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Meningococcal carriage in men who have sex with men presenting at a sexual health unit in Spain

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Abstract

Neisseria meningitidis (Nm) is asymptomatically carried in the nasopharynx of 5 to 10% adults, although certain populations, such as men who have sex with men (MSM) exhibit a higher colonization rate. Interest in Nm carriage has been renewed, owed to meningitis outbreaks within populations of MSM. The aim of this study was to characterise Nm isolates and risk factors for its carriage among MSM attending a sexual health unit. A retrospective cross-sectional study was undertaken between June 2018 and December 2021. We took anal, oropharyngeal, urethral and blood samples as part of the sexually transmitted infection screening procedures routinely implemented. Nm isolates were subjected to antimicrobial susceptibility testing; the serogroup and genogroup were determined by multi-locus sequence typing. A total of 399 subjects were recruited, ant the Nm oropharyngeal carriage rate was nearly 29%, similar among both people living with HIV (PLWH) and uninfected individuals. Nm carriage was less common in vaccinated individuals, especially those who had received the tetravalent vaccine (2.6% vs 10.6%, *p*=0.008). The most frequent serogroups were B (40%) and non-groupable (45%). Most of isolates were susceptible to ciprofloxacin (96%), and ceftriaxone (100%). However, we identified 21 strains (20%) belonging to hyperinvasive lineages (CC11, CC4821, CC32, CC41/44, CC213, and CC269), most of which belonged to serogroup B. Given that vaccination with MenACWY was associated with a low Nm carriage, we encourage routine vaccination of all MSM. Moreover, the administration of the meningitis B vaccine should also be assessed considering that several invasive lines included in serogroup B are circulating among MSM.

Introduction

Neisseria meningitidis (Nm), also known as meningococcus, is a gram-negative diplococcus which is distributed worldwide and is the leading cause of meningitis and sepsis [1]. Six serogroups (A, B, C, W, X and Y) cause most of the invasive meningococcal disease (IMD) globally. Several comorbidities increase the risk of IMD, especially those compromising the efficacy of the immune system. Congenital immunodeficiencies, human immunodeficiency virus (HIV) infection, asplenia, solid organ transplantation, some drugs (e.g., eculizumab) and complement deficiency increase the risk of IMD [2–4]**.** Nm is asymptomatically carried in the nasopharynx of 5 to 10% of adults during non-epidemic periods and transmission usually occurs by direct contact with oral or nasal secretions [5]. Nm is also frequently isolated from other mucosal sites, specifically the urogenital tract (cervix, vagina, urethra) and the rectum.

Of note, certain populations, such as men who have sex with men (MSM) exhibit a significantly higher nasopharyngeal carriage rate of around 30% [6–8]**.** Since the 2000s, several IMD outbreaks caused by serogroup C have been reported worldwide, especially among MSM. Moreover, MSM exhibit a fourfold greater risk for developing IMD compared to men who have sex with women (MSW) [9]. Research focused on Nm carriage and microbiological identification, especially in high-risk populations, is needed to better understand the transmission of Nm and to design adequate vaccination policies. The aim of this current study was to characterise Nm isolates and risk factors for Nm carriage within MSM attending the sexual health unit at the Alvaro Cunqueiro Hospital (Vigo, Spain).

Material and methods

Study design

This was a retrospective cross-sectional study undertaken at the Infectious Disease Unit in the University Hospital Complex of Vigo (Spain), a tertiary hospital serving a population of around 500,000 inhabitants. We considered the sexually transmitted infection (STI) screening procedures performed between June 2018 and December 2021 , which accounted for a total of 977 screenings of 546 patients. Only MSM aged ≥ 18 years-old were recruited, and incomplete screenings (one or more samples missing) were excluded. Thus, 399 patients were finally enrolled in this study (Figure 1). Epidemiological and clinical data were obtained from the medical records of the patients.

Sample management and the detection of sexually transmitted infections

Anal, oropharyngeal, urethral and blood samples gathered at the Infectious Disease Unit were screened for STIs as part of the standard screening procedures by taking two swabs from each anatomical site. Bacterial culture tests with Stuart medium (Copan, Italy) were performed from one swab and polymerase chain reaction (PCRs) for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) were conducted with the second swab (Cobas® PCR media, dual Swab sample kit and Cobas® CT/NG assay on the Cobas 6800 System, Roche Diagnostics, Switzerland). The blood samples were tested for HIV (Elecsys HIV Duo, Roche Diagnostics) and *Treponema pallidum* (enzyme immunoassay or ELISA, LIAISON®, DiaSorin; rapid plasma regain or RPR, Biomérieux®, France).

