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Human Immunodeficiency Virus Infection Is Associated With Increased Meningococcal Carriage Acquisition Among First-year Students in 2 South African Universities

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Background. Invasive meningococcal disease clusters occur among university students and may reflect higher carriage prevalence among this population. We aimed to measure meningococcal carriage prevalence, acquisition, and risk factors among first-year university students in South Africa.

Methods. In summer–autumn 2017, after consenting to participate, we collected oropharyngeal swabs and questionnaires on carriage risk factors and tested students for HIV at 2 universities, during registration week (survey 1) and 6–8 weeks later (survey 2). Meningococci were detected by culture and polymerase chain reaction.

Results. We enrolled 2120 students at registration. Mean age was 18.5 years, 59% (1252/2120) were female and 0.8% (16/1984) had HIV. Seventy-eight percent of students returned for survey 2 (1655/2120). Among the cohort, carriage prevalence was 4.7% (77/1655) at registration, increasing to 7.9% (130/1655) at survey 2: 5.0% (83) acquired new carriage, 2.8% (47) had persistent carriage, 1.8% (30) cleared the initial carriage, and 90.3% (1495) remained carriage free. At both surveys, nongenogroupable meningococci predominated, followed by genogroups Y, B, W, and C. On multinomial analysis, risk factors for carriage acquisition included attending nightclubs (adjusted relative risk ratio [aRRR], 2.1; 95% CI, 1.1–4.0), having intimate kissing partners (aRRR, 1.8; 95% CI, 1.1–2.9) and HIV (aRRR, 5.0; 95% CI, 1.1–24.4).

Conclusions. Meningococcal carriage among first-year university students increased after 2 months. Sociobehavioral risk factors were associated with increased carriage for all analyses. HIV was associated with carriage acquisition. Until vaccination programs become mandatory in South African universities, data suggest that students with HIV could benefit most from meningococcal vaccination.

Keywords. meningococcus; *Neisseria meningitidis*; carriage; Southern Africa; risk factors.

Neisseria meningitidis (meningococcus) is spread from human to human, the main ecological niche being the mucosa of the human oropharynx [1]. *Neisseria meningitidis* carriage is a prerequisite for ongoing transmission and invasion of the organism leading to meningococcal bacteremia or meningitis [2]. Understanding meningococcal carriage dynamics in a population is important for understanding disease epidemiology and transmission, and determining vaccination strategies for disease prevention.

The polysaccharide capsule surrounding the meningococcus is its most important virulence factor. Almost all isolates causing invasive meningococcal disease (IMD) are encapsulated, with serogroups A, B, C, W, and Y being the most frequent causes of IMD globally [3]. However, the majority of carriage isolates are nongroupable/unencapsulated and these isolates rarely cause disease, as either there is phase variation in the expression of the capsule, capsular synthesis genes have been inactivated, or there is an absence of genes required for capsule production (capsule null phenotype) [4, 5]. Carriage isolates that are sero-/genogroupable may be responsible for the spread of meningococcal disease in the community.

Invasive meningococcal disease is seasonal, peaking in May to October each year in South Africa, but it also fluctuates over periods of 10 to 15 years [2, 6]. Following the emergence of serogroup W in South Africa in 2005, IMD incidence has declined in recent years (0.2 cases per 100 000 population in 2016) [6, 7]. Human immunodeficiency virus (HIV) infection has been associated with increased risk of IMD, particularly serogroup W, which causes more severe disease and IMD in men who have

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sex with men [8–10]. Thirty-seven percent of patients with IMD in South Africa are living with HIV (in a background population HIV prevalence of 13%) [6, 11]. Recommendations for meningococcal vaccination of high-risk groups (including persons with HIV and those entering universities) have been published; however, meningococcal vaccine uptake is minimal in South Africa [12, 13]. While data on IMD incidence and risk factors are available, there are currently no published data on meningococcal carriage prevalence in any population in southern Africa or by HIV status.

Meningococcal carriage prevalence increases with age, peaking at 23% in 19- to 24-year-olds in industrialized countries [14]. However, in the African meningitis belt, an area of 26 countries in sub-Saharan Africa extending from Senegal to Ethiopia, carriage peaks at a younger age (10–14 years) and occurs at a lower rate (5%) [15, 16]. Despite this low carriage rate and huge successes with MenAfriVac controlling serogoup A IMD, periodic large-scale meningococcal epidemics still occur in this region [17, 18].

Even though carriage peaks in adolescence, carriage prevalence is often higher in young adults living in semiconfined populations such as university campuses or in army barracks [19, 20]. This may be due to mixing of diverse meningococcal strains brought in by carriers from different areas, resulting in a rapid meningococcal carriage acquisition among new students or recruits, which usually plateaus after 1 month [21]. Globally, studies on university campuses have shown varied carriage rates among students, ranging from 46% in the United Kingdom and 15% in the United States to 9% in Australia [22–24]. Spreading of new meningococcal strains is enhanced by behavioral risk factors associated with meningococcal carriage, such as smoking (active and passive), attendance at pubs/nightclubs, and intimate kissing [14, 19]. Some studies have suggested carriage of *Streptococcus pneumoniae* to be higher in adults with HIV; however, it is not known whether HIV coinfection is also a risk factor for meningococcal carriage and/or acquisition [25–27].

We aimed to describe the meningococcal carriage prevalence and risk factors for carriage among first-year university students in South Africa at 2 time points and to determine whether HIV coinfection was associated with meningococcal carriage acquisition, persistence, and clearance among students 2 months after starting university.

METHODS

We performed a cross-sectional study to determine the prevalence of meningococcal carriage among university students at 2 time points (survey 1 and survey 2), with a nested cohort study to determine the acquisition, persistence, and clearance of meningococcal carriage. The study population included first-year university students registering at the start of the 2017 academic year at 2 large universities: University of the Witwatersrand (Wits) in Gauteng Province or University of Cape Town (UCT) in Western Cape Province. The student populations are diverse and draw from all 9 provinces of South Africa. Combined, approximately 14 000 first-year students register each year at the 2 universities. The cohort of students included those participating in both cross-sectional studies: at registration (survey 1) and 6–8 weeks later (survey 2).

Sample size was calculated using OpenEpi (https://www.openepi.com/SampleSize/SSCC.htm), assuming differences in the acquisition of meningococcal carriage in a cohort of students with and without HIV coinfection. Calculations were for repeat measurements assuming an HIV prevalence of 7% and initial meningococcal carriage prevalence of 4%. Our target was to enroll 3000 students (see Supplementary Methods).

