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Genome-based study of a spatio-temporal cluster of invasive meningococcal disease due to *Neisseria meningitidis* serogroup C, clonal complex 11

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Summary *Objectives:* To describe a spatio-temporal cluster of invasive meningococcal disease (IMD) due to serogroup C meningococci, occurred in a restricted area of Tuscany between January and October 2015, and the results of whole genome sequencing (WGS).
Methods: Surveillance activities and public health measures were implemented in the Region. Bacterial isolates from IMD cases were characterized by the National Reference Laboratory of the Istituto Superiore di Sanità (ISS), and WGS was performed on available strains. The kSNP software was used to identify core genome SNPs.

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Results: Overall, 28 IMD cases due to meningococcus C were identified up to 31st October, 2015. Of them, 26 were due to meningococcus C:P1.5-1,10-8: F3-6:ST-11 (cc11) and 2 to C:P1.5-1,10-8: F3-6:ST-2780 (cc11). WGS of 13 meningococci isolated during the outbreak occurred in Tuscany in 2015 showed higher similarity when compared with those of 47 C: P1.5-1,10-8: F3-6:ST-11 (cc11) invasive strains from sporadic cases previously detected in Italy.
Conclusions: A highly aggressive meningococcal C strain was involved in the cluster of severe IMD occurred in Tuscany, a Region with high vaccine coverage among children. Whether this was due to low herd immunity related to the short duration of vaccine protection needs further investigation.

Introduction

Italy is considered a country at low incidence of invasive meningococcal disease (IMD), with predominance of the meningococcal B and C capsular serogroups.[1] However, IMD cases due to serogroup C (MenC) declined after vaccine introduction[2] consistently with the trends observed in other EU countries.[3–7] In Italy, the use of the meningococcal C conjugate (MCC) vaccine for children 13-to-15 month olds and for adolescents 11-to-18 year-olds was recommended by the Italian National Immunization Plan for the years 2012/2014.[8] However, few Regions, like Tuscany, introduced the MCC vaccine in the Regional immunization schedule since 2005. Initially, the regional plan provided three doses at 3, 5, and 13 months of age, switching to a single dose at 13 months, with a catch-up immunization until 6 years of age, in 2008.

In some Regions, a booster dose with the conjugate vaccine against meningococcal A, C, W and Y was provided to adolescents, people at risk, and travelers to endemic areas.

A wide variation in vaccination coverage between Italian Regions was observed, with values ranging between 42.72% and 88.28%; in Tuscany the coverage was around 87.2% in the birth-cohort of the year 2012 (http://www.salute.gov.it/imgs/C_17_tavole_20_allegati_iitemAllegati_2_fileAllegati_itemFile_0_file.pdf). Unfortunately, data on immunization coverage for teens and at-risk groups were not available.

Despite the extended use of the vaccine, outbreaks and smaller clusters of MenC belonging to the ST-11 clonal complex (cc11) (MenC:cc11) have continued to occur in Europe; in particular, the finetype C:P1.5-1,10-8:F3-6:ST-11 (cc11) was responsible of outbreaks in France and in Germany.[9,10]

Since meningococci belonging to cc11 are considered highly virulent and able to cause outbreaks, it is important to identify and characterize this aggressive, vaccine preventable strain. Hereby, we describe a cluster of cases of IMD due to MenC:cc11 occurred between January and October 2015 in Tuscany. In particular, we describe the epidemic dynamics and the characteristics of the strain involved in the outbreak up to that date. To this end, the results of the whole genome sequencing (WGS), and the geo/temporal distribution and characterization of the isolates compared with other strains of the same finetype identified in different geographical areas are presented.

Materials and methods

IMD surveillance and control

In Italy, IMD cases are mandatorily reported, through the Regions, to the Ministry of Health and to the Italian Institute of Public Health (Istituto Superiore di Sanità, ISS). The EU case-definition for lab-confirmed cases is utilized.[11] When a case of IMD is identified, contact tracing and chemoprophylaxis are provided by the local health authorities. In Tuscany, after the detection of an increase in the number of IMD cases, a number of actions were undertaken. Public health measures consisting in early detection (*i.e.* 24 h rapid diagnosis using molecular methods) and treatment of cases, antibiotic prophylaxis of close contacts, active offer of the tetravalent vaccine to all teenagers (up to 20 years of age), and free-of-charge vaccination of the adults (up to 45 years) living in the areas at greatest risk. We defined an "high risk area" a province with an observed number of cases in 2015 (up to October 31st) at least two-fold higher than expected (the number of cases reported in the same province in the last 5 years).

