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Observational study of nasopharyngeal carriage of *Neisseria meningitidis* in applicants to a military academy in the Russian Federation

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ABSTRACT

Objective: To determine the carriage and the serogroup distribution of *Neisseria meningitidis* in military academy applicants in the Russian Federation.
Design: This was a prospective, observational study of adults aged >18 years from a military academy; applicants who had samples taken on arrival (Day 1), and applicants who had samples taken after passing exams (Day 30) and 60 days after arrival. *N. meningitidis* serogrouping was determined by slide agglutination tests of isolates and real-time PCR.
Results: Samples were provided by 671 applicants on Day 1 and 261 applicants on Day 30, with 232 of these also providing samples on Day 60. *N. meningitidis* was detected in 16.2% of samples from Day 1, 7.7% of samples from Day 30 and 15.9% of samples from Day 60. Serogroup composition was most diverse at Day 1, with serogroups B and W dominant (40% [17/43 isolates] and 9% [4/43], respectively; 30% [13/43] ungroupable); by Day 60, there was a low diversity, with 58% (14/24 isolates) serogroup W.
Conclusions: While carriage of *N. meningitidis* in this study appeared stable, there was an increase in carriers of serogroup W in this population. Given recent increases in outbreaks attributed to serogroup W, further monitoring may be considered.

Introduction

The *Neisseria meningitidis* bacteria can lead to meningococcal disease, a contagious disease with substantial morbidity and mortality, particularly in young children; with six serogroups (A, B, C, W, X and Y) the most significant for causing disease (Rouphael and Stephens, 2012). The oropharynx mucosa in humans is the sole ecological niche of *N. meningitidis*, and person-to-person transmission requires direct contact or dispersion through respiratory droplets (Caugant and Maiden, 2009). Carriage of *N. meningitidis* has been estimated to occur in approximately 10% of the general population, the carriage rate is low in children and increases to peak in adolescents and young adults (Cartwright et al., 1987; Caugant et al., 1994; Christensen et al., 2010; Claus et al., 2005). However, the close contact that is required for transmission of *N. meningitidis* means that dense and semi-closed populations such as student campuses, military barracks and large gatherings of people (such as at camps or on pilgrimages) are at high risk for meningitis outbreaks (Bruce et al., 2001; Harrison et al., 1999; Moore et al., 1988; National Foundation for Infectious Diseases (NFID), 2017; Smith-Palmer et al., 2016).

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Understanding the carriage of *N. meningitidis* may help to understand the link between carriage and invasive disease, and the characterisation of carriage serogroups, along with those responsible for invasive disease, will also allow for the monitoring of epidemiological patterns. The aim of this study was to determine the prevalence of meningococcal carriage and the serogroup distribution of *N. meningitidis* in applicants aged >18 years to a military academy in the Russian Federation at several time points.

Materials and methods

Study design

This study was a prospective, observational study of applicants and first year students at a Military Medical Academy in the Russian Federation. Samples were taken from applicants to the academy, 18–20 years old, on their arrival to the training camp (Day 1). The training camp was located in sub-urban area in the forest and applicants were accommodated in wooden single story barracks (approx. 30 people per barrack). Between Days 25 to 30 the applicants received a medical exam, and went through physical fitness and general knowledge assessments. There was no opportunity for applicants to establish close contacts, and the population during this period can be considered as semi-closed. All applicants who successfully passed exams entered the 25 days preliminary training course. They were divided into groups (each approx. 30 people), and each group was accommodated in a separate barrack; the members of these groups were in close contact during daily activities. Second samples were taken from applicants (regardless of whether they were included in the study at Day 1) immediately after forming of groups (on Day 30, range 30–35 days). The third samples were obtained on Day 60 (range 60–65 days) from the applicants who successfully passed the preliminary training course. During the period from Day 30 to Day 60 the population can be considered as closed. Enrollment occurred between June and September 2016.

The study was approved by the local Institutional Review Boards (Ethical committee of Military Medical Academy), and the study was conducted in accordance with the guidelines of Good Epidemiological Practice. All included individuals were provided information on the study, and signed the informed consent form. On enrolment the individuals were coded and the investigators were blinded to subjects IDs, the information was decoded after the completion of the study.