The South African university year begins in late summer. Survey 1 of the study occurred during registration week at Wits from 30 January until 3 February 2017, and at UCT from 27 February until 8 March 2017. Survey 2 occurred 6–8 weeks later for each site in the autumn: 14–17 March 2017 at Wits and 18–21 April 2017 at UCT.

Study recruitment and sample collection took place on campus at both sites. There was no random selection of the students. All students were informed of the study via e-mail and the study was introduced at student welcome lectures. Study teams were centrally situated on campus with signage inviting students to participate. Interested participants were requested to sign an informed-consent form prior to enrollment. Participants were allocated a unique study number to link the questionnaire, HIV test result, and oropharyngeal swab. Questionnaires on basic demographics and risk factors for meningococcal carriage were self-administered on tablet computers using Google forms. Risk factors evaluated included the following: sex, university, home province, active and passive smoking in the previous month, club/pub/party attendance in the preceding 2 weeks, intimate kissing partners in the preceding 2 weeks, pre-existing chronic conditions, HIV serostatus, upper respiratory tract infection in the previous month, antibiotic use in the previous month, and prior meningococcal vaccine use. Human immunodeficiency virus testing was performed at survey 1 by trained nursing staff. Rapid HIV-antibody detection tests (Alere Determine HIV-1/2; Abbott) were used on finger-prick blood samples. Persons testing positive for HIV had a second confirmatory rapid HIV test performed (UniGold Recombingen HIV-1/2; Trinity Biotech). Oropharyngeal swabs were taken using flocked swabs (Copan Diagnostics), ensuring the oropharynx, both tonsillar beds, and posterior pharyngeal wall were touched using a figure-of-8 motion and avoiding the tongue or teeth.

The oropharyngeal swabs were placed directly into Todd-Hewitt broth (Media Mage Products), a selective enrichment medium, and incubated within 6–8 hours at 37°C for 24 hours [28]. In the laboratory, a 500-µL aliquot of broth was stored at −70°C for polymerase chain reaction (PCR) later. After

overnight incubation, 100 µL of broth was plated onto Thayer-Martin, New York City, and 5% blood agar (Diagnostic Media Products) and incubated for 48 hours in 5% CO_2. Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry was used to identify all isolates. Meningococcal isolates were serogrouped by slide agglutination using monovalent antibodies to capsular polysaccharide A, C, W, X, Y, and Z and monoclonal antibodies to polysaccharide B. All stored broths underwent real-time PCR to detect the *sodC* gene [29]. The *ctrA* and genogrouping real-time PCR (ABCEHWXYZ) was performed on all meningococcal isolates and *sodC*-positive swabs [30, 31]. *ctrA*-Negative and genogroup PCR-negative meningococci were considered nongenogroupable. Participants were classified as meningococcal carriers if *N. meningitidis* was cultured or the *sodC* gene was detected on PCR. No interventions to eliminate carriage were given to meningococcal colonized subjects in this nonoutbreak setting.

Data from surveys 1 and 2 (questionnaire, oropharyngeal swab, and HIV test) were linked using the unique study number. All statistical analyses were done using Stata version 14.0 (StataCorp). *P* values less than .05 were considered statistically significant. Variables with *P* values less than .2 in univariate analysis were evaluated in the multivariable models using manual backward elimination.

Carriage prevalence and risk factors for carriage of meningococci were determined from the cross-sectional study of first-year university students from survey 1. Carriage prevalence was determined by day of study enrolment, and percentage changes ([rate ratio − 1] × 100) in carriage were calculated to demonstrate the increase in carriage during registration at the universities and over time from survey 1 to survey 2. Prevalence of carriage by genogroup distribution was reported.

Univariate and multivariable logistic regression models were used to determine significant risk factors associated with meningococcal carriage at each of the 2 time points. Acquisition, persistence, and clearance of meningococcal carriage 6–8 weeks following registration were determined from the cohort at the second survey (see Supplementary Methods for definitions). Multinomial regression was performed to compare those who never carried meningococci (baseline category) with the carrier acquisition and carrier persistence groups (see Supplementary Methods).

The study was approved by the Wits Health Research Ethics Committee (Medical) (M160672) as well as that of the UCT (Ref395_2016). All participants gave written consent to be enrolled in the study.

RESULTS

Survey 1

In the summer of 2017, we enrolled 2137 first-year students into a cross-sectional meningococcal carriage study (survey 1). Seventeen students were excluded from the study; 6 did not consent to an oropharyngeal swab and 11 did not complete the questionnaire (Figure 1). The mean age of the students was 18.5 years (SD, 1.5 years) and the majority were female (59.1%, 1252/2120). Most students were from Gauteng (43%, 914/2120)

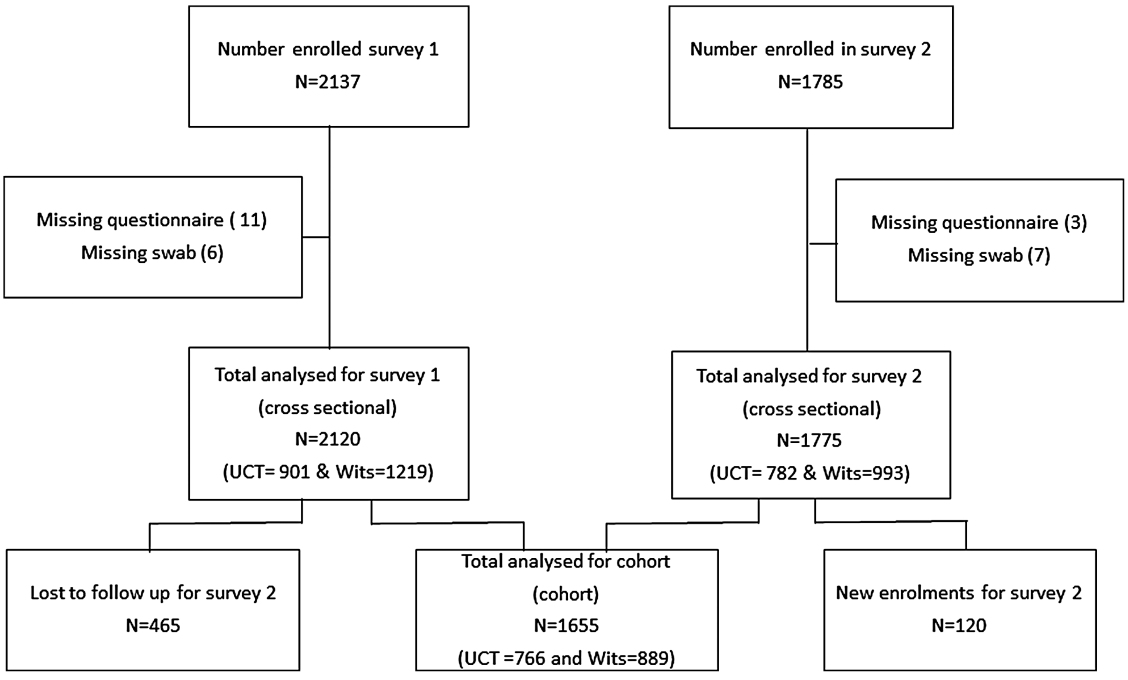

Figure 1. Flow diagram of enrollment of university students for the 2 surveys on meningococcal carriage among first-year university students: South Africa, 2017. Abbreviations: UCT, University of Cape Town; Wits, University of the Witwatersrand.

