Open Forum Infectious Diseases

MAJOR ARTICLE

Prevalence and Characteristics of Carriage of *Neisseria meningitidis* Among Young Israeli Adults

Noa Sofer-Sali,[1,a] Diana Roif-Kaminsky,[2,a] Yair Motro,[2] Boris Khalfin,[2] Eva Avramovich,[3] Inbal Galor,[3] Amir Shlaifer,[3] Adir Sommer,[4] Ran Rutenberg,[5] Yacov Sachter,[3] Avraham Yitzhak,[6] Daniel Grupel,[2,7] and Jacob Moran-Gilad[2]

[1]Braun School of Public Health and Community Medicine, The Hebrew University of Jerusalem-Hadassah Medical Organization, Jerusalem, Israel, [2]Department of Health Policy and Management, School of Public Health, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel, [3]Israel Defense Forces Medical Corps, Tel Hashomer, Ramat Gan, Israel, [4]Department of Ophthalmology, Rambam Health Care Campus, Haifa, Israel, [5]Emergency Department, Beilinson Hospital, Rabin Medical Center, Petah Tikva, Israel, [6]Surgery Division, Soroka University Medical Center, Beer Sheva, Israel, and [7]Department of Internal Medicine B, Soroka University Medical Center, Beer Sheva, Israel

Background. No updated data currently exist regarding *Neisseria meningitidis* carriage and genomic epidemiology among young Israeli adults.

Methods. Oropharyngeal swabs were collected from 1801 military recruits on the day of recruitment during 2019. *Neisseria meningitidis* was detected and identified by culture and quantitative polymerase chain reaction (qPCR). Confirmed isolates were serotyped by qPCR, and encapsulated strains underwent whole-genome sequencing. Risk factors for carriage were determined by analyzing focused questionnaires using uni- and multivariate models. Genomic typing was performed by means of core genome multilocus sequence typing.

Results. Carriage rates overall and of encapsulated strains were 20.1% and 6.7%, respectively. Genogroups B (49.2%) and Y (26.7%) were the most commonly encapsulated strains. Genogroups C, W, and X were scarce, and genogroup A was absent. The most notable clonal complexes (CCs) were CC23 (n = 30), CC32 (n = 16), and CC44/41 (n = 9). Carriage was significantly associated with smoking (odds ratio [OR], 1.82; 95% CI, 1.43–2.33) and boarding school attendance before recruitment (OR, 1.49; 95% CI, 1.14–1.96).

Conclusions. The prevalence of meningococcal carriage among young Israeli adults is high, compared with similar studies in other developed countries. This might be due to sociocultural characteristics including smoking and boarding school attendance during and after high school. The dominant genogroups and CCs found were compatible with those implicated in invasive disease in Israel.

Keywords. *Neisseria meningitidis*; carriage; epidemiology; genomics; risk factors.

Invasive meningococcal disease (IMD), caused by *Neisseria meningitidis*, is an important bacterial infection with a high case fatality rate and severe morbidity among survivors. Meningococcal serogroups are determined by the presence and type of the polysaccharide capsule, which is a key virulence factor of this bacterium. The major pathogenic serogroups are A, B, C, W, X, and Y [1, 2].

Widespread vaccination against serogroups A, C, W, and Y (MenACWY in high-income countries and MenA in low-income countries) has led to a decrease in the prevalence of IMD and to a shift in the serogroups associated with IMD, with serogroup B becoming dominant in many high-income countries, including Israel [3–8]. This is attributed, at least partially, to reduction in the prevalence of oropharyngeal carriage [4, 9]. Nevertheless, changes in serogroup dominance are also attributed to secular trends over the years, regardless of vaccination policies [3, 4, 6]. This emphasizes the need for better understanding of the connection between carriage and risk for invasive disease.

Oropharyngeal carriage of *N. meningitidis* is thought to be a prerequisite for the development of IMD, but the transition from carriage to invasive disease is not completely understood [10, 11]. Carriage prevalence differs between geographical areas and age groups [9, 12]. A survey conducted in 7 African countries demonstrated overall carriage of 3.4%, with a peak at the age of 5–14 years [8]. Surveys in high-income countries demonstrated higher carriage prevalence among the general population (10%–35%), with young adults between the ages of 19 and 24 years being the major carriers [3, 9, 13, 14]. In the United Kingdom, carriage among teenagers in the early 2000s was 16%–18% in 3 separate studies [3], while a more recent survey revealed a carriage rate of 7.23% [3]. A recent

Received 23 June 2022; editorial decision 12 September 2022; accepted 15 September 2022; published online 19 September 2022

[a]Equal contribution.

Correspondence: Jacob Moran-Gilad, MD, MPH, FESCMID, Department of Health Policy and Management, School of Public Health, Faculty of Health Sciences, Ben Gurion University of the Negev, Rager Blvd, Beer Sheva 8410501, Israel (giladko@post.bgu.ac.il).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofac482

Australian study that sampled >20 000 teenagers demonstrated a carriage prevalence of 4%–5% [5]. Similar results were found among Norwegian young adults (7.3%) [15], as well as among young adults age 13–14 and 21–23 years in the Netherlands [16]. The latter demonstrated a significantly higher carriage rate of up to 22.6% between the ages of 17 and 18 years [16]. Of note is that carriage rate estimation is also dependent on the laboratory methods used.

Closed and semiclosed groups such as college students, military personnel, and Hajj pilgrims are known to be at particular risk for IMD outbreaks and were also found to have higher carriage prevalence as compared with the general population. Previous studies have demonstrated carriage prevalence of up to 70% in college students during different time points of their studies, and an increase of >10% in carriage prevalence was shown among military personnel during training [16–19].

Genomic typing allows classification of meningococci beyond the conventional serogrouping. Until recently, multilocus sequence typing (MLST), which assigns sequence types (STs) and clonal complexes (CCs), has been the main typing method used for studying the molecular epidemiology of meningococci [20]. While *N. meningitidis* exhibits notable genetic diversity, with many thousands of STs deposited in the PubMLST database [21], epidemics worldwide have been found to be associated with a limited number of clonal complexes. Several CCs are considered hyperinvasive lineages, such as CC23, CC32, and CC41/44 and CC11 [20, 22]. CCs found among asymptomatic carriers are more diverse, and carried isolates are commonly associated with STs that rarely cause invasive disease [20, 23].

