



Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Research note

An outbreak of severe invasive meningococcal disease due to a capsular switched *Neisseria meningitidis* hypervirulent strain B:cc11

P. Stefanelli^{1,*}, C. Fazio^{1,†}, P. Vacca^{1,†}, A. Palmieri¹, L. Ambrosio¹, A. Neri¹, A. Piana², P. Castiglia², F. Argiolas³, S. Santus⁴, L. Masala⁵, G. Steri⁴, F. Riccardo¹, S. Iannazzo⁶, F.P. Maraglino⁶, C. D'Amario⁶, G. Rezza¹

¹ Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

² Department of Medical Surgical and Experimental Sciences, University Hospital of Sassari, Italy

³ Servizio promozione della salute e osservatorio epidemiologico, Assessorato dell'Igiene e Sanità e dell'assistenza sociale-Regione Autonoma della Sardegna, Italy

⁴ Public Health Service, Local Health Unit, Cagliari, Italy

⁵ Laboratory SS Trinità Hospital, Sardinian Health Service, Italy

⁶ Director General for Health Prevention, Ministry of Health, Rome, Italy

ARTICLE INFO

Article history:

Received 6 June 2018

Received in revised form

13 July 2018

Accepted 16 July 2018

Available online xxx

Editor: S. J. Cutler

Keywords:

Capsular switching

Hypervirulent

Invasive meningococcal disease

Italy

Neisseria meningitidis

Outbreak

Whole-genome sequencing

ABSTRACT

Objectives: The aim was to investigate an outbreak of invasive meningococcal disease (IMD) in Southern Sardinia.

Methods: Epidemiological and microbiological investigations were performed. The latter included antimicrobial susceptibility testing and whole-genome sequencing (WGS).

Results: Seven individuals with severe IMD were found to be infected with serogroup B (MenB) *Neisseria meningitidis* in the first quarter of 2018. Five of the seven cases (five males; mean age 19 years; range 18–21 years; CFR 40%) were due to a unique strain B:P1.5-1,10-8:F3-6:ST-11(cc11), probably switched from the hypervirulent C-cc11, as confirmed by WGS. All five patients had attended the same nightclub in the 2 weeks prior to symptom onset. Public health measures, including chemoprophylaxis of contacts and active immunization against MenB, were implemented.

Conclusions: We observed five IMD cases due to the same switched MenB strain. The hypervirulent B:P1.5-1,10-8:F3-6:ST-11(cc11) strain, probably switched from C-cc11, is of concern due to the observed high virulence and case fatality rates. All the patients shared the same place of probable exposure. The molecular characterization of the invasive strain allowed the outbreak to be confirmed, which was then controlled through timely public health action. **P. Stefanelli, Clin Microbiol Infect 2018;■:■**

© 2018 The Author(s). Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The incidence of invasive meningococcal diseases (IMD) in Italy is relatively low (0.38/100 000 inhabitants in 2016). In 2016, in the 15–64 years age group, serogroup C (MenC) was the predominantly circulating serogroup, accounting for about 50% of IMD cases, followed by serogroup B (MenB), which caused more than 30% of all cases [1].

Only one multicomponent meningococcal serogroup B vaccine (4CMenB) is currently licensed in Italy (hereby MenB vaccine): the

National Immunization Plan recommends free of charge immunization of infants (below 1 year of age) with three doses of MenB vaccine and a fourth dose (booster) after 1 year of age [2]. Before 2017, only a few Italian regions had implemented a vaccination plan against MenB.

On the Italian island of Sardinia, five cases of IMD were reported in 2016 and four in 2017 [1]. Of those nine cases, four were caused by MenB meningococci (three in 2016 and one in 2017).

Between 1 January and 31 March 2018, seven IMD cases, presenting as meningitis and septicaemia, were reported on the Italian island of Sardinia, thus exceeding the expected caseload. All cases were caused by MenB and unvaccinated against meningococcus. An investigation was conducted in order to confirm the outbreak, identify the common source of exposure, and set up timely control measures.

* Corresponding author. P. Stefanelli, Department Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome, Italy.

E-mail address: paola.stefanelli@iss.it (P. Stefanelli).

† These authors contributed equally to this work.