and Western Cape Provinces (19%, 410/2120). Twenty-eight percent (600/2120) of students lived in a university residence, and 27% (581/2120) were current cigarette smokers. Of those tested for HIV at enrollment, 0.8% (16/1984) had HIV infection.

Overall meningococcal carriage was 4.6% (98/2120) and carriage prevalence increased significantly over the 5 days of enrolment (3.2-fold increase from day 1 [1.8%] to day 5 [5.9%]) (Figure 2). Meningococci were isolated from 65 of 2120 (3.1%) swabs, with the remaining meningococci (33/2120, 1.6%) identified by PCR only. No human DNA was detected on 0.6% (13/2120) of swabs. The overall meningococcal carriage prevalence by genogroup was 1.8% (38) nongroupable, 1.1% (24) MenY, 0.8% (16) MenB, 0.2% (4) MenW, and 0.1% (2) MenC of the 2120 swabs (Table 1).

On multivariable analysis, compared with Gauteng Province, students were twice as likely to carry meningococcus if their home province was KwaZulu-Natal (adjusted odds ratio [aOR], 2.1; 95% confidence interval [CI], 1.1–4.2) and 3 times as likely for the Western Cape (aOR, 2.7; 95% CI, 1.6–4.5). Carriage was more likely if they lived in a university residence (aOR, 2.0; 95% CI, 1.2–3.4) or an apartment with other students (aOR, 2.1; 95% CI, 1.2–3.9) compared with living with their family, or if they attended a nightclub (aOR, 1.8; 95% CI, 1.1–3.0) or had at least 1 intimate kissing partner (aOR, 2.8; 95% CI, 1.8–4.5) in the previous 2 weeks (Table 2).

Survey 2

In the autumn of 2017, 6–8 weeks following survey 1, we repeated the survey at the same 2 universities. Across the 2 sites, 1785 students were enrolled, but 10 were excluded due to not completing the questionnaire (n = 3) or missing the oropharyngeal swab (n = 7) (Figure 1). Overall meningococcal carriage at survey 2 was 7.5%, a 1.6-fold increase (95% CI, 1.3–2.1) from survey 1. Three percent of swabs were culture positive (3.4%, 60/1775) and an additional 4.2% (74/1775) were detected using PCR only. No human DNA was detected on 1.5% (28/1775) of swabs. The overall meningococcal carriage prevalence by genogroup was 3.5% (63) nongroupable, 1.5% (27) MenY, 0.9% (16) MenB, 0.1% (2) MenW, 0.1% (2) MenC, 0.1% (1) MenX, and 0.1% (1) MenZ of the 1775 swabs (Table 1).

Cohort Analysis

Of the 1775 students who participated in survey 2, 93% (1655/1775) had participated in survey 1. Carriage at survey 1 for this cohort was 4.7% (77/1655) and increased 1.7-fold (95% CI, 1.3–2.3) to 7.9% (130/1655) for survey 2. Factors associated with meningococcal carriage at surveys 1 and 2 were similar for the cohort and cross-sectional study (Table 2 and Supplementary Tables 1–4).

In the cohort, 90.3% (1495/1655) of students never carried meningococcus in their oropharynx, 5.0% (83) acquired meningococcus after 6–8 weeks of university life, 2.8% (47) had persistence of meningococcal carriage during the study period, and 1.8% (30) cleared their initial meningococcal carriage by the second survey. On further analysis, only 1 student in the persistent carrier group had different genogroups at the second survey (initially genogroup B then genogroup Y).

On multivariable analysis, among the 5% who acquired meningococcal carriage, risk factors for genogroupable meningococcal carriage included pub attendance (aOR, 7.9; 95% CI, 1.8–35.3), having intimate kissing partners (aOR, 15.4; 95%

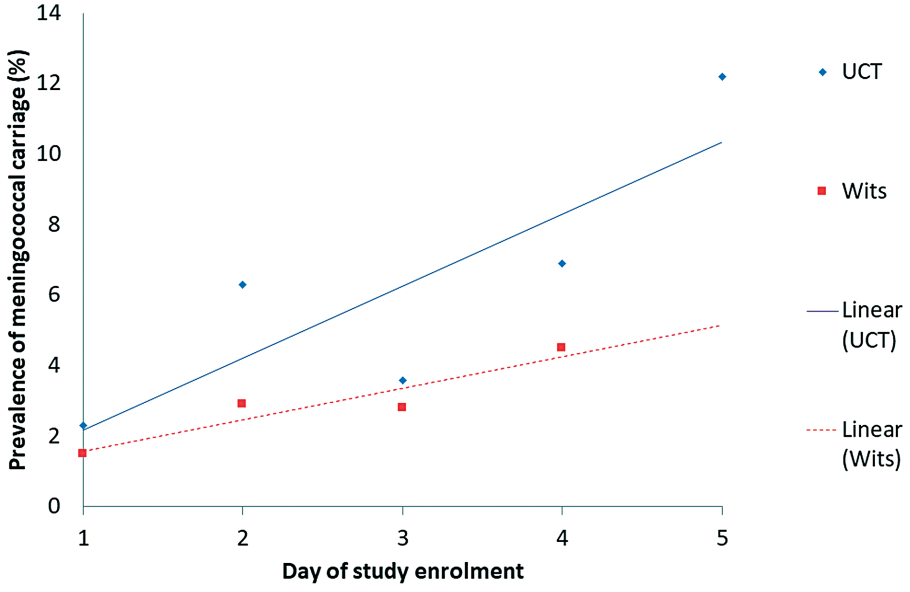

Figure 2. Trend lines depicting increasing meningococcal carriage prevalence by day of study enrollment of first-year university students during registration week: South Africa, 2017. Abbreviations: UCT, University of Cape Town; Wits, University of the Witwatersrand.