The microbiological characterization of invasive meningococcal strains was performed by the National Reference laboratory (NRL) of the ISS. Epidemiological, clinical, and microbiological data for each IMD case were managed using a dedicated database.

Microbiological analyses

Isolates were cultured following standard procedures. The serogroup was confirmed by slide agglutination with commercial antisera (Remel Europe, Ltd, UK) or by multiplex PCR.[12] Susceptibility to cefotaxime, ceftriaxone, ciprofloxacin, penicillin G, and rifampicin was determined by the MIC Test Strip Method (Liofilchem, Italy) 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, January 1, 2015 (http://www.eucast.org/).

Genomic DNA was extracted using the QiAmp mini kit (Qiagen, Hilden, Germany), according to the

manufacturer's instructions, from an overnight culture grown on Thayer Martin agar plate or directly from the clinical sample, blood, or CSF (cerebrospinal fluid).

Multilocus sequence typing (MLST), PorA and FetA typing, Bexsero antigen genes and antibiotic resistance genes were defined as described in http://neisseria.org/. The finetype is identified as follows: capsular group: *porA* (P1). VR1,VR2: *fetA* VR: ST (cc).

Whole genome sequencing

The whole genome sequencing was performed on 60 *Neisseria meningitidis* isolates at the Army Medical and Veterinary Research Center in Rome.

For each isolate, 1 ng of DNA was used to prepare the sequencing libraries, following the Nextera XT DNA protocol, according to the manufacturer's instructions. WGS of the isolates was performed by using the Illumina MiSeq platform (kit v3, 600 cycles). On average, 1.9 million paired-end reads were obtained for each sample. A first quality check of the raw sequence data was performed using FastQC.[13] Reads were trimmed to keep high quality bases (Q score >25) using the software Sickle,[14] and *de novo* assembly was carried out with the ABySS software version 1.5.2 (k parameter = 63).[15] Contigs longer than 500 bp were selected using an *ad hoc* script and kept for further analysis. The final assembly ranged from 201 to 506 (median = 247) contigs/sample covering the ~2.2 Mb of the *N. meningitidis* genome.

Genome comparison

Draft genomes were uploaded to the PubMLST.org database (http://pubmlst.org/neisseria/), which runs on the BIGSdb platform.

Genomes were analyzed and compared using the BIGSdb Genome Comparator tool implemented within the PubMLST website (http://pubmlst.org/neisseria/), through the gene-by-gene analysis approach. Phylogenetic analysis of the 60 isolates was performed firstly exploring the 53 ribosomal protein (rMLST) genes. Then, the genomes were investigated at higher resolution, analyzing the 1605 loci, defined as the core genome in the PubMLST Neisseria database (http://pubmlst.org/neisseria/), by the core genome MLST (cgMLST) approach.

Incomplete loci were automatically removed from the distance matrix calculation for the Neighbor-Net graphs. The resulting distance matrices were visualized as Neighbour-net networks, generated by SplitsTree4 (version 4.13.1).[16]

SNPs analysis

The program kSNP[17] was used to identify core genome SNPs on WGS data for each isolate. kSNP is based on k-mer analysis, therefore no multiple sequence alignment is required. Before running kSNP, the program Kchooser was used to estimate the optimum value of k-mer that for *N. meningitidis* data set was 31. After the kSNP analysis, the core_SNP_matrix output file, containing only SNP loci common to all isolates, was used for further analysis. Maximum Likelihood analysis[18] on the core SNP matrix output of kSNP was performed in MEGA 6.06,[19] using the Bootstrap Method with 100 Bootstrap replications.

Results

Epidemiological findings

From January 1st to October 31st 2015, 34 IMD cases were detected in Tuscany; of them, 28 were due to MenC. IMD cases were reported almost every week (Fig. 1) from towns and villages between Florence and Pisa. No secondary cases were identified. As already mentioned, traditional measures, including chemoprophylaxis of close contacts and vaccination of adolescents and adults, were promoted by Local Health Authorities to contain the spread of the disease. In particular, a catch-up strategy was used for unvaccinated adolescents, whereas booster doses were administered to those who had been previously vaccinated.