Sample preparation

Mucus from the back of the pharynx was sampled under fasting conditions (at least 3–4 h after a meal) by trained medical personnel using eSwabs (Copan, Brescia, Italy). After removal from the nasopharynx, the swab was placed in a transport medium maintained at 37 °C and delivered to the laboratory within six hours.

Microbiological methods

On delivery to the laboratory, mucus was inoculated on a Petri dish with Columbia agar containing 5% sheep blood, and all cultures were grown at 37 °C in an atmosphere containing about 10% CO_2. The inoculated dishes were then examined for suspected *N. meningitidis* at 24 h. Isolated colonies were selected, and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) analysis was conducted using Microflex LT controlled by FlexControl version 3.0 software (Bruker Daltonics, GmbH, Germany) under default settings. The obtained spectra were downloaded into a MALDI Biotyper 3.0 system (Bruker Daltonics, GmbH) and used for identification according to the manufacturer's instructions.

Serogrouping of N. meningitidis *isolates*

Serogrouping of meningococcal isolates was carried out by a slide agglutination test, with a set of agglutinating serogroup antisera (serogroups A, B, C, X, Y, Z, W-135, 29E) MENGRUID® (St. Petersburg Scientific Research Institute for Vaccines and Sera and the Enterprise for the Manufacture of Bacterial Preparations, Russian Federal Medical and Biological Agency) authorized to be used for this purpose in the Russian Federation. Briefly, the *N. meningitidis* culture was dissolved in physiological saline and antisera to A, B and C were added to the slide and the reaction was recorded after 1–2 min for each. If there was no reaction with one of the main serogroup antisera testing continued with other specific antisera X, Y, Z, W135, and 29E. If the *N. meningitidis* strain did not show a positive result in the agglutination reaction with a complete set of agglutinating antisera, it was classified as a nonagglutinable isolate.

Isolation of genomic DNA

Genomic DNA from pure cultures of *N. meningitidis* was isolated using the AmpliSens DNA-Sorb B kit (InterLabService, Russia) in accordance with the manufacturer's instructions. Isolation of genomic DNA from a liquid suspension from the eSwab transport systems was carried out using the MAGNO-sorb kit (InterLabService, Russia) on the automatic sample preparation station Xiril Neon 100 series (Xiril AG, Switzerland), in accordance with the manufacturer's instructions. A superoxide dismutase gene (*sodC*) was used as a target for the detection of *N. meningitidis* DNA; primers, probes and real-time PCR regimens were conducted as recommended in the WHO "Manual on Laboratory Methods for the Diagnosis of Meningitis" (Centers for Disease Control and Prevention, 2011).

PCR serogrouping

DNA from pure *N. meningitidis* cultures and suspension samples that were positive for the *sodC* gene was used for real-time PCR typing; primers, probes and PCR regimens were conducted as recommended in the WHO "Manual on Laboratory Methods for the Diagnosis of Meningitis" (Centers for Disease Control and Prevention, 2011). Cultures or DNA samples with no signal for any serogroup were designated as ungroupable.

Multilocus sequence typing (MLST)

MLST was performed *post-hoc* on a random selection of serogroup W isolates following the recommendations included in the Neisseria pubMLST website to identify sequence type (ST) and clonal complex (CC) (Jolley, 2019).

Statistical Analysis

There was no statistically powered hypothesis for the sample size in this study, however including 500 subjects with the expected carriage of 10% will give 7.51-12.95% precision on the 95% confidence interval (CI).

As this was a descriptive epidemiological study, the enrolled population was characterized by a descriptive analysis, with means (standard deviation) or median (range) for quantitative variables and proportions for qualitative variables. Frequencies (%) of carriage at the study start and acquisition during the follow-up, overall and/or stratified by the different parameters were calculated.

Results

On Day 1, 671 applicants to the military academy provided samples, with 261 applicants providing samples on Day 30, and 232 on Day 60 (Figure 1). Recruits were aged 18–20 years, more than half had 2–4 flatmates, and over 80% were smokers. *N. meningitidis* DNA was detected in 16.2% of samples from Day 1, 7.7% of samples from Day 30 and 15.9% of samples from Day 60 (Table 1). *N. meningitidis* was detected more often by PCR than by culture, but with a similar frequency across visits. There were no culture positive samples among the PCR negative samples.