A new episode of syphilis was defined as a positive ELISA test result for *T. pallidum* after a negative test or an increase in the rapid plasma regain ratio (by at least two dilutions) compared to the previous serological study. Information about HIV viral load and CD4 lymphocyte count (nadir, baseline and current) were obtained from the medical records of people living with HIV (PLWH). The study population was classified according to the isolation of Nm; the non-carrier

group comprised patients for whom Nm was not isolated in any sample, and the Nm carrier group contained patients for whom Nm was isolated in at least one oropharyngeal sample. For patients in the non-carrier group, only the first screening procedure during the study period was considered, regardless of the STI result. In addition, for patients in the Nm carrier group, only the first Nm isolate was analysed.

Neisseria meningitidis culture and antibiotic susceptibility test

Pharyngeal, rectal, and urethral swabs were cultured on Thayer-Martin medium (Poly ViteX VCAT3 agar, BioMérieux®, France) and GC agar (BD chocolate agar; Becton Dickinson, USA) and incubated for 24-48 h at 35-37 ºC in a 5% CO_2-enriched atmosphere. Nm isolates from the same plates used for NG growth were identified. The colonies with suspected *Neisseria* species were sub-cultured onto chocolate agar plates. Pure isolates that were oxidase positive were further identified using the NH API system (BioMérieux) and mass spectrometry (MALDI-TOF, Bruker, Germany). The minimum inhibitory concentrations (MICs) of penicillin, ceftriaxone, ciprofloxacin, azithromycin, trimethoprim-sulfamethoxazole, and rifampin were determined by ETES (BioMérieux) on Mueller-Hinton agar supplemented with 5% sheep blood (BD MHF agar; Becton Dickinson). The MICs for penicillin, ceftriaxone, ciprofloxacin and rifampicin were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 12.0; 2022. The MICs used for azithromycin and trimethoprim-sulfamethoxazole were those recommended by the Clinical and Laboratory Standards Institute (CLSI) 32nd ed. Supplement M100; 2022.

Characterisation of N. meningitidis isolates

Nm isolates were characterized in the *Neisseria* Reference Laboratory of the National Centre for Microbiology (ISCIII). Both the serogroup and genogroup were determined for all the isolates. The serogroup was obtained by slide agglutination with specific polyclonal antibodies against serogroups A, B, C, X, Y, W, E and Z, according to each manufacturer's instruction. The

genogroups A, B, C, E, W, X, Y, and Z were detected by different PCR amplification protocols, as previously described [10–12]. All Nm isolates were also further molecular characterized, including PorA genosubtyping and multi-locus sequence typing (MLST). PorA genotyping was done by sequencing the variable regions (VRs) of the PorA gene according to methods previously described [13] and comparing them with accessions in the *Neisseria* PorA database (http://pubmlst.org/neisseria/PorA/). MLST was performed as described by Maiden et al. [14]. PCR-amplified DNAs from 7 different housekeeping genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC*, and *pgm*) were sequenced and compared with the *Neisseria* MLST database (http://pubmlst.org/neisseria/). The sequence type (ST) and clonal complex (cc) were assigned according to the same web site.

Statistical analyses

Study data were collected and managed using REDCap electronic data capture tools hosted at the Instituto de Investigación Sanitaria Galicia Sur (IISGS). Research Electronic Data Capture (REDCap) is a secure, web-based software platform designed to support data capture for research studies. Quantitative variables are expressed as the median and interquartile range, while qualitative variables are shown as absolute values and percentages. Categorical variables were compared using χ-squared tests or Fischer exact tests as appropriate, and quantitative variables were compared using Mann-Whitney *U*-tests. An ANOVA test was used to analyse the trends of antibiotic susceptibility during the study period. In all cases, *p* values less than 0.05 were considered significant. Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) software (version 22, IBM Corp., Armonk, NY).

Results

Between June 2018 and December 2021, at least one STI screening was performed in 546 different patients, and of these, we analysed 399 cases (Figure 1). A total of 115 of the 399 patients included in the analysis tested positive for oropharyngeal Nm, accounting an overall carriage rate of 28.8%. Nm was not isolated in any of the urethral or rectal samples. During the study period, 8 cases of IMD were identified in our hospital (2 infants and 6 adults), but none of these were in patients included in our study cohort.

The clinical characteristics of the study population according to the Nm carriage status are shown in Table 1. The median patient age was 36 years for both Nm carriers and non-carriers. Moreover, patients under 30 years old exhibited a similar Nm colonisation rate to individuals aged over 30 years (29.5% vs 28.5%, respectively). Furthermore, the rate of Nm colonisation was similar between PLWH and HIV-uninfected patients (27.8% vs 30.1%, respectively). It is worth noting that most of the HIV-uninfected individuals were on a pre-exposure prophylaxis (PrEP) program for HIV (73.8%, 135 subjects).

