Neisseria meningitidis ST-11 Clonal Complex, Chile, 2012

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Serogroup W Neisseria meningitidis was the main cause of invasive meningococcal disease in Chile during 2012. The case-fatality rate for this disease was higher than in previous years. Genotyping of meningococci isolated from case-patients identified the hypervirulent lineage W:P1.5,2:ST-11, which contained allele 22 of the fHbp gene.

Neisseria meningitidis (meningococcus) is the causative pathogen of invasive meningococcal disease (IMD), which includes a set of infectious syndromes, mainly meningitis or meningococcemia (septicemia) and, less commonly, pneumonia or other infections (1). Humans are the only reservoir for meningococcus, which usually colonizes the upper respiratory tract of ≈8%–25% of persons (1).

In Chile, the incidence of IMD decreased steadily during 2000–2012 from 3.6 to 0.7 cases/100,000 inhabitants (2). However, deaths from this disease have not followed this trend; the case-fatality rate increased from 8.9% in 2009 to 14.1% in 2010, 14.7% in 2011, and 27.0% in 2012 (2,3). During this period, the distribution of meningococcal serogroups has changed. There has been a large increase in frequency of serogroup W meningococci, which has replaced serogroup B as the most common serogroup.

A total of 101 cases, 57 culture-confirmed: 42/57 (73.7%) serogroup B and 1/57 (1.8%) serogroup W were reported in 2009; 78 cases: 56 culture-confirmed 36/56 (64.3%) serogroup B and 6/56 (10.7%) serogroup W were reported in 2010; 73 cases: 63 culture-confirmed, 32/63 (50.8%) serogroup B and 22/63 (34.9%) serogroup W were reported in 2011; and 133 cases: 103 culture-confirmed, 38/103 (36.9%) serogroup B and 60/103 (58.3%) serogroup W were reported in 2012 (2–4). We conducted this study to determine whether W meningococci belonged to a hypervirulent genetic lineage of the ST-11 clonal complex (CC).

The Study

A national epidemiologic program for surveillance and control of IMD is conducted by the Department of Epidemiology of the Ministry of Health of Chile. Every national health care center must report suspected cases of IMD and send bacterial isolates to the Institute of Public Health of Chile (Santiago, Chile) or send cerebrospinal fluid samples when cultures have shown negative results. IMD cases are defined by clinical signs and symptoms (neck stiffness, altered state of consciousness, rash, meningeval irritation) and confirmed by isolation of N. meningitidis from cerebrospinal fluid, blood, or another sterile body fluid or tissue. Each case is coded according to the International Classification of Diseases, 10th Revision, as meningitis (code A39.0), meningococcemia (A39.2), Waterhouse-Friderichsen syndrome (A39.1), other meningococcal infections (A39.8), and unspecified meningococcal infections (A39.9).

In 2012, a total of 32 health care centers located throughout Chile reported 133 IMD cases. Of these cases, 103 were laboratory confirmed by bacterial isolation and biochemical identification (3). Serogroup was determined by slide agglutination with polyclonal antibodies. Genotyping was conducted by amplifying and sequencing variable regions 1 and 2 of the porA gene as described by Russell et al. (5) Variants were defined by reviewing the Neisseria PorA typing database (http://pubmlst.org/neisseria/PorA/). Sequence types (STs) were determined as described by Maiden et al. (6) on the basis of housekeeping genes abcZ, adk, aroE, fumC, gdh, pdh, and pgm. Sequences were compared with those in the Neisseria locus/sequence definition database (http://pubmlst.org/), and STs and CCs were assigned. The nomenclature used in this report, when appropriate, is serogroup: genosubtype: CC.

The fHbp genetic variant was identified as described by Brehony et al. (7). Allele numbers were assigned by querying a public database (Neisseria Factor H binding protein sequence typing; http://pubmlst.org/neisseria/fHbp/). Genotyping with higher resolution was conducted by using pulsed-field gel electrophoresis (PFGE) and restriction endonuclease SpeI. Electrophoretic profiles were analyzed by using BioNumerics software (Applied Maths NV, Sint-Martens-Latem, Belgium). A PFGE pattern was considered unique when ≥1 DNA bands in the electrophoretic migration profile differed from each other. A code was assigned to each pattern. To establish the magnitude of associations, we cross-tabulated data and calculated odds ratios (ORs) by using Med Calc software version...
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12.4.0.0 (http://www.medcalc.org/). The 95% CI was established, and p values <0.05 were considered significant.

