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SHORT REPORT
A cluster of invasive meningococcal disease revealed by the characterization of a novel serogroup B meningococcal clone

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SUMMARY

The incidence of invasive infections due to *Neisseria meningitidis* in Israel is about 1/100 000 population annually. Three cases of meningococcal meningitis were reported in employees at a single plant; the first case appeared in March 2013 and the second and third cases appeared in December, almost 9 months later. *N. meningitidis* serogroup B was isolated from cerebrospinal fluid samples. Multilocus sequence typing assigned the three meningococcal isolates to ST10418, a new sequence type and a member of the ST32 clonal complex. The clonality was confirmed by performance of pulsed-field gel electrophoresis. Post-exposure antibiotic prophylaxis was administered to close contacts of the first case. Upon the diagnosis of the additional two cases, post-exposure prophylaxis was administered to all the plant employees. This report demonstrates the importance of combining public health measures and advanced laboratory studies to confirm clonality and to prevent further disease spread in a closed setting.

Key words: Cluster, invasive meningococcal disease, molecular epidemiology, *Neisseria meningitidis*.

Neisseria meningitidis is an important pathogen causing life-threatening invasive infections. Generally, in developed countries, invasive meningococcal disease (IMD) cases occur sporadically and occasionally in clusters affecting mainly adolescents and young adults, whereas often in developing countries, cases occur in the form of epidemics [1]. In Israel, the overall annual incidence of IMD is about 1/100 000 population over the past 15 years [2, 3]. Meningococcal outbreaks are infrequent and small clusters had been reported in specific population subgroups and in closed communities [4, 5].

Most IMD cases in Israel occur in young children; infants aged <1 year present the highest age-specific incidence (9/100 000 annually) and adults aged ⩾45 years present the lowest age-specific incidence (<0·1/100 000 annually) [2, 3, 6]. Most *N. meningitidis* isolates in Israel are of serogroup B (75%) while serogroups Y (12%), C (8%), W135 (4%) and A (1%) are less frequent [2–4, 6, 7]. Meningococcal vaccines are not included in the routine childhood

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immunization programme. The recent availability of *N. meningitidis* serogroup B vaccines may affect future recommendations [3, 7]. Vaccines against serogroups A, C, Y and W135 are recommended for immunodeficient patients, army recruits and international travellers. *N. meningitidis* serogroup C vaccine has been deployed in school clusters [6].

Management of meningococcal disease clusters and outbreaks is often complicated due to their unpredictability, severity and associated public anxiety. This report describes the investigation of an unusual cluster in a single factory, combining public health investigation and molecular characterization of isolates. This event adds to the understanding that a newly introduced clone can become established in a relatively closed employment community and produce disease under suitable conditions.

IMD is a notifiable disease in Israel by law, applying both to physicians and to microbiological laboratories. Cases are reported immediately to the district health office; case confirmation requires *N. meningitidis* isolation from blood, cerebrospinal fluid (CSF) or other sterile site in a patient with a clinically compatible illness. Epidemiological investigation is performed using a standard questionnaire and post-exposure prophylaxis (PEP) is provided to close contacts in accordance with Ministry of Health guidelines. A meningococcal cluster is defined as at least two IMD cases occurring within a 6-month interval with an epidemiological link.

N. meningitidis isolates are submitted to the national reference centre for meningococci for verification, serogrouping and antimicrobial susceptibility testing. Molecular characterization has been performed at the national molecular epidemiology laboratory routinely since 2007. Multi-locus sequence typing (MLST) genotyping is performed according to the standard *N. meningitidis* protocol (http://pubmlst.org/neisseria/info). Sequence analysis is performed with Bionumerics v. 7.5 (Applied Maths, Belgium) and compared to the MLST database to determine the allele number, sequence type (ST) and clonal complex (CC). Selected isolates are also analysed by pulsed-field gel electrophoresis (PFGE). *Nhe*I digests of chromosomal DNA are separated on the CHEF-DR III system (BioRad Laboratories, USA) and DNA profiles are analysed with Bionumerics v. 7·5 (Applied Maths). Analysis includes comparison to local isolates in the PFGE profile database and determination of proximity within clusters [8–10].