In addition, the frequency of Nm colonization did not vary in patients who presented STI symptoms (e.g., dysuria, anal or urethral exudate, or inguinal adenopathy) compared to those who were asymptomatic (25.3% vs 29.6%, respectively). Furthermore, tobacco consumption was not related to a higher prevalence of Nm colonisation (25.2% vs 30.8%). Moreover, a previous diagnosis of a STI (CT, NG, or syphilis) was not associated with a higher prevalence of Nm carriage (29.2% vs 28.1%, respectively). Indeed, a simultaneous positive result for NG or CT infection was also not related to an increased prevalence of Nm carriage. Surprisingly, a positive test for syphilis was significantly associated with a lower Nm carriage rate (5.2% vs 15.1%, p=0.006). Among these patients, one subject was previously diagnosed of non-gonococcal urethritis, while 12 % (6subjects) had a prior history of gonorrhoea.

Regarding the Nm vaccine, only one patient had received the meningitis B (MenB) vaccine, 55 patients had had the meningitis C (MenC) vaccine, and 33 patients had received the tetravalent meningitis vaccine (MenACWY). The pooled analysis of all the vaccinated individuals showed no differences in Nm carriage (18.3% Nm carrier vs 24.3% non-Nm carrier), with a total of 21 individuals being Nm carriers. On the one hand, the most frequent serogroups isolated in vaccinated patients were non-groupable Nm (9 isolates), followed by serogroup B (6 isolates), other serogroups (3 isolates), serogroup C (1 isolate) and serogroup Y (1 isolate). One strain could not be processed. On the other hand, Nm carriage was less common in patients who had received the MenACWY vaccine (2.6% vs 10.6%, *p*=0.008), in a total of 3 cases. Serogroup B Nm was isolated in two patients followed by serogroup Y in one patient.

Neisseria meningitidis carriage in PLWH

A total of 216 PLWH were included in the analysis, and most of them were receiving antiretroviral treatment (ART) at the time of the STI screening procedure. In addition, the prevalence of Nm carriage did not vary between virologically suppressed (i.e., HIV viral load under 50 copies/mL) and non-supressed patients (27.4% vs 27.9%). Patient age was also similar between the two groups. Finally, the nadir CD4 lymphocyte count (357 vs 318, *p*=0.037) and CD4 lymphocyte count at the time of screening (863 vs 735, *p*=0.016) were lower in the non-carrier group (Table 1).

Antibiotic susceptibility

A total of 114 Nm isolates were tested for antimicrobial susceptibility (Table 2), with 69.4% of the Nm isolates being penicillin-susceptible (MIC<0.25mg/l). The proportion of isolates with susceptibility to penicillin was the lowest in the year 2018 (35%) and highest in 2021 (81.8%), but this trend was not statistically significant. Overall, 53.5% of the Nm isolates were susceptible to trimethoprim-sulfamethoxazole (MIC≤0.12/2.4 mg/L), and there were no significant changes during the study period. Similarly, 65% of the isolates were susceptible to azithromycin (MIC≤2

mg/L), and a significant increase in susceptibility was found over the 4-year period (35.0% in 2018 vs 86.4% in 2021, *p*=0.034). The trend analysis did not report changes in ciprofloxacin-susceptibility (95.6%; MIC≤0.03 mg/L) or rifampin susceptibility (93.9%; MIC≤0.25 mg/L) rates during the study period. All the Nm isolates were susceptible to ceftriaxone (MIC≤0.125 mg/L).

Characterization of the meningococcal isolates

Molecular characterization was performed in 106 Nm isolates. As shown in Table 3, the most common serogroup was B (*n*=42, 39.6%) followed by non-groupable (*n*=38, 33%) and self-agglutinating (*n*=10; 8.7%). MLST identified 20 STs that belonged to 14 clonal complexes, with 61 isolates (57 %) currently unassigned. The most common clonal complexes were CC213 (*n*=7, 6,6%) and CC162 (*n*=7, 6.6%). Other CCs found, in order of frequency, were CC4821 (*n*=6, 5.7%), CC53 (*n*=5, 4.7%), CC1572 (*n*=4, 3.8%) and CC11 (*n*=4, 3.8%). Three of the CC11 isolates were serogroup C, and one was serogroup W135. No other CCs accounted for more than 3% of the isolates. The serogroup B isolates included CC213 (*n*=7), CC4821 (*n*=6), CC1572 (*n*=4), CC32 (*n*=2), CC461 (*n*=2), CC41/44 (*n*=1), CC461 (*n*=1), CC269 (*n*=1), CC162 (*n*=1), and CC750 (*n*=1). The characteristics of the 106 isolates are shown in Table 3.