In the first 10 months of 2015, the incidence of IMD due to MenC in Tuscany was around 0.7 per 100,000 inhabitants, which is almost 10-fold higher than expected for the entire year in Tuscany since the introduction of MCC vaccination in 2005 (data not shown).

The median age of the patients was 25.5 years (average: 31 years), ranging from 9 to 82 years; 15 patients were females and 13 males. Meningitis and septicaemia represented the main clinical picture (14 cases), followed by septicaemia alone (13 cases), whereas only one patient presented meningitis without sepsis. The outcome was fatal for 6 patients who developed septicaemia or meningitis/septicaemia.

Two patients had received the MCC vaccine 8 years before the onset of the disease, and one patient 2 years before; all the other patients were unvaccinated. The NRL received samples from 26 of 28 IMD cases: 12 strains, 2 bacterial suspensions, and 12 clinical samples (CSF or blood). All the 12 strains were susceptible to cefotaxime, ceftriaxone, ciprofloxacin, and rifampicin, but showed a decreased susceptibility to penicillin G (range 0.094–0.25 mg/L).

Molecular analyses

Molecular analysis was performed on all 26 available samples. MLST identified the ST-11 complex (cc11) as the unique clonal complex. However, among cc11 isolates, two different sequence types (STs) were found: ST-11 (24 samples) and ST-2780 (2 samples, ID 2670 and 2691). The ST-2780 was identified in 2 strains isolated in April and May, and differed from the ST-11 for 15 nucleotide in the *fum*C gene.

Two different finetypes were identified: C: P1.5-1,10-8: F3-6: ST-11 (cc11) in 24 samples (92.3%), and C: P1.5-1,10-8: F3-6: ST-2780 (cc11) in 2 samples (7.7%).

The fHbp-1.13 variant was found in all but one analyzed strains (ID 2639) that presented a *fHbp* allele 669, encoding a truncated fHbp protein due to a frameshift mutation.

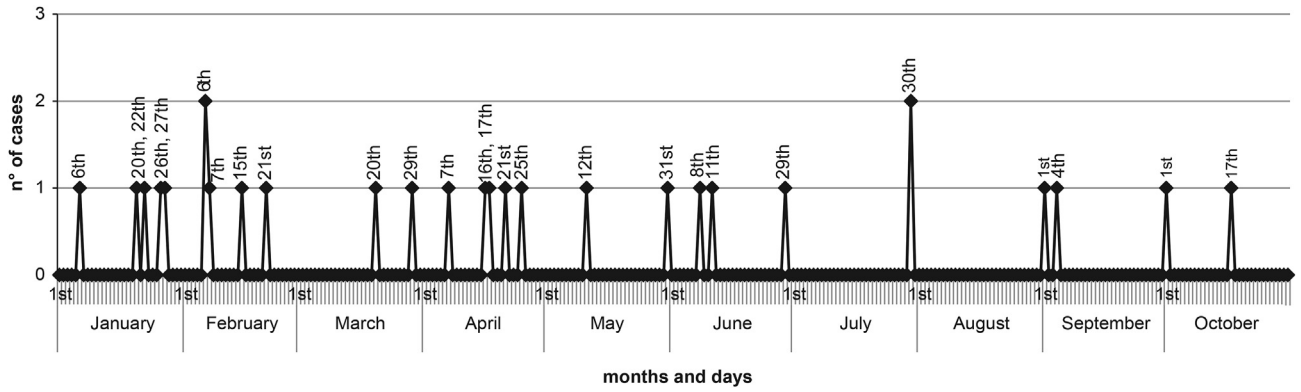

Figure 1 Timeline of serogroup C IMD cases occurred in Tuscany from January to October 31st 2015.

All meningococci showed the NHBA-20 variant and NadA with the Insertion Sequence (IS) disrupting the gene, as already described for the ET-15 meningococci.[20]

As already described,[21] a premature stop codon in the *aniA* gene was also identified in all the samples.