Whole-genome sequencing (WGS) has become the new gold standard for meningococcal typing and a main tool in epidemiological studies [20, 24]. As WGS enables the identification of clonal relationships between isolates at the strain level, it can help elucidate the origin, evolution, and spread of meningococci locally and globally. For example, WGS was an essential tool in elucidating the epidemiology of serogroup W ST11 strains after the 2000 Hajj pilgrim outbreak [20, 24]. Moreover, the use of genomic epidemiology provides insight into the carriage dynamics among populations and the link between host characteristics and carriage, thus shedding light on the risk of IMD among carriers [23].

The IMD incidence rate in Israel over the past decade was 1/10 000 in the general population and 0.25/100 000 for those older than age 15 years [7]. Clinical isolates from IMD in Israel over the last 30 years establish serogroup B as the dominant disease-causing serogroup [7, 25]. Molecular characterization of IMD-causing isolates (1998–2017) found CC32, CC41/44, CC35, CC162, and CC269 to be the most common strains [7, 25]. However, the carriage rate and serogroup distribution among carriers are largely unknown, as no carriage studies were ever conducted in Israel among the general population. The last carriage study was performed >20 years ago among soldiers near the end of their active military service, following the introduction of routine ACWY vaccination into the military vaccination policy. Overall carriage was 16%, with the majority (12%) of carriage involving serogroup B meningococci [26]. No mandatory civilian meningococcal vaccination policy currently exists in Israel.

The goal of this study was to describe the epidemiology of *N. meningitidis* carriage among a cohort of young Israeli military recruits. As Israel institutes a policy of compulsory military service, this study could partly reflect on the prevalence of carriage among the young Israeli male adult population. Moreover, the understanding of meningococcal epidemiology is crucial for the development of future public health and vaccination policy.

METHODS

Study Design and Population

This was a descriptive, prospective observational study conducted between March and December 2019. Two infantry brigades of the Israel Defense Force (IDF) served as a convenience sample, and study recruitment corresponded to the IDF enlistment cycles to combat brigades during that year (March, August, and November). All participants were recruited to the IDF for compulsory service according to national law. Participants were recruited to the study on the day of their enlistment to the IDF, which was the first day of their active service. All participants were screened for health problems before enlistment and found medically fit for combat duty. All recruits received the quadrivalent ACWY vaccine for the first time at the day of enlistment (within 24 hours of study entry) as part of the routine IDF vaccination program. None were vaccinated for serogroup B before enlistment.

Sample Collection

Samples were obtained by trained staff using a standard technique (at the military training base clinic on the day of enlistment, in conjunction with routine health examination in the clinic). Samples were obtained by swabbing the oropharynx of consenting participants using the Eswab (Copan, Brescia, Italy). Samples were stored at room temperature, transported within 24 hours [27] to Ben-Gurion University of the Negev, and were streaked and incubated immediately upon arrival.

Data Collection

Demographic and lifestyle information was collected using a questionnaire filled out by the participants immediately following study recruitment. The collected data included year of birth, place of residence, level of education, prior boarding school attendance, active and passive smoking, antibiotic use during the preceding month and over the last year, and a history of respiratory illnesses such as asthma during childhood. The place of residence was later categorized into 4 geographical

areas according to the data of the Israel Center Bureau of Statistics. The full questionnaire is provided in the supplementary.

Ethical Approval

Participation in the study was fully voluntary, and written informed consent was obtained from all participants. This study was approved by the IDF research ethics committee (approval number 1906–2018).

Laboratory Workup

All samples were streaked fresh on Martin-Lewis agar (Becton Dickinson, Sparks, MD, USA) [28, 29] and incubated for 24 hours at 35°C in ambient 5%–10% CO_2 [28, 29]. Plates were reinspected at 48 hours. Colonies suspected to be *N. meningitidis* according to morphology were subcultured for isolation and identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) [30]. Pure colonies were further studied by qPCR. Genomic DNA was extracted by heat treatment and bead beating with 425- and 600-µm glass beads (Sigma G8772) using the Precellys 24 homogenizer (Bertin Instruments, Montigny, France). Flourometric (QUBIT, QubitTM DNA HS assay, Thermo Fisher Scientific, Waltham, MA, USA) and spectrophotometric (NanoDrop, Thermo Fisher Scientific, MA, USA) assays were used to measure DNA quantity and purity (mean yield was 46 ng/µL). qPCR was performed on a minimum inhibitory concentration instrument (BMS, Brisbane, Australia). Species identification was confirmed using the gene targets *metA* and *tauE,* and presence of capsule was predicted by the presence of the gene target *ctrA,* as previously described [30–32]. Molecular genogrouping was performed for all isolates (regardless of *ctrA* presence), with qPCR using primers and probes recommended by the US Centers for Disease Control and Prevention for A, B, C, W, X, and Y [33]. Phenotypic serogrouping was not in the scope of the current study.

Whole-Genome Sequencing

Our study focused on the identification and description of invasive meningococcal strains. During the March 2019 cycle, all confirmed isolates were subject to WGS. Subsequent analysis showed *ctrA* detection by qPCR to be an excellent predictor of capsular genogroups, and thus only *ctrA*-positive isolates were subject to WGS in cycles. Consequently, WGS data in this paper are presented only for capsular genogroups (ie, A, B, C, W, X, Y, E, and Z). Library preparation was performed using the Nextera FLEX library preparation kit (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. The libraries were sequenced using Illumina NextSeq 500, producing 150-bp paired-end reads. Raw reads were checked for quality using FastQC (version 0.11.5) with MultiQC (version 1.9) and species ID using Kraken2 (version 2.0.7-beta, with the Kraken2 standard database, Jul. 2018) [34, 35]. For each isolate, reads that passed quality check (QC) and were identified as *N. meningitidis* were trimmed with Trimmomatic (version 0.39) [36] and de novo assembled using Shovill (version 1.0.4, with SPADes, version 3.13.1, as the assembler) [37, 38]. The assembled genome for each isolate underwent in silico MLST using mlst (version 2.17.6, with the *Neisseria* spp. ST profiles and clonal complex assignments from pubMLST [www.pubmlst.org], Sept. 2019) [39, 40]. In silico capsule serotyping was done with the scripts from https://github.com/ntopaz/characterize_neisseria_capsule. In silico MLST results were also obtained (as a cross-check) using meningotype (version 0.8.4).