Case 1. On 23 March 2013, a 60-year-old man was hospitalized with fever, vomiting and headache and in an acute confusional state. Serogroup B *N. meningitidis* was isolated from the CSF. Epidemiological investigation revealed that the man worked in the maintenance department of a large factory, commuting daily on the factory bus. Close contacts between his family members, department co-workers and close contacts among passengers who regularly travelled with him on the same bus received PEP, in accordance with Ministry of Health guidelines.

Case 2. About 9 months later, on 16 December 2013, a 54-year-old man was hospitalized with a similar clinical presentation and serogroup B *N. meningitidis* was isolated from the CSF. This man worked at the same factory but in another department from case 1, commuted on the factory bus with no social connection to case 1, sat regularly at a distant seat, and thus had not received PEP. Close contacts of case 2 were first defined similar to case 1. However, the scope of persons requiring chemoprophylaxis was adjusted upon the diagnosis of an additional IMD case.

Case 3. On 18 December 2013, a 31-year-old man was hospitalized with meningitis and *N. meningitidis* serogroup B was isolated from the CSF. This patient was a temporary worker who was employed in the factory for just 1 week, in a different department from cases 1 and 2, lived in another region and thus did not commute on the factory bus. His employment ended 5 days prior to disease onset. The investigation revealed one healthy employee in the department of case 3 who used the factory bus with cases 1 and 2. Although cases 2 and 3 had disease onset 2 days apart, the district health office was notified about both cases on the same day, 19 December 2013. At this point, a broad definition of contacts was applied and post-exposure chemoprophylaxis was distributed to all the 350 factory employees. Screening for carriage was not performed as they were all treated with prophylactic antibiotics immediately. The cases were hospitalized in three different hospitals and were released upon recovery. Follow-up is continuing at the factory and to date, no further IMD cases have occurred.

Isolates from the three cases were confirmed as *N. meningitidis* serogroup B and all were susceptible to the antimicrobial agents tested (penicillin, rifampicin, ceftriaxone, minocycline, ofloxacin, ciprofloxacin).

MLST analysis of the three isolates revealed a novel ST, assigned ST10418. This ST falls within ST32 CC which is the most prevalent CC found in Israel (29·2% of isolates) since 2007. Comparison of ST10418 with the national database of invasive

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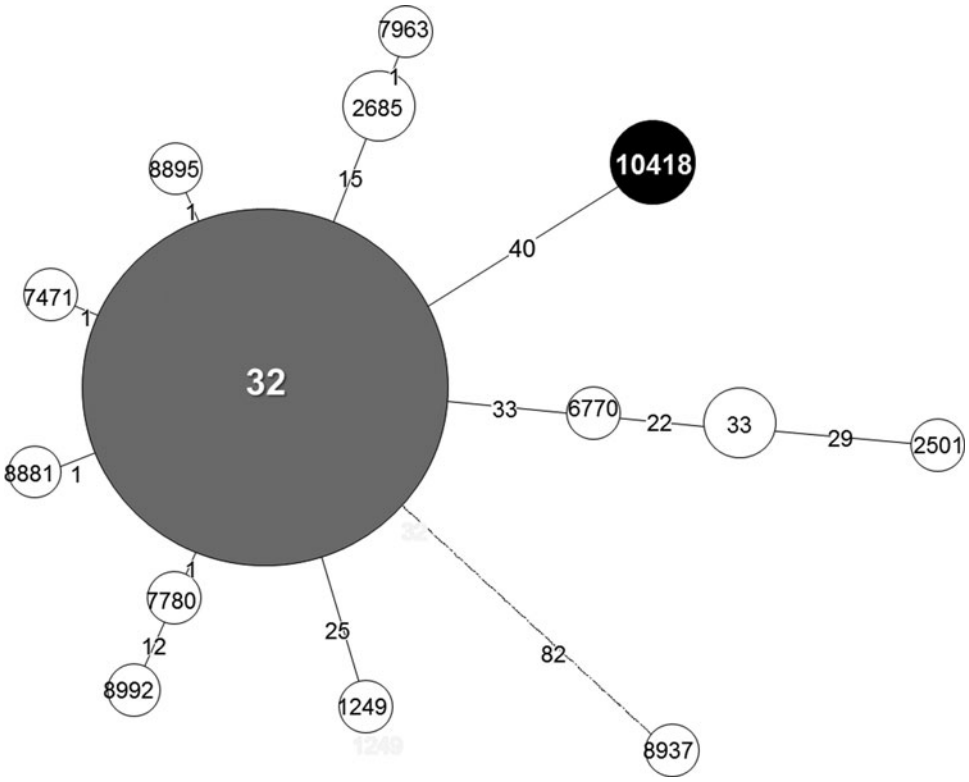