The serogroup composition of *N. meningitidis* at each of the three time points are presented in Table 2. In addition to the difference in carrier frequency at the different observation periods, there were differences in the serogroup composition. Serogroup composition of meningococci was most diverse at Day 1, through serogrouping of *N. meningitidis* cultures serogroup B was the dominant serogroup (40%; 17/43 isolates), followed by serogroup W (9%; 4/43 isolates) and ungroupable isolates (30%; 13/43 isolates). Through PCR detection the overwhelming majority of samples positive for the *sodC* gene were ungroupable (82%; 54/66 isolates). Discrepancy between the serogrouping of cultures and PCR was seen for two isolates serologically evaluated as B which were classified as ungroupable by PCR, and one isolate serologically evaluated as C and by PCR was classified as W.

At Day 30 there were few positive samples; two from *N. meningitidis* cultures, W and ungroupable, and of 13 samples positive for the *sodC* gene five belonged to serogroup W, and eight were ungroupable. There was discrepancy with PCR typing of DNA from the eSwab liquid and the pure culture in one serogroup C sample with a negative result for the eSwab suspension. Discrepancy between serogrouping of cultures and PCR was seen in five of the viable cultures; three cultures serologically identified as A were classed as W by PCR, one culture serologically identified as B was assigned to W by PCR, and one culture serologically identified as W was not groupable by PCR.

At Day 60, serogroup composition of viable cultures was distinguished by a low diversity with 58% (14/24 cultures) classed as serogroup W. Of the 13 samples positive for *sodC*, 11 belonged to serogroup W, and two were ungroupable. There was discrepancy in seven individuals.

Of the 20 carriers identified at Day 30, 11 were included at Day 1 and all had a negative result. At Day 60, however, carriage persisted in seven subjects, all having serogroup W.

Nine serogroup W isolates were selected for MLST, one isolate from Day 1 which belonged to ST11, and eight from Day 60, seven belonged to ST11 and one to ST53.

Discussion

This study identified a carriage prevalence of 16% for *N. meningitidis* in those entering a military college in the Russian Federation, and similar rates have been seen in recruits entering a military setting in Greece (15%) (Tryfinopoulou et al., 2016) and Poland (16–24%) (Tyski et al., 2001), with lower carriage rates seen for recruits entering a military setting in Turkey (4.2%) (Celal Basustaoglu et al., 2011) and Iran (8%) (Ataee et al., 2016) and professional soldiers in Poland (5.2% and 9.6%) (Korzeniewski et al., 2017; Korzeniewski et al., 2015). It was anticipated that the carriage rate would increase after recruitment while the recruits resided in a semi-closed environment as these populations are at higher risk for outbreaks of meningococcal disease (Aguilera et al., 2002; Brundage et al., 2002; National Foundation for Infectious Diseases (NFID), 2017); while a study of the change in carriage rate in students identified a rapid increase on entering university (Neal et al., 2000). However in this study carriage decreased from Day 1 to Day 30 which is more reflective of studies of carrier status in open populations where there is a consistent proportion of *N. meningitidis* carriers over time (Caugant et al., 2007; Claus et al., 2005; Glitza et al., 2008). Later when the population transformed from semi-closed to closed (between Day 30 and Day 60) the carriage increased to the levels seen at Day 1.

The serogroup composition at Day 1 was also diverse, which may be related to the large geographical catchment area for these new recruits (Claus et al., 2005). This diversity in serogroup composition was also seen for other studies of recruits entering the military setting (Ataee et al., 2016; Celal Basustaoglu et al., 2011; Tryfinopoulou et al., 2016; Tyski et al., 2001). By Day 60 the diversity of serogroup composition had reduced, with a change in serogroup distribution compared to baseline, with many of the identified isolates belonging to serogroup W. Of the serogroup W isolates that were sequenced the vast majority were ST11, and one ST53 isolate was detected at Day 60. Only two isolates of serogroup W ST53, from Africa in 2008, were found in the PubMLST data-base (https://pubmlst.org/bigsdb?page=info&db=-pubmlst_neisseria_isolates&id=12052 and https://pubmlst.org/bigsdb?page=info&db=pubmlst_neisseria_isolates&id=12076). While previously uncommon, in 2000 an outbreak of a