All assembled genomes were analyzed to determine an ad hoc cgMLST scheme using chewBBACA (version 2.0.16) [41]. The resulting ad hoc cgMLST scheme consisted of 1414 loci (at 95% presence). A minimal spanning tree (MST) was determined and visualized using the MSTreeV2 method in GrapeTree (version 1.5.0) [39]. While ad hoc cgMLST may offer a slightly higher resolution, our analysis was complemented by performing a cgMLST analysis based on the published PubMLST scheme for ensuring consistency with the literature. Genome assemblies were uploaded to pubMLST (https://doi.org/10.1186/1471-2105-11-595) and assigned pubMLST IDs (NM-ISR-[1-121]). The uploaded genome assemblies were analyzed using the *N. meningitidis* cgMLST, version 2, scheme (3098 loci; 2022-08-14) via the pubMLST Genome Comparator, version 2.7.4, plugin, and an MST was then generated and visualized via the pubMLST GrapeTree, version 1.5.0, plugin. Genome sequences are publicly available on PubMLST (PubMLST IDs NM-ISR-[1-121]).

Sample data of capsular genogroups are summarized in Supplementary Table 1.

Statistical Analysis

Statistical analysis was performed using SPSS 25.0. Risk factors for overall carriage were assessed, and a subanalysis was done for risk factors for carriage of encapsulated strains. In both analyses, a chi-square test and Fisher exact test were used for a univariate analysis. All variables with a P value <.1 were included in a multivariate analysis using binary regression to identify independent risk factors associated with carriage.

RESULTS

Population Characteristics

A total of 1809 participants were enrolled in the study, of whom 8 were excluded due to missing questionnaires. All participants were male in their young adulthood. Of the included participants, 639 (35.5%) were recruited in March, 508 (28.2%) in August, and 654 in November (36.3%). The majority of participants had a high school education, with almost a quarter

Table 1. Demographic and Lifestyle Characteristics

	March (n = 639)	August (n = 508)	November (n = 654)	Total (n = 1801)
Age, y	19.52 ± 0.76	19.1 ± 1.05	18.48 ± 0.78	19.02 ± 0.96
Full high school education	616 (96.4)	501 (98.6)	638 (97.6)	1756 (97.5)
Boarding school attendance	189 (29.6)	141 (27.8)	96 (14.5)	425 (23.6)
Area of residence[a]				
Center	231 (36.2)	196 (38.6)	271 (41.4)	698 (40.5)
Jerusalem	130 (20.3)	99 (19.5)	104 (15.9)	333 (19.3)
South	105 (16.4)	91 (17.9)	124 (19.0)	320 (18.6)
North	146 (22.8)	100 (19.7)	128 (19.6)	374 (21.7)
Active smoking	207 (32.4)	162 (31.2)	233 (35.6)	602 (33.4)
Passive smoking	254 (39.7)	219 (43.1)	300 (45.9)	773 (42.9)
Antibiotic use in the previous month	36 (5.6)	26 (5.1)	34 (5.2)	96 (5.3)
Antibiotic use in the previous year	169 (26.4)	113 (22.2)	109 (16.7)	391 (21.7)
Childhood respiratory Diseases	36 (5.6)	27 (5.3)	35 (5.4)	99 (5.5)

Data are presented as mean ± SD or No. (%).

[a]Data were available for 95.2% of the March subcohort, 95.7% of the August subcohort, and 95.9% of the November subcohort.

(23.6%) reporting boarding school attendance before recruitment. More than one-third of the cohort reported active smoking (33.4%), 5.3% used antibiotics in the month preceding recruitment, and 5.5% reported a childhood respiratory disorder. The characteristics of participants are shown in Table 1.

Carriage Prevalence

Carriage prevalence was 20.1% (361/1801) for the total cohort, and 22.7% (145/639), 21.3% (108/508), and 16.5% (108/654) for the March, August, and November subcohorts, respectively. The lower prevalence in November was statistically significant when compared with March (odds ratio [OR], 0.668; 95% CI, 0.55–0.81; $P = .004$).

Of 361 carrier-related isolates, all were positive to *tauE*, and 316 (87%) were positive to *metA*. *ctrA* presence was detected by qPCR in 120 of them (33.2%). All isolates were also subject to genogrouping using qPCR. No *ctrA*-negative isolates were found to be qPCR positive for any of the tested genogroups. Genogrouped invasive isolates were also confirmed by WGS (see below). For the March 2019 cycle, there were an additional 90 *ctrA*-negative isolates that were subject to WGS. Of those, none were found to belong to invasive genogroups or harbor the capsular region, and 71 were capsule null and carried the cnl gene.

Thus, of the total cohort, 6.7% individuals were found to be carriers of *ctrA*-positive isolates (120/1801). No statistically significant temporal changes were observed in carriage, probably due to the smaller numbers of invasive isolates. The respective prevalence rates were 8.3% (53/639), 6.7% (34/508), and 5.0% (33/654) in March, August, and November ($P = .59$).

Risk Factors for Carriage

The results of the uni- and multivariate analyses are presented in Table 2. Active smoking and boarding school attendance before recruitment were associated with higher carriage rates ($P < .001$ and $P = .004$, respectively), while antibiotic use 1 month before recruitment was inversely associated with carriage. As the reported annual incidence rate in Jerusalem is high, carriage prevalence in this region was compared with other regions in Israel [42]. Residence in Jerusalem was associated with carriage with borderline significance in the univariate analysis (OR, 1.32; $P = .05$). This trend was also evident in the multivariate analysis (OR, 1.31; $P = .072$).

Risk factors for carriage of capsular genogroups (as compared with noncarriers) were similar to those associated with carriage in general, excluding recent antibiotic use, which was not associated with loss of carriage of capsular genogroups (95% CI, 0.19–1.49; $P = .21$). Active smoking and boarding school attendance before recruitment were found to be significantly associated with carriage. Data are summarized in Table 3.

