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SHORT ARTICLE

Diversity of *Neisseria meningitidis* invasive isolates in Italy in the period 2008-2010

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Key words

Neisseria meningitidis • Molecular characterization • Invasive disease

Summary

In the period 2008-2010, 309 Neisseria meningitidis, isolated in Italy within the National Surveillance of the Invasive Meningococcal Diseases, have been tested for their phenotypic and genotypic characteristics. The main results obtained are: (a) an increase of the strains of serogroup B and a decrease of serogroup C; (b) a phenotypic and genotypic variability of the ST-41/44 clonal complex, the most frequently isolated among serogroup B strains; (c) a decrease of ST-8 clonal complex among serogroup C meningococci whereas strains belonging to ST-11 clonal complex are nowadays the most frequently isolated.

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Introduction

Neisseria meningitidis, is the aetiological agent of invasive meningococcal disease (IMD), characterized by two main clinical pictures: meningitis and sepsis. Meningococcal disease occurs worldwide in both endemic and epidemic forms. Humans are the only natural reservoir of meningococcus. As many as 10% of adolescents and adults are asymptomatic transient carriers of *N. meningitidis*, most strains of which are not pathogenic [1]. Of the 13 recognized meningococcal serogroups, 5 (A, B, C, Y, and W135) are responsible for the majority of the disease [2].
The epidemiology of meningococcal disease varies across the world [3]. In Europe and other industrialized countries, serogroups B and C are the major causes of invasive meningococcal disease with serogroup B predominantly isolated from individuals under 20 years of age. The annual incidence of confirmed cases of invasive meningococcal disease ranges from 0.13 to 3.01 per 100,000 inhabitants in different countries [4].
In Africa, serogroups A and X are the most frequently found as responsible of invasive meningococcal diseases [5].
Currently, different vaccine formulations using polysaccharides are able to prevent meningococcal cases of serogroups A, C, Y and W135, but not against serogroup B, since the B polysaccaride resembles the human neural cell adhesion molecules, resulting in poor immunogenicity response. Surface-exposed protein antigens, GNA2132 or Neisserial heparin-binding antigen (NHBA) fused with GNA1030, GNA2091 fused with factor H binding protein (fHbp), Neisserial adhesin A (NadA), and outer membrane vesicles (OMV), are the components of the next generation vaccines against serogroup B meningococci, under registration and of next release in the market [6].
Italy is currently one of the European countries with the lowest incidence, about 0.3 cases per 100,000 inhabitants per year [7]. The two main serogroups are B and C, with an increase of serogroup B since 2006.
The present study refers data collected from 2008 through 2010 describing the phenotypic and genotypic characteristics of meningococci from invasive disease. Changes in predominant serogroups and genotypes of meningococci are also here described.

Materials and methods

All strains received at the National Reference Laboratory (NRL) of the Istituto Superiore di Sanità were subcultured to confirm the serogroup by slide agglutination with commercial antisera (Remel Europe, Ltd, UK). Serotypes and serosubtypes were determined by standard whole-cell ELISA with monoclonal antibodies (purchased from NIBSC, UK) [8].
Susceptibility MIC values for penicillin, rifampicin, ciprofloxacin, and ceftriaxone were determined by Etest method (bioMérieux, Italy), according to the manufacturer's instructions.
The breakpoints were those in agreement with EUCAST breakpoints, version 2.0, January 1, 2012 [9].
Molecular characterization by MLST (Multilocus Sequence Typing), and *por*A VRs (Variable Region) typing, were performed on 188 meningococci isolated in the study period. Primers, determination of sequence alleles and designation of sequence types were those described on the MLST website [10, 11]. Variable regions (VRs) 1 and 2 were submitted to the *N. meningitidis* PorA variable regions database [12].
All data were managed and analyzed with Epi-Info version 3.3.2.

Results

Bacterial strains

Within the National Surveillance System of Invasive Meningococcal Diseases, the NRL received 60% of all *N. meningitidis* strains isolated by local hospital laboratories from January 2008 through December 2010.

Phenotypic characterization

A total of 167 strains were serogroup B, 103 serogroup C, 20 serogroup Y, 11 serogroup W135, 4 serogroup A, 1 serogroup X, and three were non-serogroupable. Figure 1 shows the distribution of serogroups by years. Serogroup B replaced serogroup C since 2006 and the proportion increased up to 58.7% in 2010, while, serogroup C decreased to 24%.

A significant increase in serogroup Y isolates has been also observed over the last three years (2.8% in 2008 vs 12% in 2010).