Discussion

Several events have led to renewed interest in Nm colonisation, especially outbreaks of IMD in MSM around the word [15,16] and genetic changes in Nm that suggest its adaptation to new niches such as the rectum and urethra [17]. In our study we found an oropharyngeal carriage rate of 29%, with no rectal or urethral cases. Similar to our experience, several studies have reported a high prevalence of Nm oropharyngeal carriage in MSM [8]. Fortunately, so far, we have not detected any cases of invasive disease in our study population. Of note, traditional risk factors for IMD (i.e., asplenia, complement deficiency, or C5 inhibitor therapy) were absent in our study cohort, and most of PLWH had an adequate immunovirological status (i.e. virologically suppressed and a CD4 lymphocyte count over 200 cells/µL). In addition, a third of the sample was healthy young males included in a PrEP program.

The prevalence of meningococcal vaccinated patients was lower than expected (less than 23%), especially with the tetravalent vaccine (8.4%). The MenC vaccine was included in the Spanish vaccination schedule in 2000s and was administered in several catchment campaigns for young adults. In addition, in 2019, MenACWY was incorporated into the vaccine calendar with a booster dose recommended for patients who received the MenC vaccine during childhood. Moreover, the administration of MenACWY is recommended in PLWH [18]. The MenACWY vaccine was related with a lower Nm carriage rate in our study population (2.6% vs 10.6%, p=0.008). This data concurs with previous studies that reported a decrease in Nm carriage among vaccinated patients [19][20]. Conversely, a recent meta-analysis performed by McMillan et al. did not demonstrate a reduction in serogroup B carriage in MenB-vaccinated patients [21]. Among our study cohort, only one patient had received the MenB vaccine because of an anatomic asplenia. Thus, we could not study the relationship between MenB vaccine and Nm carriage in this current work. In Spain, the MenB vaccine is only approved for use in high-risk populations and does not include PLWH [18].

The relationship between HIV infection and Nm carriage is equivocal, with contradictory data reported in the academic literature. For example, Rosotti et al. reported a similar prevalence of Nm carriage between PLWH and HIV-uninfected individuals (54.8% vs 56.0%) [22], while Meiring et al. reported a fivefold higher risk for Nm carriage in PLWH [23]. Focusing on MSM-PLWH, we found a lower nadir CD4 lymphocyte count in non-Nm carriers. Moreover, a CD4 nadir under 200 cells/µL was significantly associated with a lower Nm carriage rate (p=0.002). Previous research has not reported differences in the CD4 lymphocyte count between Nm carriers and non-carriers [8]. According to current HIV guidelines, severe immunocompromised patients (i.e. a CD4 lymphocyte count less than 200 cells/µL) receive long-term cotrimoxazole as a prophylaxis for *Pneumocystis jirovecii* and *Toxoplasma* infections until their CD4 lymphocyte counts remain stable above 200 cells/µL [24]. Therefore, we propose that previous treatment with cotrimoxazole might increase the chance of Nm eradication, although it is important to consider that almost 50% of the isolates were resistant to this antimicrobial in vitro. Furthermore, similar to previous research [8,22], virological suppression (i.e., HIV-RNA under 50 copies/mL) was not related to a decreased rate of Nm carriage.

Morselli et al. recently reported an intriguing interplay between Nm, NG, and the immune system in which a negative association between Nm and NG was found an the presence of pharyngeal NG was significantly higher in patients without meningococcal carriage (p=0.03) [25]. In our study, the frequency of Nm colonisation did not vary in patients who presented pharyngeal NG. Moreover, we found no differences between Nm and non-Nm carriers among patients with an acute or past gonorrhoea or an acute or past *C. trachomatis* infection (Table 2). Identical results were also obtained in a recent publication from a sexual health clinic in New York in which oropharyngeal Nm carriage in MSM (22.6%) was not associated with the presence of other STIs. Nevertheless, these authors concluded that Nm carriage was associated with the number of men kissed, performance of oral sex, and antibiotic use in the preceding 30 days [6].