Genogrouping Distribution

Genogrouping results are presented for *ctrA*-positive isolates only. Notably, molecular genogrouping using qPCR for genogroups C, B, Y, W, and X was in complete agreement with in silico predicted genogrouping using WGS. Genogroups E and Z, which were not included in the qPCR procedure, were identified using WGS only. The main genogroup was B (3.3%, 59/1801), followed by genogroup Y (1.8%, 32/1801). These 2 genogroups accounted for 49.2% and 26.7% of invasive isolates, respectively. Carriage of genogroups W (0.3%, 6/1801) and X (0.15%, 3/1801) was uncommon within the total cohort, and only 1 genogroup C isolate was detected. Genogroup A was absent in all 3 recruitment cycles. Other *ctrA*-positive isolates exhibited uncommon genogroups (ie, Z, E, and nongroupable) and accounted for 1% of the total cohort (19/1801).

Table 2. Risk Factors Associated With Carriage of *N. meningitidis*

	Carriers (n = 361), No. (%)	Noncarriers (n = 1440), No. (%)	Univariate OR [95% CI], *P* Value	Multivariate[a] OR [95% CI], *P* Value
Full high school education	357 (98.6)	1399 (97.2)	0.33 [0.083–1.38], .11	
Boarding school attendance	107 (29.6)	318 (22.1)	1.46 [1.13–1.9], .003	1.49 [1.14–1.96], .004
Area of residence[b] Jerusalem	81 (22.4)	252 (17.52)	1.32 [1.00–1.76], .05	1.31 [0.98–1.75], .072
Active smoking	159 (43.9)	443 (30.8)	1.74 [1.38–2.21], <.001	1.82 [1.43–2.33], <.001
Passive smoking	166 (46.1)	607 (42.1)	1.16 [0.92–1.47], .21	
Antibiotic use in the previous month	8 (2.2)	88 (6.1)	0.35 [0.17–0.72], .003	0.35 [0.17–0.74], .006
Antibiotic use in the previous year	73 (20.2)	318 (22.1)	0.98 [0.67–1.18], .40	
Childhood respiratory diseases	23 (6.4)	76 (5.3)	1.21 [0.75–1.95], .45	

Abbreviation: OR, odds ratio.

[a]Multivariate analysis using binary regression was performed for $P < .1$ in the univariate analysis. $P < .05$ was considered statistically significant.

[b]Residence in Jerusalem was compared with residence in all other geographical areas in Israel combined.

Genomic Typing per MLST and cgMLST

Invasive genogroup isolates belonged to 15 CCs and 37 STs. Of the 120 *ctrA*-positive isolates, 10 (8.3%) were found to have novel STs, and these were deposited in PubMLST. Another 8 isolates (6.7%) could not be assigned to any ST, known or unknown, due to technical limitations in the WGS analysis, related to sequence quality or integrity of loci. The most common CC was CC23 (25.0%, 30/120), followed by CC32 (13.3%, 16/120). CC60 (9.2%, 11/120) and CC41/44 (7.5%, 9/120) were the third and fourth most common CCs, respectively. The genogroup C isolate was assigned a new ST and thus did not belong to any known CC; the 2 genogroup X isolates belonged to CC1157 and to a new ST. Data for genogroups B, Y, W, and other uncommon genogroups (ie, Z, E, and nongroupable) are summarized in Figure 1.

Almost no overlap between STs or CCs was observed among genogroups in this cohort. ST23 (13.3%, 16/120) and ST1655 (12.5%, 15/120) were the dominant STs in the cohort; both were identified among genogroup Y isolates, and both belonged to CC23. This CC accounted for 97% of all genogroup Y isolates. ST5770 was identified in a single isolate. Genogroup B isolates were associated with 19 STs and 8 CCs, with CC32 being the most common (11.7%, 14/120). This CC consisted of 2 STs: ST7460 (n = 8) and ST32 (n = 6), comprising 6.7% and 5% of overall isolates. ST7152, which belonged to CC1572, was the second most frequent within genogroup B (5.8%, 7/120). Among genogroup W, 2 isolates belonged to CC11 and were identified as ST11 (1.6%, 2/120). The genomes of CC11/ST11 isolates were compared with those of previously reported IMD-causing isolates belonging to CC11 worldwide and were not found to be identical. The distribution of STs by genogroups is shown in Figure 2. The genomes of *ctrA*-positive, capsular genogroups (ie, B, C, Y, W, X, E, Z) were analyzed by means of an ad hoc cgMLST scheme (Figure 3). One genogroup B isolate and 1 genogroup E isolate failed QC and thus were excluded from the minimum spanning tree. Genogroups varied in diversity, with genogroup B demonstrating a much greater diversity as compared with genogroup Y, which demonstrated almost no variability between the 2 main STs that were identified among these isolates. The analysis was complemented by another minimum spanning tree, generated using the PubMLST cgMLST scheme. These data

Table 3. Risk Factors Associated With Carriage for *ctrA*-Positive Isolates

	Carriers (n = 120), No. (%)	Noncarriers (n = 1440), No. (%)	Univariate OR (CI), *P* Value	Multivariate[a] OR (CI), *P* Value
Full high school education	118 (97.5)	1399 (97.2)	1.01 [0.24–4.33], 1.00	
Boarding school attendance	36 (29.8)	318 (22.1)	1.48 [0.98–1.9], .06	1.62 [1.07–2.46], .03
Area of residence[b] Jerusalem	28 (23.1)	252 (17.52)	1.42 [0.91–2.20], .13	
Active smoking	57 (47.1)	443 (30.8)	1.98 [1.37–2.89], <.001	2.09 [1.43–3.05], <.001
Passive smoking	56 (46.3)	607 (42.1)	1.17 [0.80–1.70], .44	
Antibiotic use in the previous month	4 (3.3)	88 (6.1)	0.53 [0.19–1.46], .21	0.35 [0.17–0.74], .006
Antibiotic use in the previous year	27 (22.3)	318 (22.1)	1.02 [0.65–1.59], .95	
Childhood respiratory diseases	8 (6.6)	76 (5.3)	1.26 [0.59–2.98], .55	

Abbreviation: OR, odds ratio.

[a]Multivariate analysis using binary regression was performed for $P < .1$ in the univariate analysis. $P < .05$ was considered statistically significant.

[b]Residence in Jerusalem was compared with residence in all other geographical areas in Israel combined.