Fig. 1. Minimum spanning tree showing the sequence type (ST) distribution within the ST32 clonal complex. The ST number is shown and circle size indicates the number of strains assigned to the ST (since 2007). The numbers on the connecting lines indicate the nucleotide diversity (number of different nucleotides) between STs.

N. meningitidis and the entire MLST public database (http://pubmlst.org/neisseria/info) revealed that ST10418 differs from the ST32 CC core type ST32 by two of the seven loci examined, *abc*Z and *fum*C. The allele numbers of these ST32 genes are 4 and 4, while in ST10418, the corresponding allele numbers are 51 and 35, respectively. These differences include 22 and 18 different base pairs, respectively, and indicate a substantial difference as opposed to a point mutation.

ST10418 is the only clone identified in Israel that possesses *abc*Z allele 51, and only two other STs (ST286 and ST6572) in the public database share this allele; the latter STs differ only in single and two loci, respectively, and nave been isolated in the UK (1995) and France (2003). The other allele, *fum*C 35, has been described in the public database in 164 STs from various CCs (ST461 most prevalent) and in other meningococcal serogroups (C, Y, W135 and ungroupable strains). In Israel, *fum*C 35 has been identified previously in ST461 and ST1946, both of ST461 CC. Multiple alignment of *abc*Z and *fum*C sequences identified in Israel showed the highest similarity of *abc*Z 51 to *abc*Z 11, 12, 35 and 10 (99%) and of *fum*C 35 to *fum*C 13, 14 and 15 (99%) both of various STs.

Minimum spanning tree distribution of 14 STs within ST32 CC, identified from 84 invasive *N. meningitidis* strains, reflects the genetic distances within this CC (Fig. 1) and clearly shows that within ST32 CC, ST10481 is the most diverse clone (40 differences) from the core STs of this CC. The isolates from cases 2 and 3 were identical (100% similarity) by PFGE and differed by a single band only from the isolate of case 1. These isolates showed low similarity (56%) to other isolates of ST32, which likewise exhibited 57–73% similarity between them (Fig. 2).

We describe a cluster of three IMD cases in one factory, with nearly 9 months separating the first case from the other two, which emerged almost simultaneously. The epidemiological investigation did not reveal a close contact between the cases. Evidence of a cluster was supported by the finding of three isolates of the same *N. meningitidis* unique type by MLST which were indistinguishable by PFGE.

Phenotypic analysis and serogroup determination has insufficient discriminatory power for the unequivocal identification of *N. meningitidis* clusters. In

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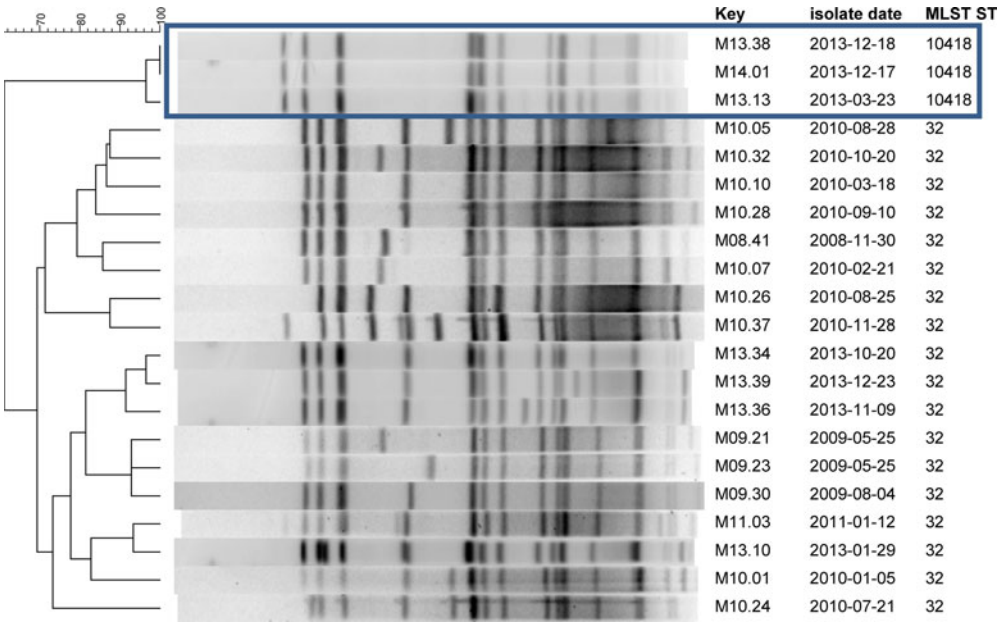