Genome comparison

Whole genome analysis

Whole genome analysis was performed on 58 C: P1.5-1,10-8: F3-6: ST-11 (cc11) strains and two C: P1.5-1,10-8: F3-6: ST-2780 (cc11) collected in Italy from 2007 to 2015; out of these strains, 6 were from an outbreak occurred in Veneto in 2007, 4 from an outbreak occurred on a cruise ship in Tuscany in 2012, and 13 from the current outbreak (Tuscany, 2015); the remaining 37 were from sporadic IMD cases.

rMLST

The analysis of 60 *N. meningitidis* isolates identified 9 ribosomal sequence type (rST). All isolates belonging to the outbreak occurred in Tuscany in 2015 and 14 isolates collected from sporadic cases in Italy between 2013 and 2015 grouped together in the same rST (data not shown).

cgMLST

Overall, 1349 of the 1605 core genome loci were included in the cgMLST analysis, while the remaining 256 loci were incompletely assembled. A total of 653 genes resulted identical in all the 60 isolates.

The 60 meningococci were resolved by cgMLST analysis (Fig. 2, panel a), revealing four groups: i) group I, comprising 12 out of 13 meningococci identified in Tuscany in 2015 and 14 meningococci isolated from sporadic cases in Italy in 2013, 2014, and 2015, respectively (Fig. 2, panel b); ii) group II, including 4 strains responsible of an outbreak occurred on a cruise ship in 2012 in Tuscany[22]; iii) group III, comprising the outbreak occurred in Veneto in 2007[23]; iv) group IV, including 1 strain isolated in Tuscany in 2015 (ID 2639) and 20 isolated from sporadic cases occurred in Italy from 2012 to 2015. Three isolates from sporadic IMD cases resulted distinct from the four groups.

Group I strains shared 1247 genes; in particular, those strains isolated in Tuscany in 2015 showed 1287 identical genes among them. The groups I and II, closer to each other, shared 1157 exclusive identical genes. Groups III and IV were in distal regions and shared respectively 1044 and 765 genes with group I.

cgMLST analysis pointed out 11 genes specific for each group. For 5 of 11 genes, the function is known: NEIS0430, hypothetically coding for a cytoplasmic axial filament protein (*cafA*); the *ctrE* gene, involved in the capsule translocation, critical for transport of the mature lipidated polymers to the meningococcal cell surface[24]; the *rpsD* gene, coding for the 30S ribosomial protein S4; the *tpsB* gene, a hemolysin activator; the *penA*, coding for the penicillin-binding protein 2 (PBP2). The *tpsB* allele "new#1" harbors a deletion of 1 adenine in the polyA stretch, causing a premature stop codon 150 bp downstream.

Table 1 listed the allelic profiles of the 11 genes for the groups I and II, together with 3 representative genomes available in the website (www.neisseria.org, last access 22nd September 2015) and identified in UK. The genomes indicated in the Table 1 include those of meningococci belonging to C:P1.5-1,10-8:F3-6:ST-11 (cc11) with the *penA* 248 allele.

Concerning the 11 genes profile specific for each groups, the main findings were:

i) 10 out of the 11 genes were identical in the group I; NEIS0430 distinguished one subgroup within group I, including all the genomes obtained from strains isolated in Tuscany in 2015, and 4 from sporadic cases occurred in Italy; ii) genomes of the group II, obtained from the outbreak strains occurred in Tuscany in 2012, were identical to each other; in particular, the genomes obtained from these strains showed 3 genes (NEIS0430, *ctrE*, and *rpsD*) identical to the genome identified in UK ID21253; moreover, they showed also the NEIS1858 gene identical to the UK genome ID 30209; iv) the genomes obtained from strains isolated in Tuscany in 2015 were identical to the UK ID 21253 genome.

SNPs analysis

A Neighbor Joining analysis was performed on 60 *N. meningitidis* strains using 2885 core SNPs obtained by the kSNP analysis of WGS data (Fig. 3). All the strains involved in the cluster occurred in Tuscany in 2015, with the exception of the strain 2639, grouped together. The strain 2639 showed a unique SNP profile, similar to those of strains