Table 1. Carriage Prevalence by University Site, Day of Enrollment, Laboratory Testing, and Genogroup for Surveys 1 and 2: South Africa, 2017

	Survey 1		Survey 2			
	Total Enrolled	NM Carriers, n (%)	Total Enrolled, n	NM Carriers, n (%)	Percentage Change From Day 1% (95% CI)	Percentage Change From Survey 1 to 2% (95% CI)
Day of study enrollment	2120					
Day 1	217	4 (1.8)	…	…	…	…
Day 2	677	32 (4.7)	…	…	156 (−9.1 to 898)	…
Day 3	341	10 (2.9)	…	…	59 (−54 to 595)	…
Day 4	477	27 (5.7)	…	…	207 (7 to 1108)	…
Day 5	408	24 (5.9)	…	…	219 (10 to 1165)	…
Total participants	2120	98 (4.6)	1775	134 (7.5)	…	63 (25 to 114)
University of Witwatersrand	1219	34 (2.8)	993	67 (6.7)	…	142 (−58 to 277)
University of Cape Town	901	64 (7.1)	782	67 (8.6)	…	21 (−16 to 73)
Laboratory test results						
Culture positive[a]	2120	65 (3.1)	1775	60 (3.4)	…	10 (−24 to 59)
PCR positive only	2120	33 (1.6)	1775	74 (4.2)	…	168 (75 to 317)
Genogroup						
A	2120	0 (0)	1775	0 (0)	…	…
B	2120	16 (0.8)	1775	16 (0.9)	…	19 (−44 to 155)
C	2120	2 (0.1)	1775	2 (0.11)	…	19 (−91 to 1548)
W	2120	4 (0.2)	1775	2 (0.11)	…	40 (−95 to 317)
X	2120	1 (0.0)	1775	1 (0.06)	…	19 (−99 to 9276)
Y	2120	24 (1.1)	1775	27 (1.52)	…	34 (−25 to 143)
Z	2120	0 (0)	1775	1 (0.06)	…	…
Nongroupable	2120	38 (1.8)	1775	63 (3.5)	…	98 (30 to 205)
Other[b]	2120	3 (0.1)	1775	2 (0.1)	…	−20 (−93 to 595)

Abbreviations: NM, *Neisseria meningitidis*; PCR, polymerase chain reaction.

[a]All culture-positive swabs were also *sodC* PCR positive.

[b]Includes 2 E and 1 non-ABCEHWXYZ from survey 1, and 1 E and 1 non-ABCEHWXYZ from survey 2. No genogroup A was detected at either time point. Ten meningococcal positive swabs in survey 1 and 20 from survey 2 were *sodC* positive yet had inconclusive genogrouping.

CI, 3.1–75.8), and having had a recent upper respiratory tract infection (aOR, 5.3; 95% CI, 1.4–19.6) (Table 3).

On multinomial analysis, compared with those who never carried meningococci, risk factors for acquiring meningococcal carriage included attending nightclubs (adjusted relative risk ratio [aRRR], 2.1; 95% CI, 1.1–4.0), having intimate kissing partners (aRRR, 1.8; 95% CI, 1.1–2.9), and having HIV (aRRR, 5.0; 95% CI, 1.1–24.4). Persistence of meningococcal carriage across the study period was associated with being male (aRRR, 2.5; 95% CI, 1.3–4.6), studying at the UCT (aRRR, 2.1; 95% CI, 1.1–4.0), and attending pubs (aRRR, 3.0; 95% CI, 1.4–6.3) (Table 4).

DISCUSSION

Meningococcal carriage among first-year university students in South Africa was 5% upon registration, increasing by 63% to 8% after 2 months on campus. At both time points, nongroupable strains predominated, followed by Y and B genogroups. Human immunodeficiency virus infection was a risk factor for meningococcal carriage acquisition; it was not associated with baseline meningococcal carriage. Significant risk factors for meningococcal carriage included home province, male gender, intimate kissing, and nightclub attendance.

Although meningococcal carriage among South African university students was lower than in studies in the United Kingdom [24, 32], it is comparable to more recent carriage studies among adolescents in Australia (6%) and Italy (5%) [22, 33]. The warmer summers and milder winters in these countries and South Africa may affect meningococcal transmission, explaining the lower prevalence as, globally, behavioral risk factors remain fairly similar [14, 19].

In our study, the risk factors associated with meningococcal carriage included being male, attending nightclubs, and having at least 1 intimate kissing partner in the previous 2 weeks. Smoking was not an independent risk factor for carriage, although the prevalence of smoking (27%) and smoke exposure (65%) was high among the students, compared with a national prevalence of 18% and 47%, respectively, among South African adults in 2012 [34]. Smoking showed collinearity with nightclub and pub attendance, which were strongly associated with carriage.

Although HIV prevalence was lower than expected, underlying HIV infection was associated with meningococcal carriage acquisition. Human immunodeficiency virus has previously been associated with increased risk of meningococcal disease and more severe disease, and some countries have included

Table 2. Multivariable Analysis of Risk Factors for Meningococcal Carriage Among First-year University Students at Registration (Survey 1): South Africa, 2017