Fig. 2. Pulsed-field gel electrophoresis analysis of ST32 and ST10418 isolates, both part of the ST32 clonal complex. Clustering results of patterns obtained with *Nhe*I digestion for 21 *N. meningitidis* strains.

a US study, 10% of meningococcal disease cases (2000–2005) could be assigned to a geo-temporal cluster (about a third of clusters – serogroup B). Molecular characterization of isolates, combined with geo-temporal analysis, was found useful in detecting the spread of virulent meningococcal clones and transmission patterns [11]. A study from Germany using a combination of molecular fine typing and spatio-temporal analysis revealed that the proportion of IMD cases in spatio-temporal clusters was 4·2% [12].

The consistent and comprehensive molecular typing of all invasive *N. meningitidis* isolates since 2007 in Israel enables tracking and monitoring of strains. It has the potential to support infectious disease prevention, epidemiology and public health as well as decisions on implementation and evaluation of immunization programmes and their consequences on the population of strains associated with invasive disease.

Bioinformatic analysis and review of local and public MLST databases showed that although the new clone, ST10418, is a member of ST32 CC, it exhibits substantial differences from the core clone ST32. The variations observed in the housekeeping genes *abc*Z and *fum*C of ST10418, are likely due to horizontal genetic exchange with other clones. Alternatively, this clone could have evolved from a hitherto unidentified carriage clone acquiring virulence factors; as such clones are not routinely monitored.

PFGE fingerprinting confirmed the high degree of relatedness; the single band difference between the first and the other two cases might be interpreted as a single genetic event occurring during person-to-person transmission [10]. PFGE analysis of all ST32 isolates from the same ST32 CC revealed 73% similarity, emphasizing the clonality of the ST10418 cluster. ST10418 was isolated for the first time, and only time, in this event. ST10418 did not belong to any previously reported hypervirulent STs. The emergence of this ST has epidemiological importance that warrants monitoring of future spread.

Occasional outbreaks or local clusters of serogroup B *N. meningitidis* occurring over prolonged intervals have been reported previously. Notably, the first case in this cluster appeared in March, towards the end of the peak meningococcal season in Israel, while the other two cases occurred in December, at the start of the next season. A similar event was reported in the 1980s in a kibbutz in northern Israel, when three lethal cases in children due to a particular group B meningococcal sero-subtype occurred over a period of 4 years, surprisingly in the summer months. This entire closed community was given chemoprophylaxis following which carriage of the implicated sero-subtype was eliminated and no further cases occurred, although this outcome could not be objectively attributed to the intervention [5]. Another IMD cluster occurred in young children in

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Jerusalem in winter 2003–2004 (10 cases, three fatalities). Compared to the other 102 cases of IMD during 1999–2005 (aged 0–14 years), both the rates of meningococcaemia (100% *vs.* 51%) and of mortality (30% *vs.* 6·9%) were higher. PFGE showed considerable variability in cluster isolates [4]. Follow-up of the IMD survivors showed that one third of them suffered long-term sequelae [7]. During this investigation, MLST was unavailable at the national laboratories but subsequent MLST analysis showed that the Jerusalem cluster was caused by an organism of ST32 (L. Valinsky, unpublished data).

Similar findings have been reported in England where identification of a serogroup B *N. meningitidis* cluster was achieved with comprehensive molecular subtyping. A rare subtype, ST1194 of CC41/44 was detected from three isolates in nursery cases and two possibly related cases. This detection supported the decision making of the epidemiological team [13]. Molecular typing of isolates was performed in an investigation of a prolonged serogroup B meningococcal outbreak which emerged in a university in the United States. The isolates from six confirmed cases had an indistinguishable PFGE pattern and belonged to a novel serogroup B strain, ST269 CC269 [14].