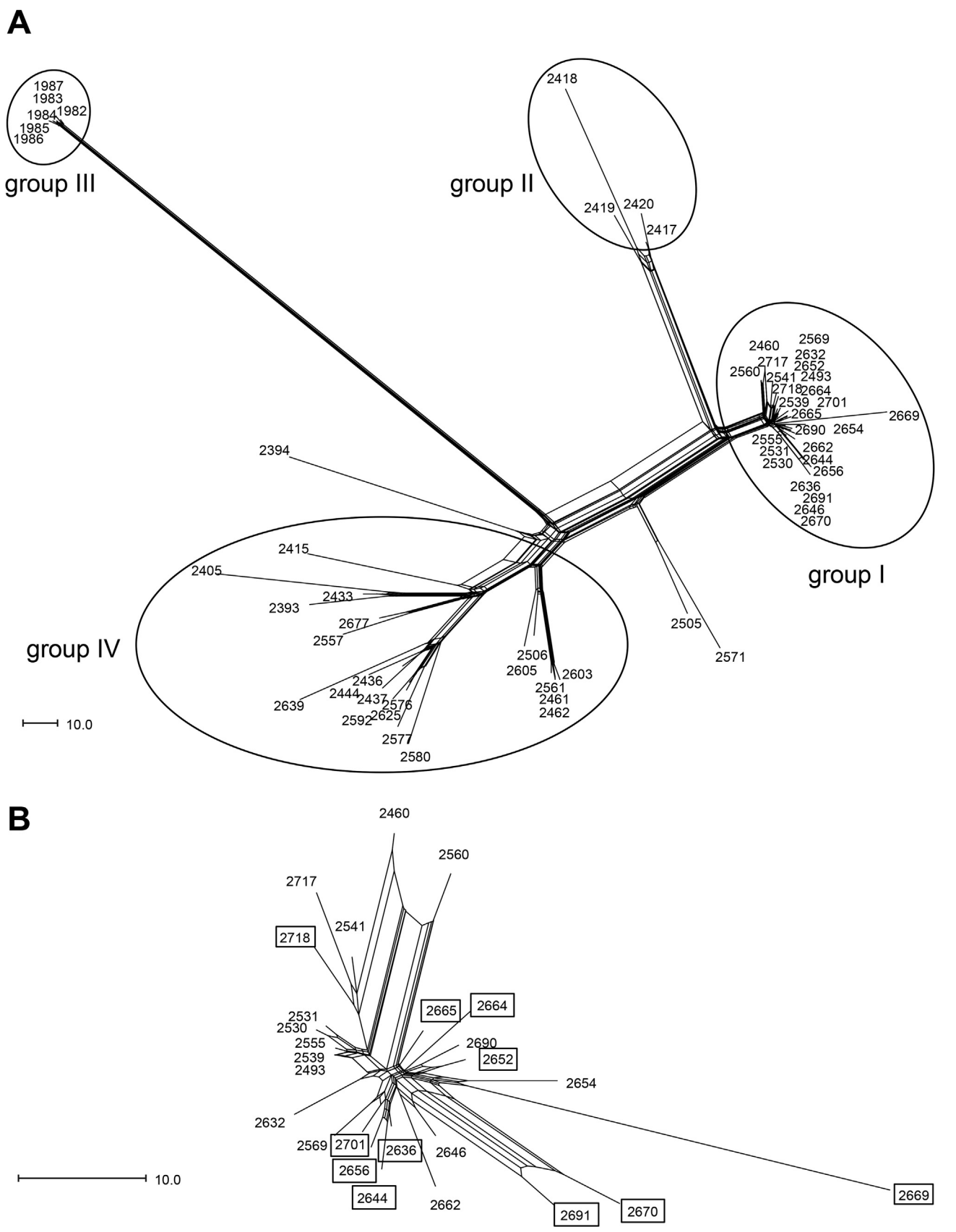

Figure 2 Panel (a), Neighbour-net phylogenetic network based on a comparison of 1349 core genome loci (cgMLST) among 58 *N. meningitidis* C: P1.5-1,10-8: F3-6: ST-11 (cc11) and 2 C: P1.5-1,10-8: F3-6: ST-2780 (cc11). Panel (b) Neighbour-net phylogenetic network based on a comparison of 1360 core genome loci (cgMLST) among 26 isolates of the group I; ID of isolates responsible for serogroup C IMD cases in Tuscany in 2015 are marked. The scale bars indicate the number of differences among the compared loci.

Table 1 Allelic profiles of 11 genes characterizing groups I and II as defined by cgMLST analysis of meningococci isolated in Italy and of 3 representative meningococcal genomes available at http://pubmlst.org/Neisseria (last access 22nd of September 2015), selected by finetype C:P1.5-1,10-8:F3-6: ST-11 (cc11) and *penA* 248 allele.