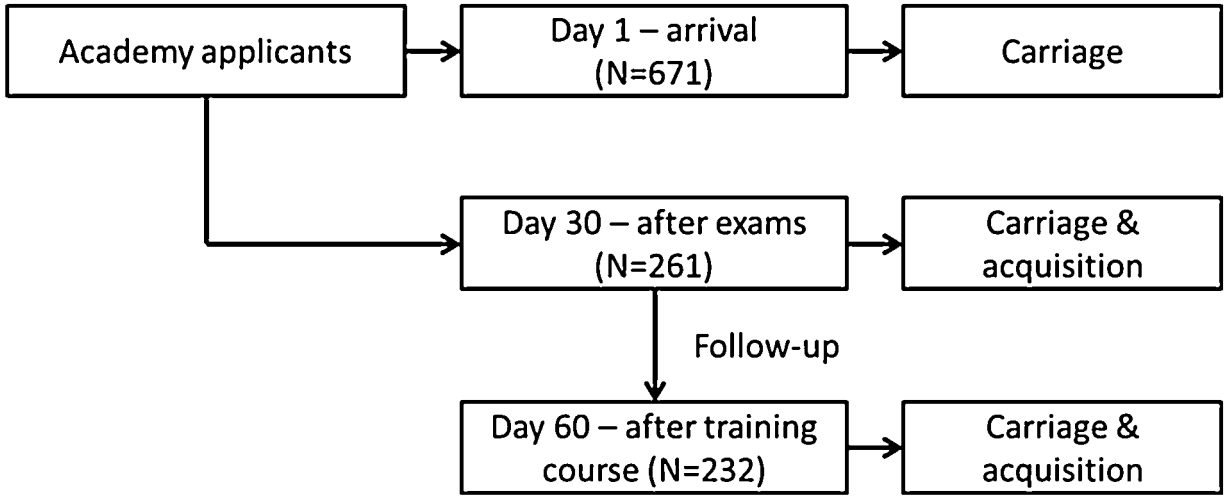

Figure 1. Flow chart showing patient enrollment by cohorts.

Table 1
Results of isolation of pure cultures and DNA of *N. meningitidis* in samples from the posterior pharyngeal wall.

	Day 1 (N = 671)		Day 30 (N = 261)		Day 60 (N = 232)	
	PCR positive	PCR negative	PCR positive	PCR negative	PCR positive	PCR negative
Total, n (%)	109 (16.2)	562 (83.8)	20 (7.7)	241 (92.3)	37 (15.9)	195 (84.1)
Culture positive	43/109	0/562	7/20	0/241	24/37	0/195
Culture negative	66/109	562/562	13/20	241/241	13/37	195/195

Table 2
Frequency of *N. meningitidis* carriage and serogroup composition identified by different methods.

	Day 1 (N = 671)			Day 30 (N = 261)			Day 60 (N = 232)		
	Viable culture, n	Only DNA, n	Total, n (%)	Viable culture, n	Only DNA, n	Total, n (%)	Viable culture, n	Only DNA, n	Total, n (%)
A	–	–	–	–	–	–	–	–	–
B	17	–	17 (15.6)	–	–	–	–	–	–
C	1	–	1 (0.9)	–	–	–	1	–	1 (2.7)
W	4	7	11 (10.1)	1	5	6 (30.0)	14	11	25 (67.5)
X	2	1	3 (2.8)	–	–	–	–	–	–
Y	2	4	6 (5.5)	–	–	–	–	–	–
Z	1	–	1 (0.9)	–	–	–	–	–	–
UG	13	54	67 (61.5)	1	8	9 (45.0)	2	2	4 (10.8)
Discrepancy	3	–	3 (2.8)	5	–	5 (25.0)	7	–	7 (18.9)
Total	43	66	109 (100)	7	13	20 (100)	24	13	37 (100)
Carriage, %	6.4	9.8	16.2	2.7	5.0	7.7	10.3	5.6	15.9

UG, ungroupable.

hypervirulent strain of serogroup W (ST11) occurred during the Hajj pilgrimage, and following this, genetically similar strains have led to outbreaks in several regions (Mustapha et al., 2015). In the Russian Federation cases due to serogroup W (ST11) were first detected in 2007, with evidence for a recent increase in cases (Borrow et al., 2016). Recent outbreaks attributed to serogroup W (ST11) in the UK, the Netherlands and Chile, have led to the introduction of vaccination programs in these countries using quadrivalent conjugate ACYW vaccines (Borrow et al., 2016; Knol et al., 2017; Knol et al., 2018).