Serogroup B meningococci were isolated from 38 (36.9%) case-patients, and serogroup W meningococci were isolated from 60 (58.2%) case-patients (4). Serogroups C and Y meningococci were rarely isolated (3 and 2 isolates, respectively). Multilocus sequencing typing showed serogroup B isolates belonged mainly to ST-32 and ST-41/44 CCs, including 4 and 6 STs respectively. Of 60 W isolates, 98% belonged to ST-11 CC (Table 1). This finding is consistent with the serogroup of CCs found in W meningococci isolated in Chile during 2010 (4/4 W isolates analyzed) and 2011 (19/21 W isolates) (8). Among this CC, 3 STs were identified, of which ST-11 was the most common (Table 1).

Genosubtyping of W:ST-11 strains obtained during 2012 indicated that 58/60 strains belonged to genosubtype P1.5,2 (Table 2). In addition, sequence analysis of the fHbp gene identified allele 22 as the most common variant; it was present in 58 (96.7%) of 60 strains that belonged to the W serogroup (Table 2). Allele 22 of fHbp was not detected in strains belonging to other serogroups, which indicated a strong association with W meningococci. These results indicated that most IMD cases reported during 2012 in Chile were caused by a hypervirulent genetic lineage of N. meningitidis serogroup W.

Serogroup W meningococci W:P1.5,2:ST-11 with allele 22 of fHbp were isolated from samples of 12 of 42 meningococci cases-patients reported during 2012 and from 38 of 51 samples from meningococemia case-patients. This association with meningococemia cases was significant (OR 5.5, 95% CI 2.4–12.9, p = 0.0001). Overall, of 103 IMD case-patients in this study, 22 died. W:P1.5,2:ST-11 meningococci carrying allele 22 of fHbp were isolated from most patients with lethal cases. However, this association was not significant (16 of 22 deaths; OR 2.5, 95% CI 0.9–6.9, p = 0.08).

Analysis of W:P1.5,2:ST-11 meningococci by PFGE identified 9 electrophoretic patterns (Table 2). PFGE showed that most hypervirulent W:ST-11 clones were closely related to each other. Meningococci with a Cl-Nm-Spe-031 pattern caused most meningococccemia cases (Table 2). However, this association was not significant (OR 1.8, 95% CI 0.59–5.38, p = 0.31). Of 16 lethal cas-

### Table 1. Clonal complexes and sequence types identified in main serogroups of Neisseria meningitidis isolated in Chile, 2012*

| Serogroup, clonal complex | No. (%) | Sequence type |
|---------------------------|---------|---------------|
| B, n = 38                 |         |               |
| ST-32                     | 17 (44.7) | NA            |
|                           | 13      | 32            |
|                           | 2       | 3822          |
|                           | 1       | 7780          |
|                           | 1       | 9918          |
| ST-41–44                  | 20 (52.6) | NA            |
|                           | 14      | 44            |
|                           | 1       | 315           |
|                           | 1       | 8528          |
|                           | 2       | 9233          |
|                           | 1       | 9354          |
|                           | 1       | 10127         |
| ST-461                    | 1 (2.6)  | NA            |
|                           | 1       | 461           |
| W, n = 80                 |         |               |
| ST-11                     | 59 (98.3) | NA            |
|                           | 51      | 11            |
|                           | 7       | 1025          |
|                           | 1       | 2961          |
| ST-22                     | 1 (1.7)  | NA            |
|                           | 1       | 184           |

*NA, not applicable.

### Table 2. Characteristics of Neisseria meningitidis serogroup W isolates from 60 patients with invasive meningococcal disease, Chile, 2012†

| Characteristic     | Genotype |
|--------------------|----------|
|                    | W:P1.5,2:ST-11, n = 58 | W:P1.5,2–53:ST-11, n = 1 | W:P1.18–1.3:ST-22, n = 1 |
| Clinical outcome†  | A39.0, n = 12 | A39.2, n = 39 | Other, n = 7 |
| fHbp gene allele  | A39.2, n = 1 |
| 16, n = 1          | 0         | 0            | 0           |
| 19, n = 1          | 0         | 1            | 0           |
| 22, n = 58         | 12        | 38           | 7           |
| PFGE pattern       |          |              |             |
| Ci-Nm-Spe-030, n = 7 | 1        | 5            | 1           |
| Ci-Nm-Spe-031, n = 33 | 5        | 24           | 4           |
| Ci-Nm-Spe-044, n = 2 | 0        | 2            | 0           |
| Ci-Nm-Spe-046, n = 8 | 2        | 5            | 1           |
| Ci-Nm-Spe-083, n = 1 | 0        | 0            | 0           |
| Ci-Nm-Spe-084, n = 3 | 2        | 1            | 0           |
| Ci-Nm-Spe-085, n = 2 | 1        | 1            | 0           |
| Ci-Nm-Spe-086, n = 1 | 1        | 0            | 0           |
| Ci-Nm-Spe-087, n = 1 | 0        | 0            | 1           |
| Ci-Nm-Spe-088, n = 1 | 0        | 1            | 0           |
| Ci-Nm-Spe-100, n = 1 | 0        | 0            | 1           |

†PFGE, pulsed-field gel electrophoresis.