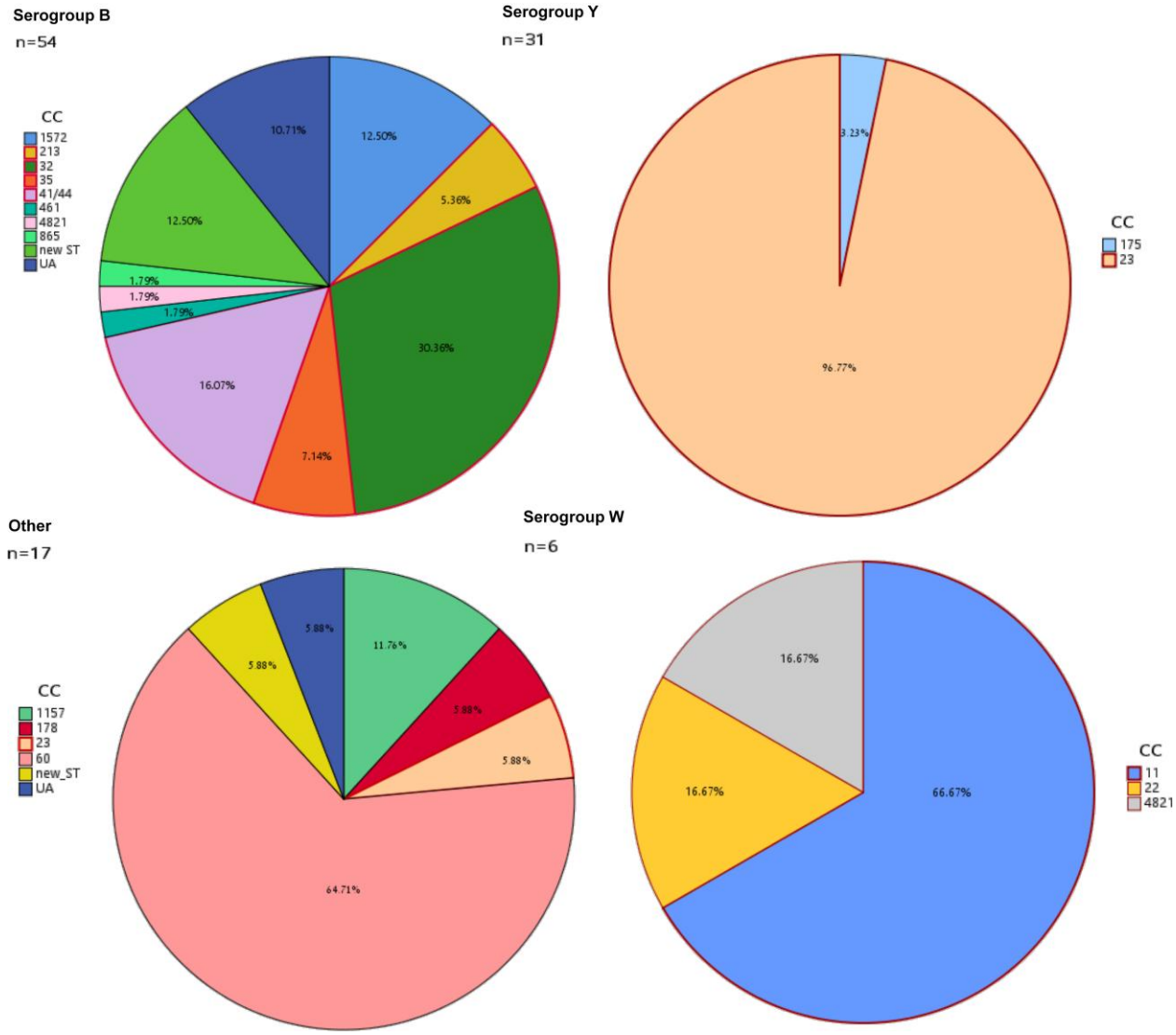

Figure 1. Distribution of clonal complexes of 108 study isolates based on MLST. Genogroups A, C, and X are not presented due to the small number of isolates (0, 1, and 3, respectively). Isolates that were not assigned an ST were excluded. Isolates that were found to have novel STs are referred to as "new ST." Isolates belonging to a known ST but not a known CC are referred to as "UA." CCs that were isolated from IMD cases in Israel over the past decade [7] are denoted in red. Abbreviations: CC, clonal complex; IMD, invasive meningococcal disease; ST, sequence type; UA, unassigned.

are presented in Supplementary Figure 1. The scheme-based tree generated similar results, with notable diversity among genogroup B isolates and notable similarity within the genogroup Y isolates. High similarity was identified among genogroup W isolates in both MSTs.

DISCUSSION

The global epidemiology of *N. meningitidis* has changed dramatically over the past 2 decades. These changes were attributed, at least partially, to the worldwide implementation of vaccination programs, as well as to secular trends [4, 6]. The understanding of global and regional carriage prevalence changes as well as serogroup distribution and molecular structure is crucial to the understanding of IMD development.

This study is the first in >20 years to investigate the prevalence of carriage of meningococci among Israeli soldiers, a risk group for IMD, and reflects on the potential introduction of hyperinvasive clones into this closed community. Moreover, as the participants were recruited to the study at the point of entry into military duty, this cohort mirrors, with some limitations, the epidemiology of carriage among young adults in Israel. This is the first study in Israel to use molecular and genomic methods to describe the genomic epidemiology of carriage. In our study, the prevalence of carriage was 20.1% for meningococci overall and 6.7% for the main invasive meningococcal genogroups. These rates are notably higher than those