Surprisingly, we found a significant association between a new, untreated diagnosis of syphilis and a lower Nm carriage rate (p=0.006). Nonetheless, there was no difference in the Nm carriage prevalence among participants with a past episode of syphilis. On the other hand, among those patients diagnosed of a new episode of syphilis, only one patient was diagnosed of non-gonococcal urethritis, while 6 subjects had a prior history of gonorrhoea. Between June 2018 and December 2019, the local protocols for gonorrhoea included a combined regimen of ceftriaxone (500 mg single intramuscular dose) and azithromycin (2 g single oral dose). A revision of the protocol in early 2020 removed azithromycin and increased the dose of ceftriaxone to a 1000 mg, owed to a decreased antimicrobial susceptibility of NG isolates. Thus, those patients diagnosed of gonorrhoeae were exposed to antimicrobial drugs with a long-lasting half-life [26, 27], maybe decreasing the risk of Nm colonisation.

Evaluation of antibiotic susceptibility revealed a high proportion of Nm isolates susceptible to penicillin (69%). The increase in the percentage of penicillin-susceptible isolates we found from 2018 (35%) to 2021 (82; Table 2) contrasts with global trends, with multiple countries reporting an increase in penicillin-intermediate and resistant Nm isolates, both in patients with IMD and in asymptomatic MSM carriers [7, 28, 29]. Only 53.5% of the Nm isolates were susceptible to trimethoprim-sulfamethoxazole; because of the early introduction of sulphonamides, resistance to these compounds is widespread [30]. Interestingly, a significant increase in azithromycin susceptibility rates was observed between 2018 (35%) and 2021 (65%; p=0.034), likely because of its removal from the treatment of gonorrhoea schedules in sexual health units in 2020. Similar to previous research [29], resistance to ciprofloxacin and rifampicin was low. These two antibiotics, as well as ceftriaxone (100% susceptibility), are used for chemoprophylaxis of meningococcal disease. Spanish guidelines recommended chemoprophylaxis only for the contacts of patients with IMD [18], and so in our study, no meningococcal carriers were treated. Although the frequency of resistant meningococci is still low, continued surveillance is required

to monitor trends in their susceptibilities and to state appropriate empirical therapy and chemoprophylaxis.

Most of the groupable meningococci were serogroup B (39.6%), which represented one of the most frequent serogroups of IMD isolates in the general population in Spain in the same period [31]. The rate of Nm serogroup B in our study was similar to the results from the carriage study performed in Italy from MSM [28]; however, it was much higher than the rate found in other carriage studies performed in New York [6] and Denmark [8] in MSM (5% and 9.8%, respectively). Conversely, most of meningococci isolated from outbreaks across Europe belong to serogroup C, while the carriage of this serogroup was scarce among our study population (5.2%; Table 2). This agrees with other reports that found that serogroup C has a low nasopharyngeal carriage prevalence [7].

In our study, the clonal analysis of the isolated meningococci revealed that clonal complexes reported both in healthy carriers and in outbreak strains. Previous research suggests that meningococcal carriage is dominated by less invasive meningococci not associated with IMD [32]. Nevertheless, we identified 21 strains (20%) that belonged to known hyperinvasive lineages (CC11, CC4821, CC32, CC41/44, CC213, and CC269; Table 3). Of note, CC11 has rapidly spread across Europe and other regions, causing outbreaks in both the general population and among MSM [33]. We also identified 6 serogroup B CC4821 meningococci. IMD outbreaks caused by this pandemic clone has been mainly limited to China so far, albeit with a recent expansion to other locations [34]. CC32, CC41/44, CC213, and CC269 are epidemic clones associated with IMD that have been circulating for many years, both in Spain and Europe. All the strains from the different clonal complexes were serogroup B [35].

Conclusions

Since vaccination with MenACWY has been associated with a low Nm carriage rate and the prevalence of meningococcal vaccinated MSM was low in our study, we encourage routine

vaccination of all MSM with MenACWY. Moreover, it may be valuable to add a MenB vaccine to the recommended vaccination program for MSM, considering that several invasive lines in serogroup B are circulating in the MSM network. MenB vaccines are unlikely to disrupt carriage but could provide immunity to infection. This carriage study provides a further understanding of the epidemiology of NM, which is important for future vaccination policies against specific serogroups given that countries may consider vaccinating the proportion of the population with the highest carriage levels and who are thus, responsible for most transmission.

Limitations

Our study has some limitations. First, because of its retrospective nature, some data could have been lost. However, to mitigate this problem, patients with missing information in their medical records were excluded from our analysis. Second, this was a single-centre study of MSM at one STI clinic, and so our findings should be validated in large patient cohorts. Finally, Nm is a fastidious organism that can be difficult to grow and diagnose using culture-based methods, and so it should be diagnosed using molecular methods in addition to cultures.