			Univariate Analysis		Multivariable Analysis	
Characteristics	All, N	Meningococcal Carriers, n (%)	OR (95% CI)	*P*	OR (95% CI)	*P*
Number of students	2120	98 (4.6)	…		…	
University						
Witwatersrand	1219	34 (2.8)	Ref		…	
Cape Town	901	64 (7.1)	2.7 (1.7–4.1)		…	
Home province						
Eastern Cape	117	7 (6.0)	1.9 (.8–4.5)	.125	1.9 (.8–4.5)	.178
Free State	51	3 (5.9)	1.9 (.6–6.5)	.301	1.8 (.5–6.5)	.344
Gauteng	914	29 (3.2)	Ref		Ref	
KwaZulu-Natal	179	15 (8.4)	2.8 (1.5–5.3)	.002	2.1 (1.1–4.2)	.032
Limpopo	183	1 (0.6)	0.2 (.1–1.2)	.080	0.2 (.1–1.3)	.094
Mpumalanga	90	3 (3.3)	1.1 (.3–3.5)	.934	1.1 (.3–3.6)	.951
Northern Cape	26	1 (3.9)	1.2 (.2–9.3)	.848	1.1 (.1–7.9)	.991
North West	54	0 (0)	…		…	
Western Cape	410	37 (9.0)	3.0 (1.8–5.0)	<.001	2.7 (1.6–4.5)	<.001
Outside South Africa	96	2 (2.1)	0.7 (.2–2.8)	.559	0.7 (.2–3.0)	.606
Median age, years	18.5	18.53	…	.451	…	
Sex						
Male	868	50 (5.8)	1.5 (1.1–2.3)	.039	1.5 (.9–2.3)	.067
Female	1252	48 (3.8)	Ref		Ref	
Living arrangements						
House/apartment, with family	1116	40 (3.6)	Ref		Ref	
House/apartment, with other students	404	23 (5.7)	1.6 (.9–2.8)	.071	2.1 (1.2–3.9)	.012
University residence/hostel/dormitory	600	35 (5.8)	1.7 (1.1–2.7)	.031	2.0 (1.2–3.4)	.007
Shares a room						
No	1268	65 (5.1)	Ref		…	
Yes	852	33 (3.9)	0.8 (.5–1.1)	.179	…	
Current cigarette smoker[a]						
No	1539	52 (3.4)	Ref		Ref	
Yes	581	46 (7.9)	2.5 (1.6–3.7)	<.001	1.2 (.7–1.9)	.533
Smoke exposure[a]						
No	741	20 (2.7)	Ref		Ref	
Yes	1379	78 (5.7)	2.2 (1.3–3.6)	.002	1.3 (.7–2.2)	.385
Nightclub attendance[b]						
No	1714	56 (3.3)	Ref		Ref	
Yes	406	42 (10.3)	3.4 (2.3–5.2)	<.001	1.8 (1.1–3.0)	.018
Pub/bar attendance[b]						
No	1646	58 (3.5)	Ref		…	
Yes	474	40 (8.4)	2.5 (1.7–3.8)	<.001	…	
Party attendance[b]						
No	1502	55 (3.7)	Ref		Ref	
Yes	618	43 (7.0)	2.0 (1.3–3.0)	.001	0.8 (.5–1.3)	.375
Intimate kissing[b]						
No	1260	29 (2.3)	Ref		Ref	
Yes	860	69 (8.0)	3.7 (2.4–5.8)	<.001	2.8 (1.8–4.5)	<.001
Recent upper respiratory tract infection[a]						
No	1215	61 (5.0)	Ref		…	
Yes	779	32 (4.1)	0.8 (.5–1.3)	.346	…	
Antibiotic use[a]						
No	1651	86 (5.2)	Ref		…	
Yes	359	10 (2.8)	0.5 (.3–1.1)	.055	…	
HIV						
Uninfected	1968	90 (4.6)	…		…	

Table 2. Continued

Characteristics	All, N	Meningococcal Carriers, n (%)	Univariate Analysis OR (95% CI)	*P*	Multivariable Analysis OR (95% CI)	*P*
Infected	16	0 (0)	…	.747	…	
Received a meningococcal vaccine						
No	1492	57 (3.8)	Ref		…	
Yes	83	6 (7.2)	2.0 (.8–4.7)	.130	…	
Chronic lung disease						
No	2012	93 (4.6)	…		…	
Yes	108	5 (4.6)	1.0 (.4–2.5)	.997	…	
Diabetes						
No	1744	80 (4.6)	…		…	
Yes	9	0 (0)	…	.954	…	

N = 2120.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio; Ref, reference.

[a]In the previous month.

[b]In the previous 2 weeks.

HIV in the criteria for meningococcal vaccination requirement [35, 36]. However, an association between HIV infection and increased risk of carriage acquisition has not been previously reported. This new finding, if validated in other studies and settings, may be important in emphasizing the need for IMD prevention through vaccination in population groups with a high HIV prevalence.

There were significant geographic differences in carriage prevalence among participants, especially in the initial survey. These differences reflect the higher incidence of meningococcal disease in the provinces with higher carriage. The provinces with higher disease are all coastal. Some provincial differences may be related to variations in lifestyle and living conditions. For example, residing in the Western Cape Province was associated with higher odds of carrying meningococcus. Other studies have shown this province to have the highest incidence of meningococcal disease, as well as the highest prevalence of smokers (37%) nationally [6, 34].

As with other carriage studies, nongroupable meningococci were the most prevalent among our participants [28, 37, 38]. Genogroup Y was the next most predominant. Serogroup Y is the fourth most predominant cause of invasive disease in South Africa and is associated with HIV coinfection, raising concern for those persons with HIV who are at higher risk of acquiring carriage [6]. Genogroup B was the third most predominant in causing carriage in this survey and the most predominant cause of invasive disease in 2016 [6]. Odds were higher for acquiring genogroupable meningococci if students attended pubs, had intimate kissing partners, or had a recent upper respiratory tract infection. Although no cases of meningococcal disease were reported among the study population during our surveys, acquisition of genogroupable meningococci is thought to drive risk of disease.

Currently, only quadrivalent conjugate meningococcal vaccines targeting serogroups A, C, W, and Y are available in South Africa. Knowing the circulating geno-/serogroups driving carriage and invasive disease in South Africa will assist policymakers to decide which meningococcal vaccine(s) to include in future vaccination campaigns should meningococcal B vaccines be registered for use in South Africa.

Limitations

Peak carriage prevalence among students in our study was 8%, much lower than university settings in the Western world [23, 24]. By only targeting first-year students, we have no reliable data for South Africa on how carriage rates change with age. Considering the lower age seen in the meningitis belt, it is possible that the observed relatively low carriage rate is a reflection of carriage peaking at a younger age in our setting [15]. Overall, 1% (41/3895) of swabs did not contain human DNA, an indicator of poor-quality specimens. These swabs may have missed meningococcus in those students. In light of previous HIV surveys at university campuses, we expected a higher HIV prevalence among our target population [39]. The prevalence of HIV in first-year students on admission to university may be lower than in the university population as a whole [39]. Even with this low HIV prevalence and not meeting our target sample size, we found a significant association between HIV and carriage acquisition.