<https://doi.org/10.1016/j.cmi.2018.07.014>

1198-743X/© 2018 The Author(s). Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Please cite this article in press as: Stefanelli P, et al., An outbreak of severe invasive meningococcal disease due to a capsular switched *Neisseria meningitidis* hypervirulent strain B:cc11, Clinical Microbiology and Infection (2018), <https://doi.org/10.1016/j.cmi.2018.07.014>

Methods

Isolates from all seven IMD cases were sent to the National Reference Laboratory (NRL) for IMD at the Istituto Superiore di Sanità (ISS), where whole-genome sequencing (WGS) using the NextSeq 500 platform (Illumina, San Diego, CA, USA) (NextSeq500 Mid Output kit, 300 cycles) was performed. Antimicrobial susceptibility testing was carried out using the MIC Test Strip Method (Liofilchem, Roseto degli Abruzzi, Italy), referring to EUCAST breakpoints (version 8.0, 2018-01-01). Genomes were compared with the BIGSdb Genome Comparator [3]. Core genome multilocus sequence typing (cgMLST) was performed on the five MenB:cc11 genomes from Sardinia, on 11 genomes representative of C:P1.5-1,10-8:F3-6:ST-11(cc11) sporadic cases that occurred in Italy (2015–2018), and on 14 B:P1.5-1,10-8:F3-6:ST-11(cc11) genomes available in the PubMLST Neisseria database (<https://pubmlst.org/neisseria>) (Fig. 1). Moreover, four MenB genomes of the clonal complexes (ccs) more frequently associated with MenB in Italy in 2017 (cc41/44, cc162, cc213, cc269) were included in cgMLST analysis [4,5]. The resulting distance matrices were visualized as Neighbor-net networks by SplitsTree4 (version 4.13.1) [4].

Public health measures were implemented in accordance with ISS and the Italian Ministry of Health. Contact tracing was performed by local health authorities, and post-exposure chemoprophylaxis and MenB vaccine were administered to all close contacts.

Ethics approval was not required, as clinical isolates were collected, processed, and stored as part of routine clinical care by the hospitals participating at the IMD surveillance system activities.

Results

Of the seven IMD cases (Table 1) reported during the first quarter of 2018, all of them unvaccinated against meningococcus, the first two occurred in the province of Nuoro in north-east

Sardinia. Of the remaining five cases, two occurred during the second week of January and three between the 15 and 31 March 2018. All five cases had attended the same nightclub near Cagliari (southern Sardinia) in the 2 weeks prior to symptom onset and were not epidemiologically linked to the first two cases. The five patients were males and their median age was 19 years (range 19–21 years). The median time between the assumed date of exposure (nightclub attendance) and symptom onset was 6 days (range 2–12 days). Two of them died.

As shown in the Table 1, WGS allowed us to clearly distinguish the first two MenB isolates in Nuoro from the cluster of the five MenB sharing an identical genotype, namely, B:P1.5-1,10-8:F3-6:ST-11(cc11). Core genome phylogeny showed that all MenB:cc11 genomes included in the analysis grouped with MenC:cc11 genomes (from epidemic strain) in a distal region from the four Italian MenB:non-cc11 genomes (Fig. 1). In particular, the five genomes isolated in Sardinia, clustered together with seven endemic MenC:cc11 isolates from other areas of Italy detected in 2015–2018 and with one MenB:cc11 isolated in the UK in 2016. The five meningococcal isolates from Sardinia showed a high proximity among them, with a mean value of three out of 1281 different loci.

All isolates were susceptible to cefotaxime, ceftriaxone, ciprofloxacin, and rifampicin and intermediately susceptible to penicillin G (PenI), (MIC values 0.125–0.19 mg/L). The sequence of a 402-bp DNA fragment of the 3' part of *penA* encoding for a peptide with five amino acid alterations in the C-terminal region of PBP2 was obtained and associated with *penA* allele 248.

Vaccination against MenB was actively offered to about 400 close contacts. Chemoprophylaxis and vaccination against MenB were also recommended to young adults who had social contacts within the nightclub that had been identified as the place of exposure ('community contacts') to prevent new cases linked to a potential persistence of bacterium circulation in the community. Overall, about 1050 close and community contacts were