ID (ID http://pubmlst.org/Neisseria)	Country	Year	Group in the cgMLST tree	NEIS0430	*ctr*E	*rps*D	*tps*B	NEIS0263	NEIS0264	NEIS0265	NEIS0745	NEIS1594	NEIS1858	*pen*A
2539 (37600)	Italy	2013	I	6	355	29	new#1	144	10	28	176	271	157	248
2493 (37594)	Italy	2013	I	6	355	29	new#1	144	10	28	176	271	157	248
2541 (38867)	Italy	2013	I	6	355	29	new#1	144	10	28	176	271	157	248
2460 (38847)	Italy	2013	I	46	355	29	new#1	144	10	28	176	271	157	248
2560 (36461)	Italy	2014	I	46	355	29	new#1	144	10	28	176	271	157	248
2530 (37597)	Italy	2014	I	6	355	29	new#1	144	10	28	176	271	157	248
2531 (37598)	Italy	2014	I	6	355	29	new#1	144	10	28	176	271	157	248
2555 (37601)	Italy	2014	I	6	355	29	new#1	144	10	28	176	271	157	248
2662 (36766)	Italy	2014	I	6	355	29	new#1	144	10	28	176	271	157	248
2569 (36765)	Italy	2014	I	398	355	29	new#1	144	10	28	176	271	157	248
2632 (36771)	Italy	2015	I	6	355	29	new#1	144	10	28	176	271	157	248
2636* (36448)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2644* (36450)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2652* (36451)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2654* (36386)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2656* (36769)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2664* (36452)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2665* (36453)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2669* (36402)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2670* (36844)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2691* (36767)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2701* (36784)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2718* (38839)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2646 (36770)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2690 (36768)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2717 (38838)	Italy	2015	I	398	355	29	new#1	144	10	28	176	271	157	248
2417** (36444)	Italy	2012	II	6	209	1	1	1	1	1	1	1	3	248
2418** (36445)	Italy	2012	II	6	209	1	1	1	1	1	1	1	3	248
2419** (36446)	Italy	2012	II	6	209	1	1	1	1	1	1	1	3	248
2420** (36447)	Italy	2012	II	6	209	1	1	1	1	1	1	1	3	248
M11 241039 (21253)	UK	2011	na	6	209	1	new#1	144	10	28	176	271	157	248
M13 240628 (30209)	UK	2013	na	6	209	1	new#1	144	10	28	176	271	3	248
M13 240155 (28093)	UK	2013	na	6	355	29	new#1	144	10	28	176	271	157	248

*Tuscany 2015; ** Tuscany 2012; na: not applicable.