Our study found less carriage acquisition than other university-based studies, especially among the students studying in the Western Cape Province. The delayed start of the academic year due to student protests disrupting examinations from the previous year meant that mixing of the students may have occurred prior to the official registration week—hence, the higher initial carriage rate at UCT [40]. However, we were able to show

Table 3. Multivariable Analysis of Risk Factors for Carriage Acquisition of Nongenogroupable Versus Genogroupable Meningococci Among First-year University Students 2 Months After Registration (Survey 2 Cohort): South Africa, 2017

			Univariate Analysis		Multivariable Analysis	
Characteristics	Nongenogroupable Meningococci, n (%)	Genogroupable Meningococci, n (%)	OR (95% CI)	*P*	OR (95% CI)	*P*
Number of students	57 (69)	26 (31)	…		…	
University						
Witwatersrand	42 (89)	5 (11)	Reference		…	
Cape Town	15 (42)	21 (58)	11.8 (3.8–36.8)	<.001	…	
Sex						
Male	29 (81)	7 (19)	0.4 (.1–1.0)	.045	…	
Female	28 (60)	19 (40)	Reference		…	
Living arrangements						
House/apartment, with family	24 (75)	8 (25)	Reference		…	
House/apartment, with other students	15 (94)	1 (6)	0.2 (.02–1.8)	.147	…	
University residence/ hostel/dormitory	18 (51)	17 (49)	2.8 (1.0–8.0)	.049	…	
Shares a room						
No	35 (66)	18 (34)	Reference		…	
Yes	22 (73)	8 (27)	0.7 (.3–1.9)	.492	…	
Current cigarette smoker[a]						
No	45 (80)	11 (20)	Reference		…	
Yes	12 (44)	15 (56)	5.1 (1.9–14.0)	.001	…	
Smoke exposure[a]						
No	19 (76)	6 (24)	Reference		…	
Yes	38 (66)	20 (34)	1.7 (.6–4.8)	.347	…	
Nightclub attendance[b]						
No	45 (80)	11 (20)	Reference		…	
Yes	12 (44)	15 (56)	5.1 (1.9–14.0)	.001	…	
Pub/bar attendance[b]						
No	51 (80)	13 (20)	Reference		Reference	
Yes	6 (32)	13 (68)	8.5 (2.7–26.7)	<.001	7.9 (1.8–35.3)	.007
Party attendance[b]						
No	41 (80)	10 (20)	Reference		…	
Yes	16 (50)	16 (50)	4.1 (1.5–10.9)	.005	…	
Intimate kissing[b]						
No	36 (92)	3 (8)	Reference		Reference	
Yes	21 (48)	23 (52)	13.1 (3.5–49.1)	<.001	15.4 (3.1–75.8)	.001
Recent upper respiratory tract infection[a]						
No	37 (86)	6 (14)	Reference		Reference	
Yes	18 (49)	19 (51)	6.5 (2.2–19.1)	.001	5.3 (1.4–19.6)	.013
Antibiotic use[a]						
No	42 (67)	21 (33)	Reference		…	
Yes	12 (75)	4 (25)	0.7 (.2–2.3)	.524	…	
HIV						
Uninfected	52 (68)	25 (32)	…		…	
Infected	2 (100)	0 (0)	…	.330	…	
Received a meningococcal vaccine						
No	38 (75)	13 (25)	Reference		…	
Yes	4 (67)	2 (33)	1.5 (.2–8.9)	.681	…	
Chronic lung disease						
No	53 (69)	24 (31)	Reference		…	
Yes	4 (67)	2 (33)	1.1 (.2–6.4)	.912	…	
Diabetes						
No	57 (69)	26 (31)	…		…	
Yes	0 (0)	0 (0)	…		…	

N = 83. Genogroupable indicates any capsular genogroup. Nongenogroupable indicates cnl, NG on polymerase chain reaction, neg ACWYZYXHE.

Abbreviations: CI, confidence interval; cnl, capsular null locus; HIV, human immunodeficiency virus; neg, negative; NG, nongenogroupable; OR, odds ratio.

[a]In the previous month.

[b]In the previous 2 weeks.

Table 4. Multinomial Analysis of Factors Associated With Meningococcal (*Neisseria meningitidis*) Carriage Status Among First-year University Students 2 Months After Registration: South Africa, 2017

		Never an NM carrier	Acquired NM Carriage			Retained NM Carriage		
Characteristics	All Students, N	Never an NM Carrier, n or n/N (%)	n or n/N (%)	RRR (95% CI)	aRRR (95% CI)	n or n/N (%)	RRR (95% CI)	aRRR (95% CI)
Number of students	1625	1495 (92.0)	83 (5.1)	…		47 (2.9)	…	
University								
Witwatersrand	881	818/881 (92.9)	47/881 (5.3)	Ref		16/881 (1.8)	Ref	
Cape Town	744	677/744 (90.8)	36/744 (4.8)	0.9 (.6–1.4)	0.8 (.5–1.3)	31/744 (4.2)	2.3 (1.3–4.3)	2.1 (1.1–4.0)
Sex								
Male	638	573/638 (89.8)	36/638 (5.6)	1.2 (.8–1.9)	1.3 (.8–2.1)	29/638 (4.6)	2.6 (1.4–4.7)	2.5 (1.3–4.6)
Female	987	922/987 (93.4)	47/987 (4.8)	Ref		18/987 (1.8)	Ref	
Living arrangements								
House/apartment, with family	647	599/647 (92.6)	32/647 (5.0)	Ref		16/647 (2.5)	Ref	
House/apartment, with other students	413	382/413 (92.5)	16/413 (3.9)	0.8 (.4–1.4)	…	15/413 (3.6)	1.5 (.7–3.0)	…
University residence/hostel/dormitory	565	514/565 (91.0)	35/565 (6.2)	1.3 (.8–2.1)	…	16/565 (2.8)	1.2 (.6–2.4)	…
Shares a room								
No	937	856/937 (91.4)	53/937 (5.7)	Ref		28/937 (3.0)	Ref	
Yes	688	639/688 (92.9)	30/688 (4.4)	0.8 (.5–1.2)	…	19/688 (2.8)	0.9 (.5–1.6)	…
Current cigarette smoker[a]								
No	1138	1056/1138 (92.8)	56/1138 (4.9)	Ref		26/1138 (2.3)	Ref	
Yes	487	439/487 (90.1)	27/487 (5.5)	1.2 (.7–1.9)	…	21/48 7 (4.3)	1.9 (1.1–3.5)	…
Smoke exposure[a]								
No	521	484/521 (92.9)	25/521 (4.8)	Ref		12/521 (2.3)	Ref	
Yes	1104	1011/1104 (91.6)	58/1104 (5.3)	1.1 (.7–1.8)	…	35/1104 (3.2)	1.4 (.7–2.7)	…
Nightclub attendance[b]								
No	1250	1165/1250 (93.2)	56/1250 (4.5)	Ref		29/1250(2.3)	Ref	
Yes	375	330/375 (88.0)	27/375 (7.2)	1.7 (1.1–2.7)	2.1 (1.1–4.0)	18/375 (4.8)	2.2 (1.2–4.0)	0.7 (.3–1.6)
Pub/bar attendance[b]								
No	1234	1148/1234 (93.0)	64/1234 (5.2)	Ref		22/1234 (1.8)	Ref	
Yes	391	347/391 (88.8)	19/391 (4.9)	0.9 (.6–1.7)	0.5 (.3–1.0)	25/391 (6.4)	3.8 (2.1–6.8)	3.0 (1.4–6.3)
Party attendance[b]								
No	1103	1026/1103 (93.0)	51/1103 (4.6)	Ref		26/1103(2.4)	Ref	
Yes	522	469/522 (89.9)	32/522 (6.1)	1.4 (.9–2.2)	…	21/522 (4.0)	1.8 (.9–3.2)	…
Intimate kissing[b]								
No	968	908/968 (93.8)	39/968 (4.0)	Ref		21/968 (2.2)	Ref	
Yes	657	587/657 (89.4)	44/657 (6.7)	1.7 (1.1–2.7)	1.8 (1.1–2.9)	26/657 (4.0)	1.9 (1.1–3.4)	1.4 (.7–2.6)
Recent upper respiratory tract infection[a]								
No	828	761/828 (91.9)	43/828 (5.2)	Ref		24/828 (2.9)	Ref	
Yes	720	662/720 (91.9)	37/720 (5.1)	0.9 (.6–1.6)	…	21/720 (2.9)	1.1 (.6–1.8)	…
Antibiotic use[a]								
No	1302	1199/1302 (92.1)	63/1302 (4.8)	Ref		40/1302 (3.1)	Ref	
Yes	238	216/238 (90.8)	16/238 (6.7)	1.4 (.8–2.5)	…	6/238 (2.5)	0.8 (.3–2.0)	…