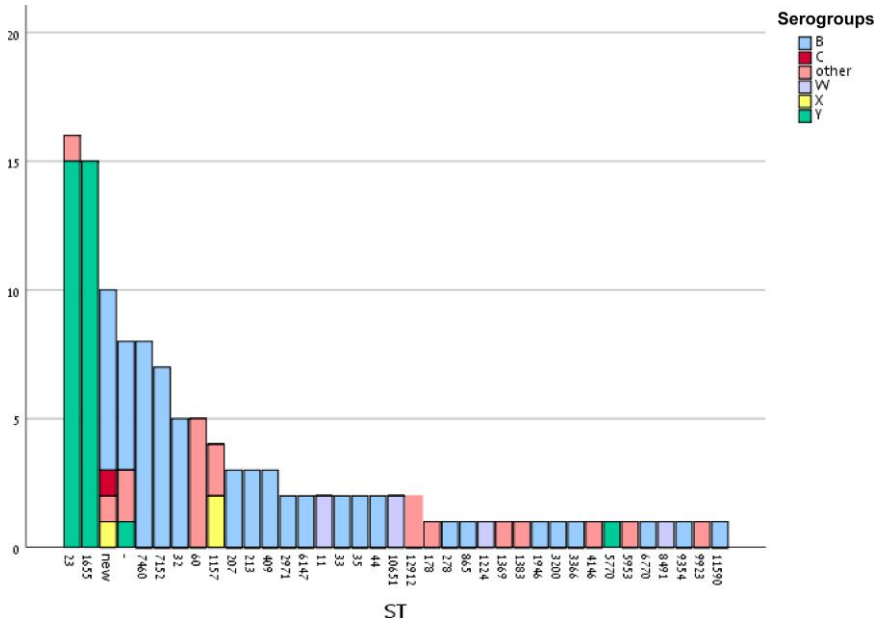

Figure 2. ST distribution by genogroup, shown in a decreasing order of frequency. Data refer to 120 *ctrA*-positive isolates. "Other" refers to serogroups E and Z and nongroupable isolates. Isolates that were found to have novel STs are referred to as "new." Isolates that were not assigned any ST due to technical limitations are denoted as "-." Abbreviation: ST, sequence type.

found in 2 of the largest carriage prevalence studies conducted in the past 5 years (in the United Kingdom and Australia), which demonstrated an overall prevalence of carriage of 4%–7.2% [3, 5]. Moreover, previous studies among military recruits demonstrated lower carriage rates compared with those found in our study, for example, 16% among military recruits in Russia [19], 15% in Greece [43], and only 2.2% in Finland [17].

The relatively high prevalence of carriage found in our study may be related to the study population. This study differs in age group and sex distribution from other nonmilitary carriage studies in late adolescence or young adulthood, due to our cohort being slightly older and male-only, both being known risk factors for meningococcal carriage [9, 14].

In addition, the rate of smoking reported in our study was 33.4%, much higher than that reported among the general population (15% among Israeli adolescents and 20.1% in the general population) [44, 45], which further increases the risk for carriage. It is worth mentioning that smoking of electronic cigarettes [3, 15] was not surveyed in this study, as it was widely acknowledged as a significant risk factor after the study commenced.

Almost a quarter of the participants in our cohort reported boarding school attendance before their enlistment, including residency in "boarding school–like" facilities after high school and before recruitment, as part of voluntary community service or advanced religious studies. As living in closed and semiclosed groups such as college students living in dormitories is a known risk factor for carriage [10], boarding school attendance should be considered a risk factor for carriage.

Taken together, young Israeli adults have several characteristics at the time of recruitment, compared with other studied populations, that make them more prone to meningococcal carriage. It is important to note that 2 important groups (Israeli Arabs and ultra-orthodox jews) were not represented in this study as most members of those communities do not enroll into military service. Whether the carriage rate among these subpopulations is similar or lower deserves further study [42]. Known risk factors such as participation in social events (such as attendance at pubs and nightclubs) and intimate kissing [46] were not addressed in this study. Moreover, our cohort was male-only. These limitations could hamper comparison of our study with previous studies and should be addressed in future studies.

Among invasive genogroups, genogroup B was the most common (50.8% of invasive isolates, 3.4% of participants), followed by genogroup Y (26.7% and 1.8%, respectively). While it is highly likely that invasive genogroup isolates confirmed by qPCR and WGS express a capsule, phenotypic confirmation was not performed in the scope of the current study, which is a limitation. The dominant CCs among genogroup B isolates were CC32 and CC41/44, while CC23 accounted for 97% of genogroup Y isolates. These CCs were previously found to be hyperinvasive [23]. Interestingly, in a national survey of IMD, CC32, CC23, and CC41/44 were reported to cause >61% of IMD cases in Israel between 1998 and 2017 [7].

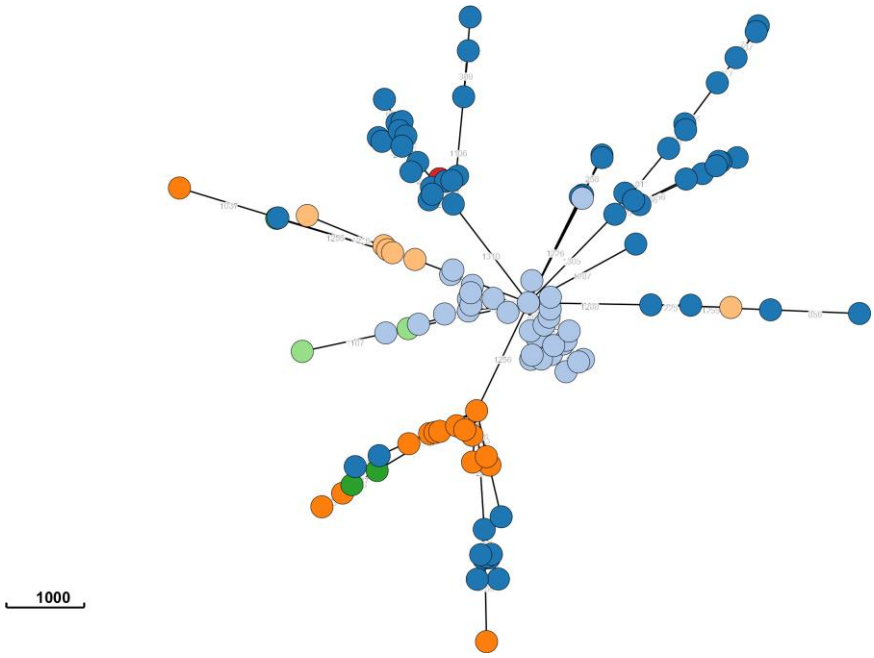

Figure 3. An MST featuring an ad hoc cgMLST analysis of 117 *ctrA*-positive isolates (isolates that failed quality check or nongroupable isolates are not presented in this tree). The ad hoc cgMLST scheme consisted of 1412 loci. Color coding denotes serogroup assignment. Node size is proportional to the number of isolates assigned to clone types (in that case, all types were singletons). Numbers denote the allelic distances between nodes. Abbreviation: MST, minimum spanning tree.