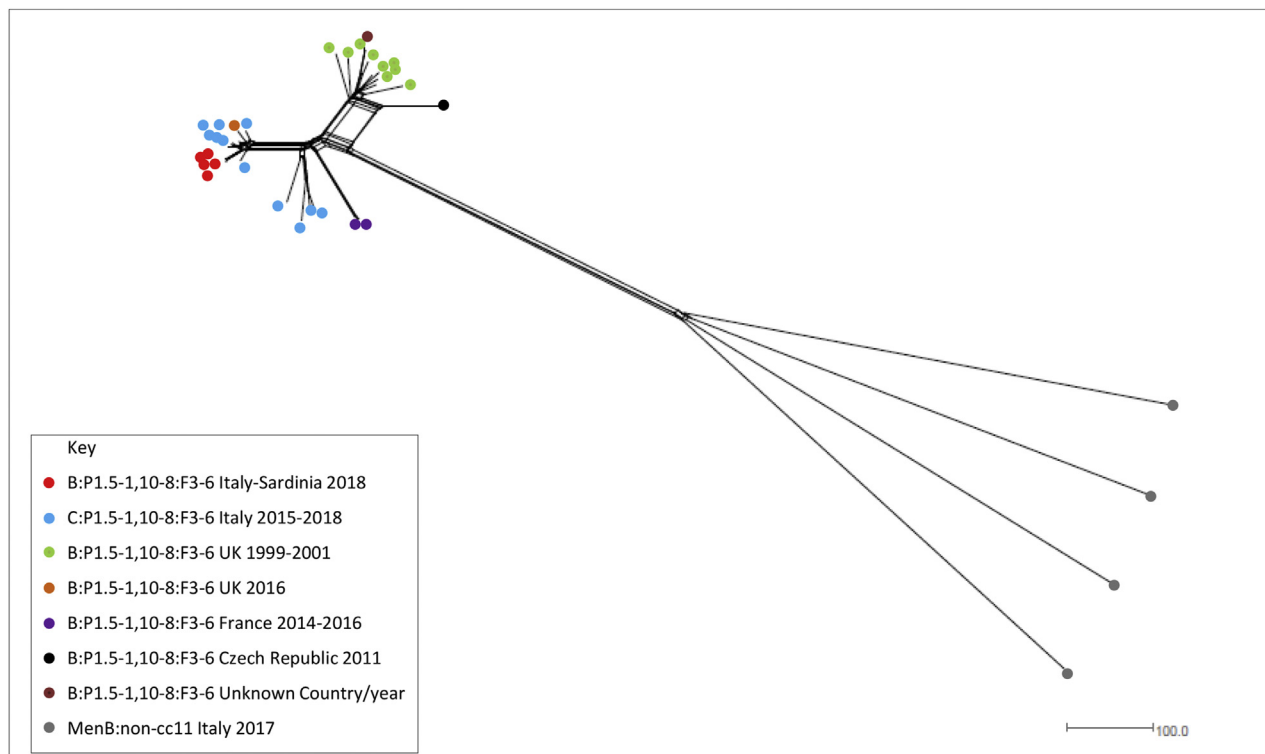


Fig. 1. Neighbor-net phylogenetic network based on a comparison of 1281 core genome loci (cgMLST) of MenB:cc11 ($n = 19$ of which 5 from Sardinia), MenC:cc11 ($n = 11$), MenB:non-cc11 ($n = 4$) genomes. The scale bar indicates the number of differences among the compared loci.

Table 1
Invasive meningococcal disease cases occurred in Sardinia in 2018 and associated genotype

ID	Onset date	Place of exposure	Age (years)	Sex	Outcome	Genotype
1	01/01/2018	Nuoro	50	Male	Alive	B:P1.7,30:F1-5:ST-41(cc41/44)
2	04/01/2018	Nuoro	50	Female	Alive	B:P1.7,30:F1-5:ST-41(cc41/44)
3	07/01/2018	Cagliari	19	Male	Dead	B:P1.5-1,10-8:F3-6:ST-11(cc11)
4	12/01/2018	Cagliari	20	Male	Alive	B:P1.5-1,10-8:F3-6:ST-11(cc11)
5	18/03/2018	Cagliari	19	Male	Dead	B:P1.5-1,10-8:F3-6:ST-11(cc11)
6	22/03/2018	Cagliari	18	Male	Alive	B:P1.5-1,10-8:F3-6:ST-11(cc11)
7	24/03/2018	Cagliari	21	Male	Alive	B:P1.5-1,10-8:F3-6:ST-11(cc11)

identified and treated. Most of them were vaccinated both against MenB and against MenACWY. People who had received a dose of MenACWY conjugate vaccine more than 5 years before were revaccinated.

Conclusions

Following an increase in the number of IMD cases because of the MenB in Sardinia, we conducted an outbreak investigation in order to confirm the outbreak, identify a common exposure, and implement timely and adequate public health actions. Two distinct transmission events were identified: a small family cluster in the north-east of the island, and an outbreak because of a capsular switched *N. meningitidis* strain B:cc11 among five individuals attending the same nightclub. The first small cluster, fuelled by household transmission, had been contained through chemoprophylaxis of close contacts, while the larger cluster required more extensive control measures.

Although IMD incidence rate in general, and the incidence of serogroup B and C IMD in particular, in Italy shows cyclical fluctuations, the overall annual incidence remains quite low [1]. The serogroup distribution is similar to what is reported in most European countries, with serogroup B and C responsible for the majority of disease, followed by serogroup Y, W [1].

Recently, an increase in the incidence of IMD was observed in Italy, mostly sustained by MenC [6], and to a lesser extent by different MenB clonal complexes. The increase of MenC IMD was due to the occurrence of an outbreak in Tuscany, a region in central Italy [6]. The outbreak was caused by a highly virulent MenC:cc11 strain [6], detected also in other countries [7].

