38 were *lpxL1* type XVII, seven type VI, two type V and one type XVI. However, no associations between a specific *lpxL1* type, ST and age group were identified. Moreover, no differential association with the clinical picture of meningitis and septicaemia was found. Isolates belonging to cc167

TABLE

Epidemiological and clinical data of patients with invasive meningococcal disease due to serogroup Y compared with other serogroups, Italy, 2007–2013 (n=902)

Variable	Serogroup Y patients (N=81) n (%)	Other serogroups patients (N=821) n (%)	OR (95% CI)	Total patients (N=902) n (%)
Age group in years ^a				
<5	8 (10)	274 (33)	1	282 (31)
5–14	28 (35)	113 (14)	8.49 (3.56–20.91) ^b	141 (16)
15–24	12 (15)	166 (20)	2.48 (0.92–6.78)	178 (20)
≥24	33 (41)	266 (32)	4.25 (1.84–10.17) ^b	299 (33)
Sex ^a				
Male	45 (56)	420 (51)	1.19 (0.73–1.93)	465 (52)
Female	36 (44)	399 (49)	1	435 (48)
Clinical picture				
Meningitis	44 (54)	371 (45)	1.21 (0.69–2.15)	415 (46)
Septicaemia	22 (27)	225 (27)	1	247 (27)
Meningitis + septicaemia	14 (17)	223 (27)	0.64 (0.30–1.35)	237 (26)
Other	1 (1)	2 (1)	NA	3 (1)
Geographical area				
North	44 (54)	541 (66)	1	585 (65)
Centre	18 (22)	173 (21)	1.28 (0.69–2.35)	191 (21)
South and islands	19 (23)	107 (13)	2.18 (1.18–4.029) ^b	126 (14)

CI: confidence interval; NA: not applicable; OR: odds ratio.

^a Age and sex were unknown in two cases.

^b $p \leq 0.01$.

showed a *lpxL1* sequence identical to the reference (GenBank accession number: AE002098.2).

The *lpxL1* genotype was analysed in a subsample of non-serogroup Y meningococci. Among 20 serogroup C strains only one showed the mutation type III in *lpxL1* gene.

Discussion

As already reported, a stable increase of MenY cases has been observed in Italy since 2004 [10]. Noteworthy, the proportion of MenY among IMD cases increased almost eight times between 2007 (2%) and 2013 (17%).

Previous studies reported that, relative to other *N. meningitidis* serogroups, MenY is usually found in older patients [15–17]. Nevertheless, recent data analyses from several countries yield conflicting results as to the principal age groups affected by MenY: 20 to 29 years in Sweden (2000–2010) [9], ≥45 years in England and Wales (2007–2009) [13] and <5 years in South Africa (2003–2007) [18]. In Italy, the most affected age group comprised 45 to 64 year-olds until 2007, shifting to the five to 14 years age group from 2008 onwards; in this regard, from 2007 to 2013, ca 20% of patients in the latter age group were affected by MenY.

Of note, since 2011, an increase of septicaemia cases attributed to serogroup Y was observed. Overall, the case fatality ratio among IMD cases caused by serogroup Y was six per cent. As already reported [10], a

high proportion (42%) of MenY isolates with decreased sensitivity to penicillin was found.

Several reports indicated the cc23 as one of the most frequently detected in invasive MenY cases: in particular, it was responsible for an increase of IMD incidence in the 1990s in the US [1] and was associated with 94% of serogroup Y meningococci isolated between 2000 and 2005 [19]. From 1999 to 2003, in Canada 65.7% of invasive MenY strains were cc23 [20], whereas in Taiwan this cc characterised 11 of 13 MenY causing disease between 2001 and 2002 [21]. In South Africa, during the years 2003 to 2007, 11% of invasive MenY belonged to cc23 [18]. In Europe, during the 1990s, the cc23 was isolated more frequently from healthy carriers than from invasive meningococcal cases [22,23]. Nevertheless, in Sweden, from 2000 to 2010, the cc23 was identified in the three major clones responsible for the increased number of IMD cases [9] and in England it was found in the 56% of MenY causing IMD during the years from 2007 to 2009 [13]. In Italy, cc23 was the main cc among invasive MenY: it was detected in 89% of MenY samples from 1998 to 2006 [10], and in 54 of 56 (96%) of samples from 2007 to 2013. However, the identification in this study of 25 different finetypes suggests that more than a single strain is responsible for the MenY increase in Italy.

All the cc23 isolates analysed in this work harboured a mutation in the *lpxL1* gene, and in particular, the mutation XVII was the most frequently found (79%). Several

studies have demonstrated the presence of *lpxL1* mutations in *N. meningitidis* carrier strains cc23 and in meningococci isolated from cases of chronic meningococcaemia and meningitis [13,24-27]. In contrast, as shown from the results reported here, MenY cc23 with a mutated *lpxL1* was associated indifferently with meningitis or septicemia.

In conclusion, in Italy, IMD due to serogroup Y is steadily increasing, especially among five to 14 year-old patients, with predominance of isolates belonging to cc23 and harbouring *lpxL1* mutation. Overall, these results have significant public health implications. They support the potential utility of vaccination with the quadrivalent-meningococcal vaccine (ACWY) and/or the opportunity of a booster dose with this vaccine among children and young adolescents previously immunised with the MCC vaccine. More than 10 years since the beginning of vaccination with the MCC vaccine, there is evidence of a different epidemiology of IMD in Italy. The results are consistent with those of other studies that reported an increase of MenY and, more recently, of MenW infections [6,28] and provide further information which can be used to decide if and when the quadrivalent vaccination should be introduced. The quadrivalent meningococcal vaccine (ACWY) is safe and immunogenic; however, the cost-effectiveness of a booster with MCC vs the latter vaccine is still debated. In Italy, the use of quadrivalent vaccine is currently recommended for people at risk and for people who live in or travel to countries where meningococcal disease is hyperendemic or epidemic; this policy is likely to change. In fact, the dynamic nature of IMD epidemiology is well known [29]. In this respect, monitoring changes in the trend of the different serogroups and the microbiological features of meningococci is key to generate scientific evidence which is essential for producing appropriate vaccine recommendations.

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Conflict of interest

None declared.

Authors' contributions

Cecilia Fazio provided insight on microbiological investigation and drafted the manuscript. Arianna Neri contributed in the molecular analyses and provided insight into interpretation of results. Giovanna Renna and Paola Vacca carried out the laboratory analyses. Raffaele Antonetti, Anna Maria Barbui, Laura Daprai, Paolo Lanzafame, Lucia Rossi, Iolanda Santino, Carlo Tascini and Caterina Vocale were involved in the invasive meningococcal diseases at the local level. They were in charge of the data collection and management. Paola Stefanelli designed the purpose of this article and drafted the manuscript. All authors participated in the drafting and revision of this manuscript and gave their final approval of this version.

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