A notable genetic diversity was demonstrated among genogroup B isolates in our cohort (19 different STs). Importantly, >50% of these STs were previously reported as causing disease in Israel [7]. Only 1 genogroup C isolate was found in this cohort, and none genogroup A. Genogroup W isolates were rare, with a carriage rate of 0.3%, including 4 isolates belonging to CC11 and 2 isolates belonging to ST11 [22]. As ST-11 is known to be hyperinvasive and the cause of outbreaks in different countries, this finding may be concerning regarding the introduction of hyperinvasive strains into an at-risk population [22]. However, none of the isolates were genetically related to the CC11 strains that were previously associated with IMD outbreaks.

These data highlight the likely connection between meningococcal carriage and IMD development as the most carried strains in our cohort are those commonly implicated in IMD. As genogroup B was found to be the dominant invasive genogroup, implementation of MenB vaccinations into the military vaccination policy should be discussed, taking into account considerations such as the IMD incidence among military recruits and servicemen and cost-effectiveness analyses, to name a few. However, these analyses are beyond the scope of this paper.

Our cohort lays the foundation for elucidation of meningococcal carriage in young adults in Israel and highlights the need in further carriage studies among the civilian population. Moreover, this cohort reflects on the genogroups and strains being introduced by the constant influx of new recruits into military servicemen populations. The extent to which those strains are cross-transmitted during military service and the risk factors for such transmission, including interactions with the respiratory microbiome and possible health implications, deserve further study.

CONCLUSIONS

This study is the first in 20 years to assess the prevalence of meningococcal carriage among Israeli soldiers. These new data suggest that carriage prevalence of meningococci among this age group is higher than that reported in similar populations in other high-income countries. This carriage rate is likely associated with several epidemiological characteristics such as young male predominance, smoking, and boarding school attendance.

While unencapsulated isolates (per qPCR) constitute the majority of carriage, carriage rates of invasive genogroup isolates, especially B and Y, were notable. Our genomic analysis demonstrated that the majority of invasive strains were previously reported as disease-causing among the Israeli population in national surveillance studies, including hyperinvasive strains. The introduction of disease-causing strains into a vulnerable closed community such as military recruits commencing basic training is noteworthy, and the effect of high carriage on IMD incidence rates, as well as the dynamics of carriage over time, cross-transmission, and risk for disease, warrants future

study. The data presented herein are expected to inform future policy development with respect to IMD prevention among young adults and in the military setting.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

We thank the IDF recruits who volunteered to participate in this study and the military base personnel who facilitated this study. We would also like to thank Mr. Omer Grufinkel and Ms. Kelly Vaknin for their commitment and contribution to the field work in the study and the members of the Moran-Gilad laboratory at Ben Gurion University of the Negev for their assistance.

Financial support. The principal investigator, Prof. Moran-Gilad, received funding support from the Israeli Defense Forces Medical Corps and an independent grant (Investigator Initiated Research, project #53234197) from Pfizer Inc.

Potential conflicts of interest. All authors reported no conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Patient consent. This study was approved by the IDF research ethics committee (approval number 1906–2018). Study enrollment followed the procedures set in the IDF General Staff Directives. Each participant was approached with written informed consent and provided a verbal explanation of the study purposes and procedures. Participants had the opportunity to review the written consent form, consult a family member, and ask questions before signing.