The outbreak of five cases of IMD because of the hypervirulent MenB:cc11 strain was confirmed by combining an epidemiological and microbiological investigation, using molecular typing including WGS. The cgMLST confirmed both the close similarity among the five outbreak isolates and to MenC:cc11 strains circulating in Italy in the same period. The close similarity among the genomes of B and C capsular serogroups, strongly suggests capsular switching from C to B strains, even though we cannot definitely rule out the possibility that the strain was imported from abroad. All infected individuals attended the same nightclub in the province of Cagliari, identifying this social and behavioural context as an amplifier of hypervirulent meningococci transmission. The five cases occurred in two waves, the first in January and the second in March. It is likely that the circulation of the MenB strain involved in this small outbreak persisted in the community attending the nightclub during the month of February.

We estimated the incubation time based on the assumption that the five individuals belonging to the same cluster were exposed in the nightclub. However, we cannot rule out that cases might have attended the same club multiple times. Further, we calculated the time until the date of IMD-specific symptom onset. While this is a reasonable choice, we cannot exclude that the onset of IMD might have occurred after an episode of pharyngitis. These factors could have led to an under or over estimation of incubation times.

Both the epidemiological and the microbiological evidence support the hypothesis that the two cases identified at the beginning of January in the province of Nuoro were unrelated to this outbreak. Further, the only case of MenB IMD reported in Sardinia in 2017 occurred in the area of Cagliari at the end of December in an 18-year-old girl who did not attend the nightclub and was caused by a different MenB strain.

Two of the five outbreak patients died, suggesting a high lethality of the disease because of the B:cc11 strain. Capsular switching from MenC to MenB among cc11 meningococci is of concern due to disease severity. Switching events are not rare within the cc11 meningococci [3]. MenB:cc11 outbreaks have been documented in different countries and an increase in cases caused by this strain has been reported in France from 2002 [8]. In Italy, only an isolated case due to the same strain was reported in 2017 from the Emilia Romagna region (north of Italy) (data not shown).

No subsequent cases due to the same strain were detected among individuals attending the same nightclub in the island, suggesting that both contact tracing and antibiotic prophylaxis were effective. Furthermore, vaccination against MenB was offered to close and community contacts to reduce the risk of persistent circulation of the hypervirulent strain.

In conclusion, WGS in outbreak investigations facilitated the rapid and accurate identification of the pathogen. It also allowed to confirm and define the true size of the outbreak and supported towards a more focused investigation.

Transparency declaration

All authors report no conflicts of interest relevant to this article. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The data on cgMLST analysis were made available via the Neisseria Multi Locus Sequence Typing website (<http://pubmlst.org/neisseria>) developed by Keith Jolley and sited at the University of Oxford.

References

- Italian National Health Institute. (Istituto Superiore di Sanità, ISS). Dati di sorveglianza delle malattie batteriche invasive aggiornati Aprile. 2018. Available from: <http://old.iss.it/binary/mabi/cont/InterimReport2017.pdf>.
- Italian Ministry of Health. National immunization plan. 2017–2019. Available from: <http://www.salute.gov.it/portale/vaccinazioni/dettaglioContenutiVaccinazioni.jsp?lingua=italiano&id=4828&area=vaccinazioni&menu=vuoto>. [Accessed 23 May 2018].
- Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinform* 2010;11:595. <https://doi.org/10.1186/1471-2105-11-595> PMID: 21143983.
- Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 2006;23:254–67. <https://doi.org/10.1093/molbev/msj030> PMID: 16221896.
- Bratcher HB, Corton C, Jolley KA, Parkhill J, Maiden MC. A gene-by-gene population genomics platform: de novo assembly, annotation and genealogical

- analysis of 108 representative *Neisseria meningitidis* genomes. *BMC Genom* 2014;15:1138. <https://doi.org/10.1186/1471-2164-15-1138>.
- [6] Stefanelli P, Miglietta A, Pezzotti P, Fazio C, Neri A, Vacca, et al. Increased incidence of invasive meningococcal disease of serogroup C/clonal complex 11, Tuscany, Italy, 2015 to 2016. *Euro Surveill* 2016;21. <https://doi.org/10.2807/1560-7917.ES.2016.21.12.30176>. PMID: 27035155.
- [7] Lucidarme J, Hill DM, Bratcher HB, Gray SJ, du Plessis M, Tsang RS, et al. Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. *J Infect* 2015;71:544–52. <https://doi.org/10.1016/j.jinf.2015.07.007>.
- [8] Lancellotti M, Guiyoule A, Ruckly C, Hong E, Alonso JM, Taha MK. Conserved virulence of C to B capsule switched *Neisseria meningitidis* clinical isolates belonging to ET-37/ST-11 clonal complex. *Microbe. Infect* 2006;8:191–6. <https://doi.org/10.1016/j.micinf.2005.06.012>.