‡Codes from International Classification of Diseases, 10th Revision. Clinical outcomes: meningitis (A39.0); meningococccemia (A39.2); Other: Waterhouse-Friederichsen syndrome (A39.1); meningococccemia unspecified (A39.4); other meningococcal infections (A39.8) and nonspecified invasive meningococcal disease (A39.9).
Conclusions
We showed that most W meningococci belonged to a hypervirulent genetic lineage of the ST-11 CC. Hypervirulent serogroup W meningococci W:P1.5,2:ST-11, which has the fHbp gene allele 22, was the main cause of IMD in Chile during 2012. Its presence was associated with meningococcemia cases and partially accounted for more deaths during 2012 than in previous years. These isolates have a genetic profile similar to that of isolates from the first outbreak of IMD attributed to serogroup W, which affected the Hajj pilgrimage in Saudi Arabia in 2000, and to that of isolates from a larger outbreak in Burkina Faso in 2002 (9). However, these isolates from Chile have allele ID 22 of fHbp instead of alleles ID 9 or ID 23. This allele has also been found in a hypervirulent W:P1.5,2:ST-11 strain in Mali in 1994 (10), but we have not found more instances of its presence.

Some genotypes of these isolates have been detected in serogroup W strains obtained in previous years, specifically genosubtype (8), allele 22 of the fHbp gene (17 strains obtained in 2011), and specific PFGE patterns (9 strains were Cl-Nm-Spe-030, 4 were Cl-Nm-Spe-031, and 3 were Cl-Nm-Spe-046). These results indicate that hyperinvasive clones are circulating in Chile.

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References
1. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and Neisseria meningitidis. Lancet. 2007;369:2196–210. http://dx.doi.org/10.1016/S0140-6736(07)61016-2
2. Department of Epidemiology, Ministry of Health, Government of Chile. Epidemiological situation of meningococcal disease. September 2012 data update [in Spanish] [cited 2014 Oct 21]. http://www.sochipe.cl/subidos/noticias2/docs/Meningococo_Minsal.pdf
3. Valenzuela MT, Moreno G, Vaquero A, Seoane M, Hormazábal JC, Bertoglia MP, et al. Emergence of W135 meningococcal serogroup in Chile during 2012 [in Spanish]. Rev Med Chil. 2013;141:599–67 http://dx.doi.org/10.4067/S0034-98872013000800001.
4. Gallegos D, Maldonado A, Cáceres K, Seoane M. Epidemiologic status and fulfillment of surveillance indicators of meningococcal disease. El Vigia [in Spanish]. Epidemiological Bulletin, Ministry of Health, Government of Chile 2012; 27: 59–63 [cited 2014 Oct 21]. http://epi.minsal.cl/epi/0notransmisibles/revista/vigia27/articulo_12.pdf
5. Russell JE, Jolley KA, Feavers IM, Maiden MC, Suerk J. PorA variable regions of Neisseria meningitidis. Emerg Infect Dis. 2004;10:674–8. http://dx.doi.org/10.3201/eid1004.030247
6. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A. 1998;95:3140–5. http://dx.doi.org/10.1073/pnas.95.6.3140
7. Brechon C, Wilson DJ, Maiden MC. Variation of the factor H-binding protein of Neisseria meningitidis. Microbiology. 2009;155:4155–69. http://dx.doi.org/10.1099/mic.0.027995-0
8. Barra GN, Araya PA, Fernandez JO, Gabastu JM, Hormazábal JC, Seoane M, et al. Molecular characterization of invasive Neisseria meningitidis strains isolated in Chile during 2010–2011. PLoS ONE. 2013;8:e66006 http://dx.doi.org/10.1371/journal.pone.0066006.
9. Kelly D, Pollard AJ. W135 in Africa: origins, problems and perspectives. Travel Med Infect Dis. 2003;1:19–28. http://dx.doi.org/10.1016/S1477-8939(03)00019-X
10. Pajon R, Fergus AM, Koeberling O, Caugant DA, Granoff DM. Meningococcal factor H binding proteins in epidemic strains from Africa: implications for vaccine development. PLoS Negl Trop Dis. 2011;5:e1302 http://dx.doi.org/10.1371/journal.pntd.0001302.

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