Table 4. Continued

Characteristics	All Students, N	Never an NM carrier	Acquired NM Carriage			Retained NM Carriage		
		Never an NM Carrier, n or n/N (%)	n or n/N (%)	RRR (95% CI)	aRRR (95% CI)	n or n/N (%)	RRR (95% CI)	aRRR (95% CI)
HIV								
Uninfected	1552	1430/1552 (92.1)	77/1552(5.0)	Ref		45/1552 (2.9)	Ref	
Infected	10	8/10 (80.0)	2/10 (20.0)	4.6 (.9–22)	5.0 (1.1–24.4)	0/10 (0.0)	…	…
Received a meningococcal vaccine								
No	1060	986/1060 (93.0)	51/1060 (4.8)	Ref		23/1060 (2.2)	Ref	
Yes	85	77/85 (90.6)	6/85 (7.1)	1.5 (.6–3.6)	…	2/85 (2.4)	1.1 (.3–4.8)	…
Chronic lung disease								
No	1531	1410/1531 (92.1)	77/1531 (5.0)	Ref		44/1531 (2.9)	Ref	
Yes	94	85/94 (90.4)	6/94 (6.4)	1.3 (.5–3.1)	…	3/94 (3.2)	1.1 (.3–3.7)	…
Diabetes								
No	1616	1486/1616 (92.0)	83/1616 (5.1)	Ref		47/1616 (2.9)	Ref	
Yes	9	9/9 (100)	0/9 (0.0)	…	…	0/9 (0.0)	…	…

Abbreviations: aRRR, adjusted relative risk ratio; CI, confidence interval; HIV, human immunodeficiency virus; NM, *Neisseria meningitidis*; Ref, reference; RRR, relative risk ratio.

[a]In the previous month.

[b]In the previous 2 weeks.

a rapid increase in carriage during the first survey at both universities, as seen in other studies conducted at registration [21]. Our swabbing techniques and detection of meningococci were standard across the sites and surveys; therefore, these factors are unlikely to be the cause for the observed difference. Persistence of carriage was not confirmed by whole-genome sequencing of the isolates; therefore, estimates of meningococcal persistence could have overestimated the actual rates.

Conclusions

Neisseria meningitidis carriage among first-year university students in South Africa was low initially, with a moderate acquisition of carriage between the 2 surveys. Nongroupable strains predominated, followed by Y, B, C, and W genogroups. Significant risk factors for meningococcal carriage at both time points included male sex, intimate kissing, and nightclub attendance. Human immunodeficiency virus is known to be associated with increased risk of IMD and more severe IMD; however, this is the first study that has shown an association between HIV infection and meningococcal carriage acquisition. These data suggest that students with HIV could benefit most from receiving meningococcal vaccination.

Supplementary Data

Supplementary materials are available at *Clinical 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.

Notes

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References

1. Caugant DA, Kristiansen PA, Wang X, et al. Molecular characterization of invasive meningococcal isolates from countries in the African meningitis belt before introduction of a serogroup A conjugate vaccine. PLoS One **2012**; 7:e46019.
2. Mandell GL, Bennett JE, Dolin R. Principles and practice of infectious diseases. Philadelphia, PA: Churchill Livingstone, **2010**.