References

1. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. Vaccine **2009;** 27:B51–63.
2. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. Lancet **2007;** 369:2196–210.
3. MacLennan JM, Rodrigues CMC, Bratcher HB, et al. Meningococcal carriage in periods of high and low invasive meningococcal disease incidence in the UK: comparison of UKMenCar1–4 cross-sectional survey results. Lancet Infect Dis **2021;** 21:677–87.
4. Whittaker R, Dias JG, Ramliden M, et al. The epidemiology of invasive meningococcal disease in EU/EEA countries, 2004–2014. Vaccine **2017;** 35:2034–41.
5. Marshall HS, McMillan M, Koehler AP, et al. Meningococcal B vaccine and meningococcal carriage in adolescents in Australia. N Engl J Med **2020;** 382:318–27.
6. Baccarini C, Ternouth A, Wieffer H, Vyse A. The changing epidemiology of meningococcal disease in North America 1945–2010. Hum Vaccin Immunother **2013;** 9:162–71.
7. Stein-Zamir C, Shoob H, Abramson N, et al. Invasive meningococcal disease epidemiology and characterization of *Neisseria meningitidis* serogroups, sequence types, and clones; implication for use of meningococcal vaccines. Hum Vaccin Immunother **2019;** 15:242–8.
8. Ali O, Aseffa A, Bedru A, et al. The diversity of meningococcal carriage across the African meningitis belt and the impact of vaccination with a group a meningococcal conjugate vaccine. J Infect Dis **2015;** 212:1298–307.
9. Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: a systematic review and meta-analysis. Lancet Infect Dis **2010;** 10: 853–61.
10. Peterson ME, Mile R, Li Y, Nair H, Kyaw MH. Meningococcal carriage in high-risk settings: a systematic review. Int J Infect Dis **2018;** 73:109–17.
11. Caugant DA, Maiden MCJ. Meningococcal carriage and disease—population biology and evolution. Vaccine **2009;** 27:B64–70.
12. Pelton SI. The global evolution of meningococcal epidemiology following the introduction of meningococcal vaccines. J Adolesc Health **2016;** 59(2 Suppl):S3–11.
13. Findlow H, Campbell H, Lucidarme J, et al. Serogroup C *Neisseria meningitidis* disease epidemiology, seroprevalence, vaccine effectiveness and waning immunity, England, 1998/99 to 2015/16. Euro Surveill **2019;** 24:1700818.
14. Cartwright KAV, Stuart JM, Jones DM, Noah ND. The Stonehouse Survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol Infect **1987;** 99:591–601.
15. Watle SV, Caugant DA, Tunheim G, et al. Meningococcal carriage in Norwegian teenagers: strain characterization and assessment of risk factors. Epidemiol Infect **2020;** 148:e80.
16. van Ravenhorst MB, Bijlsma MW, van Houten MA, et al. Meningococcal carriage in Dutch adolescents and young adults; a cross-sectional and longitudinal cohort study. Clin Microbiol Infect **2017;** 23:573.e1–7.
17. Jounio U, Saukkoriipi A, Bratcher HB, et al. Genotypic and phenotypic characterization of carriage and invasive disease isolates of *Neisseria meningitidis* in Finland. J Clin Microbiol **2012;** 50:264–73.
18. Andersen J, Berthelsen L, Bech Jensen B, Lind I. Dynamics of the meningococcal carrier state and characteristics of the carrier strains: a longitudinal study within three cohorts of military recruits. Epidemiol Infect **1998;** 121:85–94.
19. Sidorenko S, Zakharenko S, Lobzin Y, et al. Observational study of nasopharyngeal carriage of *Neisseria meningitidis* in applicants to a military academy in the Russian federation. Int J Infect Dis **2019;** 81:12–16.
20. Caugant DA, Brynildsrud OB. *Neisseria meningitidis*: using genomics to understand diversity, evolution and pathogenesis. Nat Rev Microbiol **2020;** 18: 84–96.
21. *Neisseria* isolates database. Available at: https://pubmlst.org/bigsdb?db=pubmlst_neisseria_isolates. Accessed April 12, 2022.
22. Lucidarme J, Hill DMC, Bratcher HB, et al. Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. J Infect **2015;** 71:544–52.
23. Joseph SJ, Topaz N, Chang H-Y, et al. Insights on population structure and within-host genetic changes among meningococcal carriage isolates from U.S. universities. mSphere **2020;** 5:e00197–20.
24. Retchless AC, Fox LM, Maiden MCJ, et al. Toward a global genomic epidemiology of meningococcal disease. J Infect Dis **2019;** 220:S266–73.
25. ירושלים. המעבדות המרכזיות ירושלים. Available at: https://www.health.gov.il/PublicationsFiles/LAB_JER2019.pdf. Accessed January 17, 2022.
26. Mimouni D, Gdalevich M, Mandel Y, et al. Meningococcal polysaccharide vaccination of military recruits in Israel: preliminary assessment of vaccine effect. Scand J Infect Dis **1998;** 30:263–4.
27. Phillips I, Humphrey D, Middleton A, Nicol CS. Diagnosis of gonorrhoea by culture on a selective medium containing vancomycin, colistin, nystatin and trimethoprim (VCNT). A comparison with gram-staining and immunofluorescence. Br J Vener Dis **1972;** 48:287–92.
28. Gasparini R, Comanducci M, Amicizia D, et al. Molecular and serological diversity of *Neisseria meningitidis* carrier strains isolated from Italian students aged 14 to 22 years. J Clin Microbiol **2014;** 52:1901–10.
29. Jang KS, Kim YH. Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. J Microbiol **2018;** 56:209–16.
30. Diene SM, Bertelli C, Pillonel T, et al. Comparative genomics of *Neisseria meningitidis* strains: new targets for molecular diagnostics. Clin Microbiol Infect **2016;** 22:568.e1–7.
31. Zhang Z, Xu X, Chen K. PCR for detection and characterization of bacterial meningitis pathogens: *Neisseria meningitidis, Haemophilus influenzae,* and *Streptococcus pneumoniae*. **2014**. Available at: http://www.cdc.gov/meningitis/lab-manual/chpt10-pcr.html.
32. Centers for Disease Control and Prevention. Meningitis lab manual: PCR detection and characterization. Available at: https://www.cdc.gov/meningitis/lab-manual/chpt10-pcr.html. Accessed July 19, 2021.
33. Babraham Bioinformatics. FastQC: a quality control tool for high throughput sequence data. Available at: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Accessed July 16, 2021.
34. Ewels P, Magnusson M, Lundin S, Käller M. Multiqc: summarize analysis results for multiple tools and samples in a single report. Bioinformatics **2016;** 32: 3047–8.
35. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **2014;** 30:2114–20.
36. Seeman T, Edwards R, Goncalves da Silva A, Kiil K. GitHub—Tseemann/Shovill: assemble bacterial isolate genomes from Illumina paired-end reads. Available at: https://github.com/tseemann/shovill. Accessed July 16, 2021.
37. Nurk S, Bankevich A, Antipov D, et al. Assembling genomes and mini-metagenomes from highly chimeric reads. In: Deng M, Jiang R, Sun F, et al, eds. Lecture Notes in Computer Science. Springer; **2013**:158–70.
38. GitHub. Tseemann/MLST: scan contig files against PubMLST typing schemes. Available at: https://github.com/tseemann/mlst. Accessed July 16, 2021.

39. Jolley KA, Maiden MC. Bigsdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinf **2010;** 11:595.
40. Silva M, Machado MP, Silva DN, et al. Chewbbaca: a complete suite for gene-by-gene schema creation and strain identification. Microbial Genomics **2018;** 4:e000166.
41. Stein-Zamir C, Abramson N, Zentner G, Shoob H, Valinsky L, Block C. Invasive meningococcal disease in children in Jerusalem. Epidemiol Infect **2008;** 136: 782–9.
42. Tryfinopoulou K, Kesanopoulos K, Xirogianni A, et al. Meningococcal carriage in military recruits and university students during the pre MenB vaccination era in Greece (2014–2015). PLoS One **2016;** 11:e0167404.
43. Zarka S, Levine H, Rozhavski V, et al. Original investigation smoking behavior change during compulsory military service in Israel, 1987–2011. Nicotine Tob Res **2017;** 19:1322–9.
44. גבע-הספיל ג. דו"ח שר הבריאות על עישון בישראל. **2020**. Available at: https://www.health.gov.il/PublicationsFiles/smoking_2020.pdf. Accessed May 05, 2022.
45. MacLennan J, Kafatos G, Neal K, et al. Social behavior and meningococcal carriage in British teenagers. Emerging Infect Dis **2006;** 12:950–7.
46. Mustapha MM, Marsh JW, Shutt KA, et al. Transmission dynamics and microevolution of *Neisseria meningitidis* during carriage and invasive disease in high school students in Georgia and Maryland, 2006–2007. J Infect Dis **2021;** 223:2038–47.