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Author contribution

APG and CP conceptualised the study, APG planned the statistical analysis with input from CP and RC. APG, AAA, and AO designed the database and performed the data curation. RA and CN performed the Nm identification and molecular characterisation. CP and RC performed the cultures and antimicrobial susceptibility test of Nm isolates. APG wrote the original manuscript draft, and EP and RA revised it for important intellectual content. All the authors have approved the final version of this article.

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval: All the procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional research committee at Galicia and complied with the 1964 declaration of Helsinki and its later amendments or comparable ethical standards. This work was approved by the Ethics Committee of Pontevedra-Vigo-Ourense wit reference number 2021/311.

Consent to participate: Informed consent was waived because of the retrospective design of this study.

Competing interests: The authors declare no competing interests.

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Table 1. Baseline characteristics of our study population according to their *Neisseria meningitidis* carriage status

	Nm carriers *n*=115 (28.8%)	**Non-Nm carriers *n*=284 (71.2%)**	**Total *n*=399**	***p*-value**
Gender, n (%)				
• Men	112 (97.4%)	274 (96.5%)	386 (96.7%)	*p*=0.765
• Transgender women	3 (2.6%)	10 (3.5%)	13 (3.3%)	
Age (in years)	36 (17)	36 (14)	36 (15)	*p*=0.792
HIV infection	60 (52.2%)	156 (54.9%)	216 (54.1%)	*p=0.617*
STI symptoms				
• Asymptomatic	96 (83.5%)	228 (80.3%)	324 (81.2%)	*p*=0.459
• STI-associated symptoms	19 (16.5%)	56 (19.7%)	75 (18.8%)	
Tobacco consumption				
• Active smoker	35 (30.4%)	104 (36.6%)	139 (34.8%)	*p*=0.240
• Former smoker	5 (4.3%)	29 (10.2%)	34 (8.5%)	
• Never smoker	50 (43.5%)	103 (36.3%)	153 (38.3%)	
• Missing data	25 (21.7%)	48 (16.9%)	73 (18.3%)	
Simultaneous STI	33 (28.7%)	97 (34.2%)	130 (32.6%)	*p*=0.292
• Gonorrhoea	13 (11.3%)	43 (15.1%)	56 (14.0%)	*p*=0.318
• *Chlamydia trachomatis* infection	16 (13.9%)	33 (11.6%)	49 (12.3%)	*p*=0.527
• Syphilis	6 (5.2%)	43 (15.1%)	49 (12.3%)	*p*=0.006
• Oropharynx NG or CT	10 (8.7%)	23 (8.1%)	33 (8.3%)	*p*=0.566
Previous STI	74 (64.3%)	179 (63.0%)	253 (63.4%)	*p*=0.804
• Gonorrhoea	23 (20.0%)	48 (16.9%)	71 (17.8%)	*p*=0.463
• *Chlamydia trachomatis* infection	16 (13.9%)	25 (8.8%)	41 (10.3%)	*p*=0.146
• Syphilis	48 (41.7%)	118 (41.5%)	166 (41.6%)	*p*=0.972
• Non-gonococcal urethritis	11 (3.9%)	4 (3.5%)	15 (3.8%)	*p*=1
Nm vaccine, any vaccine	21 (18.3%)	69 (24.3%)	90 (22.6%)	*p*=0.234
MenACWY vaccine	3 (2.6%)	30 (10.6%)	33 (8.3%)	*p*=0.008
MenC vaccine	18 (15.7%)	37 (13.0%)	55(13.8%)	*p*=0.522
HIV subgroup analysis (n=216)				

• AIDS defining ilness, n (%)	6 (10.0%)	25 (16.0%)	31 (14.4%)	*p*=0.288
• Nadir CD4 lymphocyte count (cells/µL)	357 (217)	318 (320)	342 (293)	*p*=0.037
• Nadir CD4 under 200 cells/µL, n (%)	5 (9.4%)	39 (30.2%)	44 (24.2%)	*p*=0.002
• Baseline CD4 lymphocyte count (cells/µL)	467 (355)	413 (405)	440 (396)	*p*=0.178
• CD4 lymphocyte count at the screening (cells/µL)	863 (451)	735 (549)	769 (535)	*p*=0.016
• HIV RNA < 50 copies/mL,n (%)	43 (71.7%)	111 (71.2%)	154 (71.3%)	*p*=0.941

Quantitative variables are expressed as median and interquartile range (IQR), qualitative variables are expressed as numbers and percentages. Nm: ***Neisseria meningitidis***; HIV: human immunodeficiency virus; STI: sexually transmitted infection; NG: ***Neisseria gonorrhoeae***; CT: ***Chlamydia trachomatis***; AIDS: acquired immunodeficiency syndrome.