3. Borrow R, Alarcón P, Carlos J, et al; Global Meningococcal Initiative. The Global Meningococcal Initiative: global epidemiology, the impact of vaccines on meningococcal disease and the importance of herd protection. Expert Rev Vaccines **2017**; 16:313–28.
4. Johswich KO, Zhou J, Law DK, et al. Invasive potential of nonencapsulated disease isolates of *Neisseria meningitidis*. Infect Immun **2012**; 80:2346–53.
5. Ganesh K, Allam M, Wolter N, et al. Molecular characterization of invasive capsule null *Neisseria meningitidis* in South Africa. BMC Microbiol **2017**; 17:40.
6. Meiring S, Cohen C, de Gouveia L, et al. Declining incidence of invasive meningococcal disease in South Africa: 2003–2016. Clin Infect Dis **2018**; 69:495–504. doi:10.1093/cid/ciy914.
7. von Gottberg A, du Plessis M, Cohen C, et al; Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa. Emergence of endemic serogroup W135 meningococcal disease associated with a high mortality rate in South Africa. Clin Infect Dis **2008**; 46:377–86.
8. Cohen C, Singh E, Wu HM, et al; Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA). Increased incidence of meningococcal disease in HIV-infected individuals associated with higher case-fatality ratios in South Africa. AIDS **2010**; 24:1351–60.
9. Simmons RD, Kirwan P, Beebeejaun K, et al. Risk of invasive meningococcal disease in children and adults with HIV in England: a population-based cohort study. BMC Med **2015**; 13:297.
10. Folaranmi TA, Kretz CB, Kamiya H, et al. Increased risk for meningococcal disease among men who have sex with men in the United States, 2012-2015. Clin Infect Dis **2017**; 65:756–63.
11. Statistics South Africa. Mid-year population estimates, South Africa, **2016**. STATS-SA Available at: http://www.statssa.gov.za/publications/P0302/P03022016.pdf. Accessed 17 July 2017.
12. Department of Health. Guidelines for the management, prevention and control of meningococcal disease in South Africa. vol. 2017, **2011**. Available at: https://www.nicd.ac.za/assets/files/DoH%20Meningococcal%20Disease%20Guidelines%202011.pdf. Accessed 21 May 2020.
13. Meiring S, Hussey G, Jeena P, Parker S, von Gottberg A. Recommendations for the use of meningococcal vaccines in South Africa. South African J Infect Dis **2017**; 32: 82–6.
14. MacLennan J, Kafatos G, Neal K, et al; United Kingdom Meningococcal Carriage Group. Social behavior and meningococcal carriage in British teenagers. Emerg Infect Dis **2006**; 12:950–7.
15. Kristiansen PA, Ba AK, Sanou I, et al. Phenotypic and genotypic characterization of meningococcal carriage and disease isolates in Burkina Faso after mass vaccination with a serogroup a conjugate vaccine. BMC Infect Dis **2013**; 13:363.
16. MenAfriCar Consortium. 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**; 212:1298–1307.
17. Trotter C, Lingani C, Fernandez K, et al. Impact of MenAfriVac in nine countries of the African meningitis belt, 2010–15: an analysis of surveillance data. Lancet Infect Dis **2017**; 17:867–72. doi:10.1016/ S1473-3099(17)30300–6.
18. Nnadi C, Oladejo J, Yennan S, et al. Large outbreak of Neisseria meningitidis serogroup C—Nigeria, December 2016-June 2017. MMWR Morb Mortal Wkly Rep **2017**; 66:1352–6.
19. 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.
20. 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.
21. Neal KR, Nguyen-Van-Tam JS, Jeffrey N, et al. Changing carriage rate of *Neisseria meningitidis* among university students during the first week of term: cross sectional study. BMJ **2000**; 320:846–9.
22. McMillan M, Walters L, Mark T, et al. B Part of It study: a longitudinal study to assess carriage of *Neisseria meningitidis* in first year university students in South Australia. Hum Vaccin Immunother **2018**; 15: 987–94. doi:10.1080/21645515.2018.1551672.
23. Breakwell L, Whaley M, Khan UI, et al. Meningococcal carriage among a university student population—United States, 2015. Vaccine **2018**; 36:29–35.
24. Oldfield NJ, Cayrou C, AlJannat MAK, et al. Rise in group W meningococcal carriage in university students, United Kingdom. Emerg Infect Dis **2017**; 23:1009–11.
25. Glennie SJ, Banda D, Gould K, et al. Defective pneumococcal-specific Th1 responses in HIV-infected adults precedes a loss of control of pneumococcal colonization. Clin Infect Dis **2013**; 56:291–9.
26. Kobayashi M, Bigogo G, Kim L, et al. Impact of 10-valent pneumococcal conjugate vaccine introduction on pneumococcal carriage and antibiotic susceptibility patterns among children aged <5 years and adults with human immunodeficiency virus infection: Kenya, 2009–2013. Clin Infect Dis **2020**; 70:814–26. doi: 10.1093/cid/ciz285.
27. Heinsbroek E, Tafatatha T, Phiri A, et al. Persisting high prevalence of pneumococcal carriage among HIV-infected adults receiving antiretroviral therapy in Malawi: a cohort study. AIDS **2015**; 29:1837–44.
28. Manigart O, Okeakpu J, Odutola A, et al. Alternative molecular methods for improved detection of meningococcal carriage and measurement of bacterial density. J Clin Microbiol **2016**; 54:2743–8.
29. Dolan Thomas J, Hatcher CP, Satterfield DA, et al. sodC-Based real-time PCR for detection of *Neisseria meningitidis*. PLoS One **2011**; 6:e19361.
30. Wang X, Theodore MJ, Mair R, et al. Clinical validation of multiplex real-time PCR assays for detection of bacterial meningitis pathogens. J Clin Microbiol **2012**; 50:702–8.
31. Diallo K, Trotter C, Timbine Y, et al. Pharyngeal carriage of *Neisseria* species in the African meningitis belt. J Infect **2016**; 72:667–77.
32. Ala'Aldeen DAA, et al. Carriage of meningococci by university students, United Kingdom. Emerg Infect Dis **2011**; 17:1761–3.
33. Terranova L, Principi N, Bianchini S, et al. Neisseria meningitidis serogroup B carriage by adolescents and young adults living in Milan, Italy: prevalence of strains potentially covered by the presently available meningococcal B vaccines. Hum Vaccin Immunother **2018**; 14:1070–4.
34. Reddy P, Zuma K, Shisana O, Kim J, Sewpaul R. Prevalence of tobacco use among adults in South Africa: results from the first South African National Health and Nutrition Examination Survey. S Afr Med J **2015**; 105:648–55.
35. MacNeil JR, Rubin LG, Patton M, Ortega-Sanchez IR, Martin SW. Recommendations for use of meningococcal conjugate vaccines in HIV-infected persons—Advisory Committee on Immunization Practices, 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:1189–94.
36. Advisory Committee on Immunization Practices. Updated recommendations for use of meningococcal conjugate vaccines—Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Morb Mortal Wkly Rep **2011**; 60:72–6.
37. Moreno J, Sanabria O, Saavedra SY, Rodríguez K, Duarte C. Phenotypic and genotypic characterization of *Neisseria meningitidis* serogroup B isolates from Cartagena, Colombia, 2012-2014. Biomedica **2015**; 35:138–43.
38. MenAfriCar Consortium. Household transmission of *Neisseria meningitidis* in the African meningitis belt: a longitudinal cohort study. Lancet Glob Heal **2016**; 4:e989–95.
39. Higher Education HIV and AIDS Programme (HEAIDS). HIV prevalence and related factors: Higher Education Sector Study South Africa 2008–2009. **2009**. Available at: http://www.heaids.org.1. Accessed 19 November 2016.
40. Dandara C, Chimusa ER, Wonkam A. South African student protests, 2015 - 2016: the aftermath viewed through medical science honours students at the University of Cape Town. South African Med J **2017**; 107:723.