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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

P. Stefanelli [a,*], C. Fazio [a,h], A. Neri [a,h], A. Ciammaruconi [b], E. Balocchini [c], A. Anselmo [b], C. Azzari [d], G.M. Rossolini [e,f,g], P. Vacca [a], A. Fortunato [b], A. Palozzi [b], S. Fillo [b], F. Lista [b], M. Moriondo [d], F. Nieddu [d], G. Rezza [a]

[a] *Department of Infectious, Parasitic & Immuno-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy*
[b] *Molecular Biology Section, Army Medical and Veterinary Research Center, Rome, Italy*
[c] *Living Environment, Food and Veterinary Prevention and Safety Office, Tuscany Region, Florence, Italy*
[d] *Laboratory of Immunology and Infectious Diseases, Anna Meyer Children's University Hospital, University of Florence, Florence, Italy*
[e] *Department of Medical Biotechnologies, University of Siena, Italy*
[f] *Department of Experimental and Clinical Medicine, University of Florence, Italy*
[g] *Microbiology and Virology Unit, Florence Careggi University Hospital, Italy*

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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.

* Corresponding author. Department of Infectious, Parasitic & Immune-mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. Tel.: +39 06 49902126; fax: +39 06 49387112.
E-mail address: paola.stefanelli@iss.it (P. Stefanelli).
[h] These authors contributed equally to this article.

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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

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