The most common phenotypes identified within serogroup B isolates were: B:15:P1.4 (14.6%) and B:NT:P1.14 (7.1%).

Among serogroup C the most common phenotypes identified were: C:2a:P1.5 (31.1%), C:2b:P1.2,5 (14.6%) and C:NTNST (9.7%).

A total of 275 meningococci were analyzed for the antimicrobials susceptibility. All isolates were susceptible to ceftriaxone; 3 isolates were resistant to rifampicin with MIC = 32 mg/L; 2 were resistant to ciprofloxacin with MIC = 0.19 mg/L and MIC = 0.064 mg/L, respectively. Notably, the 46.5% of the meningococci showed a reduced susceptibility to penicillin with a MIC range of 0.094-0.19 mg/L and one isolates was resistant (MIC = 0.5 mg/L) according to the most recent breakpoint [9].

Genotypic analysis

Table I summarizes the molecular characterization of 188 meningococci. MLST revealed a total of 17 already known clonal complexes (cc_s) and 56 different Sequence Types (STs). The ST-41/44 cc was the most common, with 24 different STs and 62 (96.8%) serogroup B isolates. This clonal complex remained stable among serogroup B (55%) from 2008 through 2010.

The ST-11 cc was the most important among serogroup C (36 isolates out of 38) during the 3 years period (Tab. I). The ST-8 cc and the ST-334 cc were identified exclusively among serogroup C isolates. In particular, ST-8 cc is one of the most common identified until 2008, and then it decreased in favour of others clonal complexes as the ST-334 cc, accounting for 30.8% of the serogroup C isolates.

MLST showed the highest heterogeneity among serogroup B (Tab. I). Moreover, ST-865 cc and ST-162 cc have been rapidly increased: from 3% in 2008 to 7% in 2010, and from 6% in 2008 to 9.5% in 2010, respectively (data not shown).

Fig. 1. Distribution of meningococcal serogroups detected from IMD cases from 2008 to 2010.

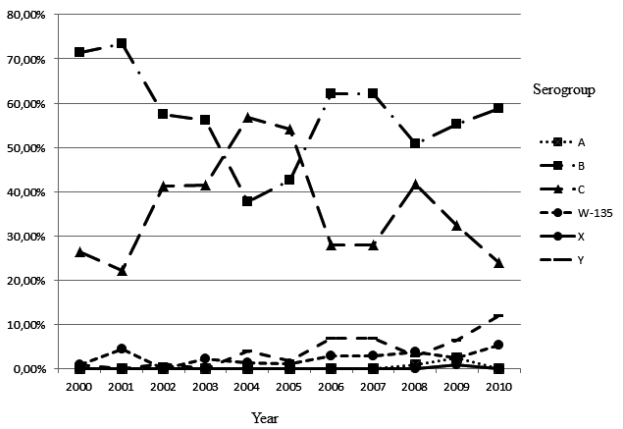
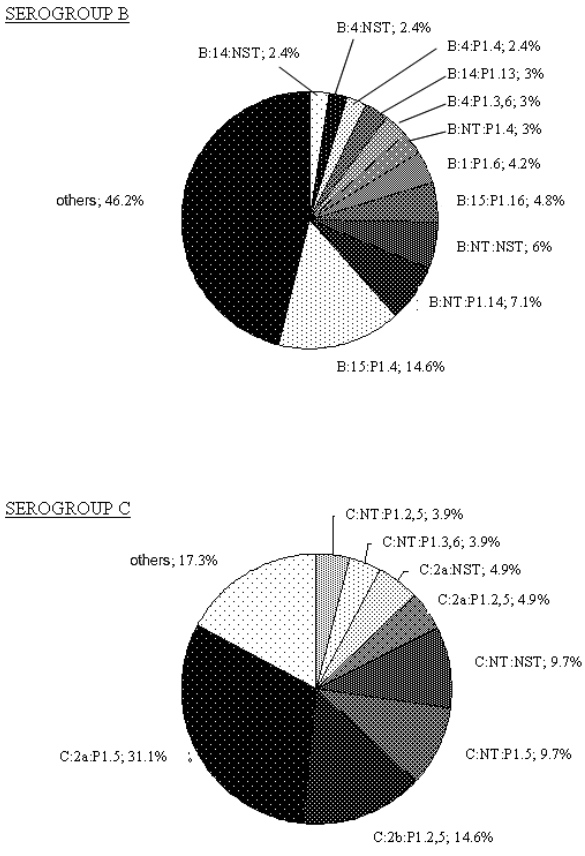


Fig. 2. Percentage of phenotypes belonged to serogroups B and C meningococci isolated in Italy from 2008 to 2010.