Table 2. Antimicrobial susceptibility and serogroup distribution of *Neisseria meningitidis* isolates

Number of susceptible isolates, *n*=114

	Penicillin	Ceftriaxone	TMP-SMX	Azithromycin	Ciprofloxacin	Rifampin
2018	7 (35)	20 (100)	10 (50)	7 (35)	20 (100)	20 (100)
2019	21 (63.6)	33 (100)	19 (57.6)	21 (63.6)	32 (97)	30 (90.9)
2020	32 (82.0)	29 (100)	23 (59)	27 (69.2)	39 (100)	35 (89.7)
2021	18 (81.8)	22 (100)	9 (41)	19 (86.4)	18 (81.8)	22 (100)
Total	78 (68.4)	114 (100)	61 (53.5)	74 (65)	109 (95.6)	107 (93.9)

***Neisseria meningitidis* serogroup distribution**

	B	NG	C	W135	Z	Y	E	X
Number of isolates, n (%)	42 (39.6%)	48 (45%)	6 (5.2%)	3 (2.8%)	3 (2.8%)	2 (1.9%)	1 (0.9%)	1 (0.9%)

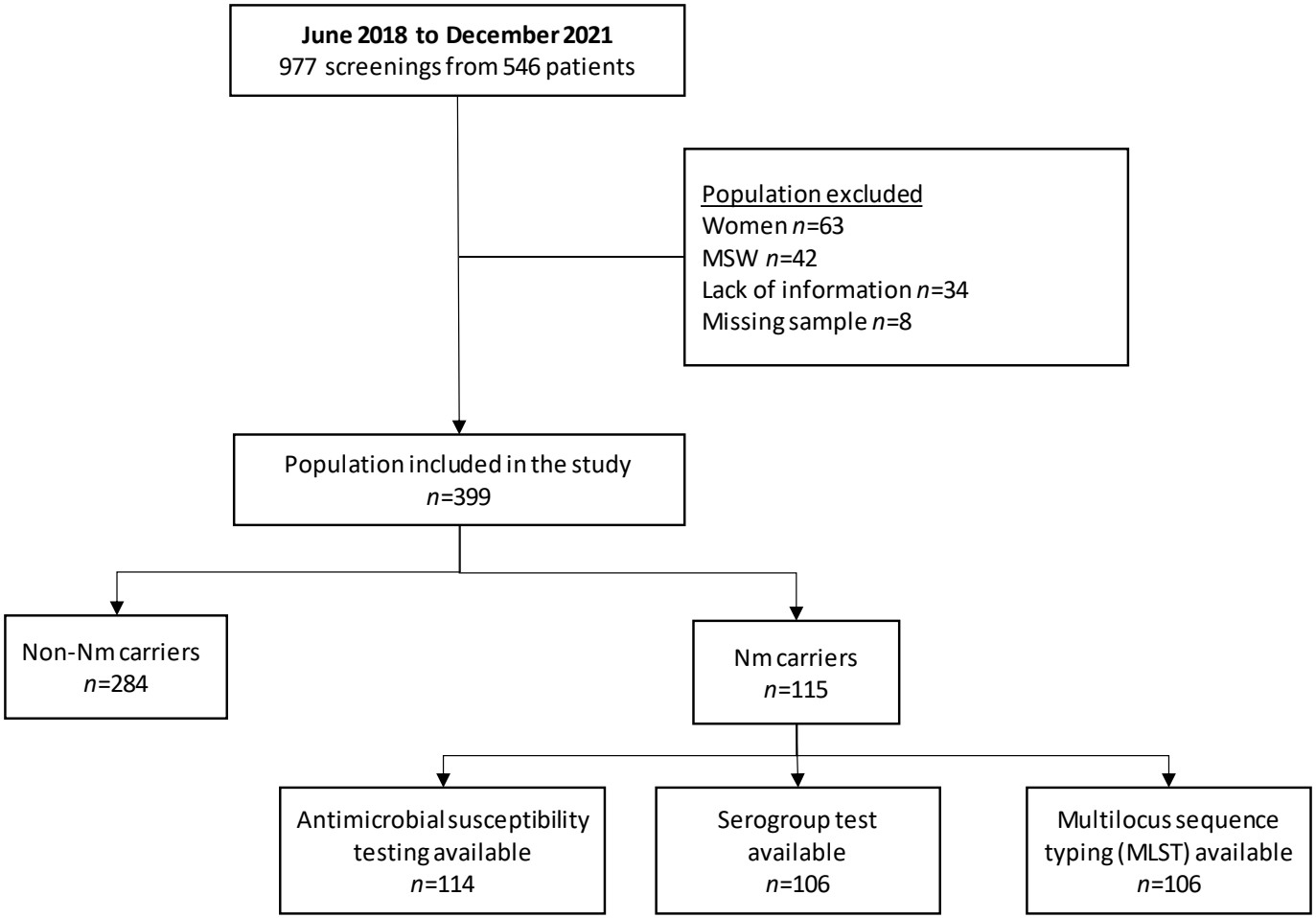
Isolates are expressed as numbers and percentages; TMP-SMX: trimethoprim-sulfamethoxazole; NG: non-groupable