Introduction of a vaccination program has been shown to reduce carriage of *N. meningitidis*. The UK has had a universal mass vaccination program with MenC vaccines since 1999, carriage studies before and after the initiation of the vaccination program demonstrated a significant reduction in meningococcal serogroup C carriage (Maiden et al., 2008). Regarding the military environment, a study in Poland has demonstrated the effectiveness of quadrivalent meningococcal conjugate vaccine in suppressing *N. meningitidis* carriage (Korzeniewski et al., 2015). Currently the Russian Federation does not have a mass vaccination program against *N. meningitidis*, surveillance of cases and carriage rates with the potential introduction of a vaccination program with serogroup ACYW vaccine may help to reduce carriage and outbreaks of meningococcal disease.

There were a number of limitations to this study. There has been shown to be seasonal variations in the incidence of meningitis peaking in the winter months (Paireau et al., 2016; Palmgren, 2009), and a similar winter peak in the carriage rate in new recruits (Tyski et al., 2001), in this study the samples were collected outside of the winter period and so carriage may be lower. Immediate plating of swabs has been shown to result in higher detection of carriage (Caugant et al., 2007; Christensen et al., 2010; Cunningham et al., 2001; Roberts et al., 2009), however in this study swabs were plated up to 6 h after sampling, which may have led to lower observed carriage rates. This study was also only conducted at 3 time points and was not a continuous longitudinal sampling study, and so provides only a limited understanding of the dynamics of carriage changes in this population, and cannot provide information on transmission patterns. Additionally, many of the isolates were ungroupable, which has been seen before in carrier studies (Caugant et al., 2006; Celal Basustaoglu et al., 2011; Glitza et al., 2008; Korzeniewski et al., 2015; Tyski et al., 2001) and previous evidence has shown challenges in serogrouping of isolates from carriage studies due to reduced or no capsule expression in the carriage state (Jones et al., 2016). Similarly genetic plasticity of the capsule can effect PCR assay sensitivity (Jones et al., 2016) and may have resulted in some of the discrepancy seen between the assays. MLST and whole genome sequencing (WGS) are more precise methods for characterizing isolates, however they were not used in this study to characterize all of the positive samples. The use of these advanced methods can help our understanding of carriage and transmission of *N. meningitidis*, as well as identify more virulent or invasive strains, although their use may be limited in countries with limited resources (Mustapha et al., 2015; Rodrigues and Maiden, 2018).

Conclusion

In this study of applicants to a military academy in the Russian Federation the carriage rate for *N. meningitidis* appeared to stable over the 3 months following entrance. Of particular interest is the increase in the proportion of carriers of serogroup W in this population, which has also been linked to recent outbreaks in several world regions, and may warrant further surveillance. Our study data can be used for guiding vaccination strategies to reduce transmission of *N. meningitidis* in military settings and other similar semi-closed populations in the Russian Federation.

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The authors employed by Sanofi Pasteur were involved in the design of this study, the interpretation of the data and the writing of the report.

Ethical approval

The study was approved by the local Institutional Review Boards (Ethical committee of Military Medical Academy), and the study was conducted in accordance with the guidelines of Good Epidemiological Practice.

Author contribution

SS and MHK were involved in the study design, SZ, YL, KZ, EM, VG, AM, MV, OK, LG and AG were involved in data collection, SS and MHK conducted the data analysis and interpretation, MHK wrote the manuscript and all authors provided critical revision. All authors provided final approval of the manuscript.

Conflicts of interest

MHK was an employee of Sanofi Pasteur at the time this study was conducted.

AG is an employee of Sanofi Pasteur.

SS, VG, YL, MV, OK received grants from Sanofi Pasteur during the conduct of the study.

SZ, KZ, EM, AM, LG declare no conflicts of interest.

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