The ST-23 cc was predominant among serogroup Y strains whereas only 1 serogroup Y strain belongs to ST-167 cc (Tab. I).

The P1.7-2,4 PorA variants represented the 36% of serogroup B strains belonging to ST-41/44 cc and P1.5,2 PorA variants represented the 39.4% of serogroup C/ ST-11 cc.

Tab. I. Distribution of clonal complexes within 188 invasive meningococcal isolates collected from 2008 through 2010 in Italy.

Clonal complex	No. of STs	No. of isolates	Serogroup(s) (no. isolates)	Most common PorA type[a] (no. of isolates)
ST-41/44	24	64	B(62) C(1) Y(1)	P1.7-2, 4 (23)
ST-11	1	38	C(36) B(1) W135(1)	P1.5, 2 (15)
ST-8	2	14	C(14)	P1.5, 2 (7)
ST-32	6	12	B(12)	heterogenous
ST-23	3	11	Y(11)	P1.5-2, 10-2 (4)
ST-162	1	9	B(9)	P1.22, 14 (9)
ST-269	5	7	B(6) C(1)	P1.19-1, 15-11(3)
ST-334	1	6	C(6)	P1.7-4, 14-6 (6)
ST-461	2	5	B(5)	heterogenous
ST-865	1	5	B(5)	P1.21, 16-36(2)
ST-22	2	5	W135(5)	P1.18-1, 3(5)
ST-5	2	4	A(4)	P1.20, 9 (2)
ST-213	2	4	B(4)	P1.22, 14 (3)
ST-35	1	1	B(1)	P1.22-1, 14(1)
ST-167	1	1	Y(1)	P1.5-1, 10-8(1)
ST-18	1	1	B(1)	P1.22, 14(1)
ST-60	1	1	B(1)	P1.21, 16(1)

[a] PorA type=VR1, VR2.

Discussion

Invasive infections caused by *N. meningitidis* are a serious public health problem worldwide, given the ability to spread easily and quickly. Public health management of the disease requires bacterial typing information, by knowing the epidemiological and microbiological characteristics of strains isolated throughout the country.
Meningococcal disease is characterized by a remarkable variation in the incidence and serogroup distribution [13]. Although the incidence of IMD in Italy has been reported of a mean value of 0.27 per 100,000 inhabitants [7], meningococcal serogroups showed a dynamic evolution. Generally, meningococcal serogroups define a change between C and B serogroups every 3-4 years. What we observed in the last 3 years was a rapidly decline of serogroup C meningococci probably due to the primary vaccination of infants adopted by the majority of the Italian Regions (17 of 21) [14].
As reported in the U.S.A. [15] and England [16], serogroup Y increased significantly; this trend was also observed in Italy accounting for 2.8% in 2008 and 12% in 2010.
Typing results of the Italian strains showed a wide range of phenotypes among serogroup B compared to serogroup C. In particular, the two most prevalent phenotypes were B:15:P1.4, as the predominant since 1999, and B:NT:P1.14, more recently identified [17].
The majority of serogroup C were C:2a:P1.5,2. These strains were hyper-endemic and responsible for an increased number of fatal cases of septicemia and for two clusters of IMD in young adults in the North of Italy [18]. In addition, the results revealed a proportion of strains belonging to C:NT:NST (9.7%), suggesting a lower power of discrimination of the monoclonal commercial antibodies possibly due to recent changes of these surface proteins.
We found a strong association between some clonal complexes and serogroups, as reported in previous studies [13]. ST-41/44 cc was significantly associated with serogroup B, while the ST-23 cc showed a strong association with serogroup Y. It is also worth mentioning that serogroup C meningococci, belonging to ST-11 cc is the most frequently identified (61%) which has replaced the previously predominant ST-8 clonal complex (23%).
PorA type P1.7-2,4 was detected in 36% of serogroup B meningococci which show a high variability.
Few cases due to resistant strains to ciprofloxacin and rifampicin have been found and almost half of strains (46.5%) showed a reduced susceptibility to penicillin. Although rifampicin is the antibiotic of choice in the prophylaxis of close contacts there is a very low rate of resistance reported in the literature [19, 20].
The results of the present study suggest that a careful monitoring of phenotypical and genotypical changes among the circulating meningococci is required, especially after the introduction of MenC vaccine and before the introduction of MenB vaccines in the country.

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