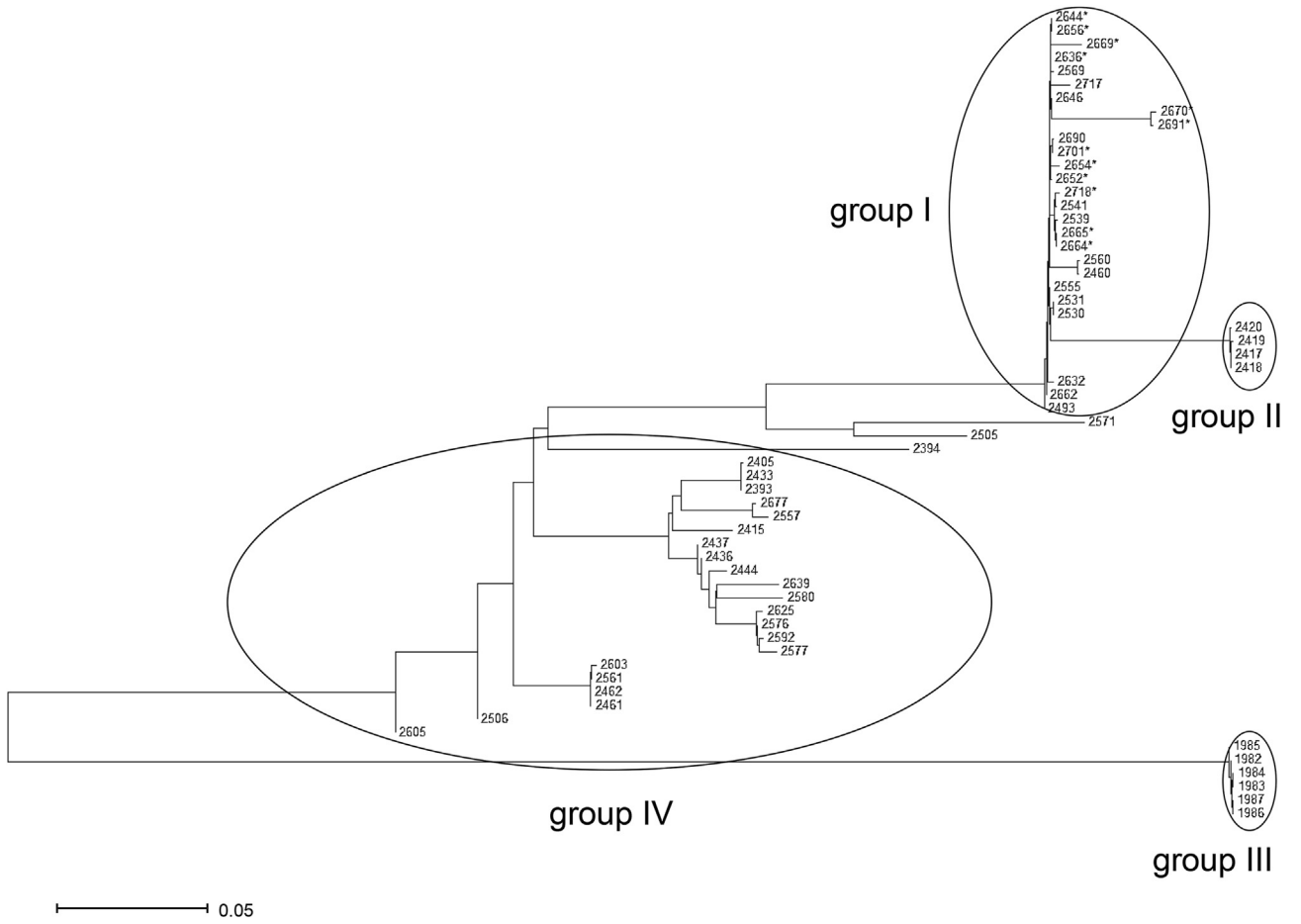

Figure 3 Maximum Likelihood-based tree of 58 *N. meningitidis* C: P1.5-1,10-8: F3-6: ST-11 (cc11) and 2 C: P1.5-1,10-8: F3-6: ST-2780 (cc11) constructed on 2885 core SNPs. ID of isolates responsible for serogroup C IMD cases in Tuscany in 2015 are marked with an asterisk.

from sporadic cases from other Italian Regions. The Between Groups Mean Distance was 0.038 between the Tuscany outbreak strains (Number core SNPs: 177) and the isolates 2691 and 2670, which showed a different Sequence Type (ST 2780).

The relatedness between the MenC strains isolated in Tuscany in 2015 and in the 2012 outbreak was supported by the number of base substitutions per site (Between Groups Mean Distance in MEGA6) of only 0.071.

Discussion

In a 10 months period, 28 IMD cases due to serogroup C within the cc11/ET-15 clonal complex were reported in a geographically restricted area of Tuscany. All but two isolates belonged to the finetype C:P1.5-1,10-8:F3-6:ST-11 (cc11). This strain had been already identified and characterized in several European countries,[9,10] including two smaller outbreaks occurred in Italy.[22,23]

The number of IMD cases due to MenC reported in the first 10 months of 2015 in Tuscany was higher than expected. For comparison, in 2014, a total of 156 lab-confirmed IMD cases was reported in Italy, and only two were due to MenC; the majority of cases was due to meningococcus B, as a consequence of the decline of serogroup C cases among children 0—5 years old, following the introduction of childhood vaccination. In Tuscany, the incidence of IMD cases due to MenC was 0.2 cases/100.000 inhabitants before the introduction of MCC (2000—2005), declining to 0.07 after the introduction of vaccination (2006—2014), then showing this unexpected peak of 0.7 in 2015. This was to some extent surprising, since the coverage of MCC vaccination in the Region was high.

Traditional measures, including chemoprophylaxis of close contacts and vaccination of adolescents and young adults, were implemented to contain the spread of IMD cases. In particular, a catch-up strategy, boosting adolescents who had been vaccinated and immunizing those who had not been vaccinated before, was first implemented. In fact, the lack of a catch-up program targeting adolescents and young adults, who are considered the principal responsible for carriage and transmission, is likely to explain at least in part the excess of IMD cases observed in Tuscany. Since several cases were reported among young adults, it was then decided to offer the vaccine to all unvaccinated individuals, up to 45 years old, living in the affected area. Up to October 31st 2015, about 180,000 individuals have been vaccinated (data not shown).