The described cluster was confined to a closed setting and prophylactic antibiotics were provided to all workers immediately after the second and third cases. Therefore, a carriage study was not performed. In a cross-sectional study from France, carriage was evaluated at the peak of a clonal meningococcal B outbreak. Of 3522 volunteers, 196 (6·46%) were carriers, with only five having the outbreak strain (B:14:P1·7,16/ST32). The low carriage rate of the strain was in contrast to the high virulence [15].

The significance of advanced laboratory studies in epidemiological investigation and implementation of preventive measures is high. Mass chemoprophylactic intervention was applied considering that the new strain had been probably circulating among the workers during the period of low meningococcal activity (summer and autumn), with two systemic cases at the onset of the next season. The intervention objectives were to eradicate or suppress the new variant; however, further follow-up and prevalence studies are necessary. Our findings highlight the value of coordination of comprehensive epidemiological surveillance, conventional laboratory work-up and molecular analysis, to monitor trends and events and manage public health challenges.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Cohn AC, *et al.*** Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity and Mortality Weekly Report* 2013; **62**: 1–28.
2. **Block C, *et al.*** Forty years of meningococcal disease in Israel 1951–1990. *Clinical Infectious Diseases* 1993; **17**: 126–132.
3. **Ben-Shimol S, *et al.*** Dynamics of childhood invasive meningococcal disease in Israel during a 22-year period (1989–2010). *Infection* 2013; **41**: 791–798.
4. **Stein-Zamir C, *et al.*** Invasive meningococcal disease in children in Jerusalem. *Epidemiology and Infection* 2008; **136**: 782–789.
5. **Block C, *et al.*** Re-emergence of meningococcal carriage in a kibbutz population after whole-community chemoprophylaxis: a three year follow-up. *European Journal of Clinical Microbiology and Infectious Diseases* 1993; **12**: 505–511.
6. **Paret G, *et al.*** Invasive meningococcal disease: patient and strain characteristics set new challenge for prevention and control. *Infection* 1999; **27**: 261–264.
7. **Stein-Zamir C, *et al.*** The clinical features and long term sequelae of invasive meningococcal disease in children. *Pediatric Infectious Disease Journal* 2014; **33**: 777–779.
8. **Birtles A, *et al.*** Multilocus sequence typing of *Neisseria meningitidis* directly from clinical samples and application of the method to the investigation of meningococcal disease case clusters. *Journal of Clinical Microbiology* 2005; **43**: 6007–6014.
9. **Vazquez JA, *et al.*** An interlaboratory comparison of agar dilution and Etest methods to determine the antimicrobial minimum inhibitory concentration of antibiotics used in management of *Neisseria meningitidis* infections. *Antimicrobial Agents and Chemotherapy* 2003; **47**: 3430–3434.

10. **Tenover FC, *et al.*** Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology* 1995; **33**: 2233–2239.
11. **Wiringa AE, *et al.*** Geotemporal analysis of *Neisseria meningitidis* clones in the United States: 2000–2005. *PLoS ONE* 2013; **8**: e82048.
12. **Elias J, *et al.*** Spatiotemporal analysis of invasive meningococcal disease, Germany. *Emerging Infectious Diseases* 2006; **12**: 1689–1695.
13. **Chatt C, *et al.*** Four-month outbreak of invasive meningococcal disease caused by a rare serogroup B strain, identified through the use of molecular PorA subtyping, England, 2013. *Eurosurveillance* 2014; **19** (44): pii=20949.
14. **Mandal S, *et al.*** Prolonged university outbreak of meningococcal disease associated with a serogroup B strain rarely seen in the United States. *Clinical Infectious Diseases* 2013; **57**: 344–348.
15. **Delbos V. *et al.*** Meningococcal carriage during a clonal meningococcal B outbreak in France. *European Journal of Clinical Microbiology and Infectious Diseases*. 2013; **32**: 1451–1459.
16. **Jolley KA, Maiden MCJ.** BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC *Bioinformatics* 2010; **11**: 595.