Table 3. Molecular characterisation of *Neisseria meningitidis* isolates

Clonal complex	Sequence type	Genosubtype (PorA)	Number of isolates	Serogroup
11 (*n*=4)	11	5-1/10-8	1	C
	12394	5-2	1	C
	12394	7-4/1	1	C
	11	5/2	1	W135
32 (*n*=2)	11527	22/14-68	1	B
	11527	22-42/14-6	1	B
41/44 (*n*=1)	2922	12-1/13-1	1	B
53 (*n*=5)	53	7-64/30	1	NG
	53	7/30-2	2	NG
	53	7-30-3	2	NG
103 (*n*=1)	14405	5-1/10-46	1	Y
162 (*n*=7)	2153	19/15-1	1	SA
	2153	7-2/13-33	1	SA
	2153	19/15-1	1	SA
	2153	19-2/13-15	1	NG
	2153	12-1/23-30	1	NG
	2153	22/14-68	1	NG
	12483	17-2/4	1	B
198 (*n*=3)	823	18/25-14	1	NG
	823	18-22/25-15	1	NG
	16132	18/25-44	1	NG
213 (*n*=7)	213	22/14	4	B
	3496	22/14	2	B
	3496	22-1/1	1	B
254 (*n*=1)	254	5-1/2-2	1	E
269 (*n*=1)	10864	19/15-1	1	B
461 (*n*=2)	1946	19-2/13-15	2	B
750 (*n*=1)	15642	22/1	1	B
1572(*n*=4)	1572	7-12/14	4	B
4821(*n*=6)	3200	17-6/23	1	B
	3200	12-1/23-2	1	B
	3469	17-6/23	1	B
	11311	17-6/23	3	B
Unassigned CC (*n*=61)	897	12-1/16-75	2	B
	1111	12-1/13-1	1	B
	1768	21/4	2	W135
	1768	21/4-18	1	SA
	2888	19/15	1	X
	2888	19/15	1	NG
	2888	19/15	1	SA
	3882	5-2/10-62	1	NG
	4221	17-1/23-2	1	NG
	4221	19/15	1	NG
	4221	7-2/1	1	NG
	4221	19/15	1	NG
	4221	12-1/23-2	1	NG
	5953	18-1/30-28	1	NG

	5953	18-1/13-81	1	NG
	5953	18-1/30-8	1	SA
	6119	12-8/16-119	1	NG
	8930	22/9	1	NG
	8930	5/22	1	NG
	8930	17-6/23-7	1	SA
	8939	18-1/30-2	1	SA
	9191	22/9-18	1	B
	10866	22/14-3	2	NG
	10866	22/14-3	1	NG
	11312	18-1/13-8	1	NG
	11506	12-35/16-11	1	B
	11506	12-14/23	1	B
	11562	22/1	1	NG
	11562	22/1	1	SA
	11563	21-2/23	2	C
	12395	12/16-11	1	B
	13018	12-1/16-75	1	B
	13506	12/16-75	3	B
	13741	21-19/16-16	1	Z
	14404	18-4/25	1	NG
	14406	12-8/16-119	1	NG
	14724	17-6/23-18	1	B
	14969	19/15	1	NG
	14970	19-1/13-9	1	NG
	14973	7-2/13-6	1	NG
	14977	18-1/30-4	1	SA
	14979	12/16-175	1	B
	15347	21/16	1	Y
	15348	12/16-75	3	B
	15349	21-19/16-16	1	NG
	15880	22/14-69	1	Z
	15582	19-1/13-9	1	NG
	15588	12-6/13-3	1	NG
	15643	7-2/13-43	1	C
	15644	12-8/16-119	1	NG
	15658	19-1/13-9	1	NG
	16236	12-1/23-7	1	B
	16024	21-19/16-16	1	Z

Figure 1. Algorithm for the inclusion and exclusion of patients in this study.



MSW: men who have sex with women; Nm: Neisseria meningitidis; MLST: multilocus sequence typing