Unfortunately, data on the prevalence of MenC carriage by age category during the outbreak were not available. Thus, the vaccination strategy was based on the assumption of a rather short duration of protection among already vaccinated infants and young children, and a rapid circulation of MenC strains among unvaccinated adolescents and young adults.[25,26] To this regard, the MCC vaccine is likely to provide high levels of direct protection in the short term[26,27] and to reduce the prevalence of serogroup carriage, improving the herd immunity in the overall population.[28,29] However, in areas where children are the main vaccination target, a relatively short duration of protection, combined with low vaccine coverage in the adolescence, may explain the occurrence of IMD cases among unvaccinated individuals in older age-groups. In this context, adolescents and young adults are known to have the highest *N. meningitidis* carriage rates.[30]

As mentioned above, the outbreak was caused by the hypervirulent clone C:P1.5-1,10-8:F3-6:ST-11 (cc11). To support the analysis of the epidemic dynamics, whole genome sequencing was performed to characterize the *Neisseria* pathogenome, and to compare the genomic characteristics of the 2015 Tuscany outbreak strains with those of meningococci of the same finetype isolated in Italy over the last years. Our findings indicate that all the C:P1.5-1,10-8:F3-6:ST-11 (cc11) strains with *penA*248 isolated in Italy show high similarity. This strain was identified for the first time during the outbreak occurred on a cruise ship docking in the port of Livorno, Tuscany, in 2012.[22] Whether this event led to the introduction and spread of this strain is matter of debate.

WGS may greatly contribute to outbreak investigation, allowing the identification of specific lineages causing disease in specific age-groups.[31] Using conventional methods able to characterize the finetype of each isolate, strains collected from IMD cases occurred in Tuscany in 2015 appear similar to the other strains with the same finetype, which were responsible of sporadic cases occurring throughout the country over the years. WGS and, in particular, core genome sequencing demonstrated that meningococci isolated in 2015 in Tuscany were closely related to each other, whereas they differed from the strains sharing the same finetype which had been isolated in other Italian Regions. The SNP analysis confirmed the findings of cgMLST, identifying the two strains with the same finetype but a different ST (ST-2780).

As already mentioned by Lucidarme et al.,[32] there are several globally spread cc11 strains belonging to different lineages. Our analysis suggests the relationship between the 2015 Tuscany outbreak strains and the cc11/ET15 sublineage 11.2 described by Lucidarme et al.[32] Interestingly, several C:P1.5-1,10-8:F3-6:ST-11 (cc11) isolated in the UK from 2011 to 2015[32] showed allelic profiles similar to those identified in the two outbreaks occurred in Tuscany in 2012 and 2015. The same sublineage 11.2 was also responsible of outbreaks among MSM in France and UK. It seems that the meningococci of cc11/ET15 are rather homogeneous, but different strains may emerge in different regions of the world.

Before drawing conclusions, possible limits of the study should be mentioned. First of all, data on the carriage of MenC strains involved in the outbreak are not available. To this regard, although several studies showed low rates of MenC carriage during outbreaks of MenC disease, a study from Brazil found relatively high rates.[33] The same study suggested that the polysaccharide vaccine had no effect on carriage, whereas the MCC was able to reduce it. Secondly, information on risk factors was not routinely and systematically collected. Thus, circulation dynamics and infection modalities in the affected population remain largely undefined. However, the characteristics of the cases suggest a widespread circulation of MenC:cc11 among unvaccinated age cohorts. For this reason, it was decided to vaccinate adolescents and young adults, who are likely to sustain MenC circulation and represent the population where IMD cases were mostly concentrated.

In conclusion, our findings show that increased incidence rates of IMD due to hypervirulent meningococcal strains may occur, unpredictably, also in areas with high vaccination coverage among infants and young children. WGS supports the hypothesis that C:P1.5-1,10-8:F3-6:ST-11 (cc11) is an hypervirulent, outbreak-associated, and evolving strain, which is now spreading globally. Detailed molecular information is key to better understand epidemic and hyper-endemic dynamics, and to re-modulate vaccination strategies.

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Conflict of interest

The authors declare no conflict of interest.

Transparency declaration

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