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ORIGINAL ARTICLE

# Genomic characteristics of meningococcal carriage amongst young adults in Malta

Paul Torpiano, Marlene Farrugia, Sabrina Goodlip, Julie Haider, Graziella Zahra, David Pace

**Background:** Invasive meningococcal disease has an incidence of 0.4-3/100,000 in Malta. Meningococcal carriage studies shed light on the epidemiology, transmission, and pathogenesis of invasive meningococcal disease. This research aimed to investigate the prevalence and genomic characteristics of meningococcal carriage in young adults aged 18-24 years attending the only university in Malta.

**Methods:** Two posterior pharyngeal swabs were taken from 404 university students, and tested for *Neisseria meningitidis* (Nm) by culture and polymerase chain reaction. Isolates were assigned a serogroup and genogroup, and underwent whole genome sequencing to identify sequence type (ST), clonal complex (CC) and Bexsero antigen sequence type (BAST). Diversity amongst carried isolates was assessed using Simpson's Index of Diversity (D).

**Results:** Twenty-five students (6.2%; 95% C.I. 4-9%) were carriers for Nm While most meningococcal isolates were non-serogroupable (n=14; 66.7%; 95% C.I. 43-85.4%), the predominant genogroup was B (n=9; 36%; 95% C.I. 18–57.5%), followed by Y (n=6; 24%; 95% C.I. 9.4 – 45.1%). Fourteen different ST distributed among 9 CC, and demonstrating 17 different BAST, were identified amongst the carried meningococcal isolates. There was a high degree of BAST diversity (D=0.98). CC53, CC23 and the hyperinvasive CC41/44 accounted for 4 (19%; 95% C.I. 5.4-41.9%), 4 (19%; 95% C.I. 5.4-41.9%) and 3 (14.3%; 95% C.I. 3-36.3%) of isolates respectively.

**Conclusions:** Our findings demonstrate a wide biodiversity in meningococcal carriage, with CC23, CC41/44 and CC53 important. The introduction of glycoconjugate MenACWY vaccines on the national immunisation schedule could reduce meningococcal carriage and potentially IMD in Malta.

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Invasive meningococcal disease (IMD) is a devastating illness, with a 10% mortality rate, and morbidity in 8-20% that includes sensorineural deafness, limb amputations, and neurological disability.[1] *N. meningitidis* (Nm), the causative agent of IMD, is often carried asyptomatically in the throat, with rates varying from 4.5% in infants to 23.7% in adolescence.[2] The rarity of IMD compared with oropharyngeal meningococcal carriage (OMC), and the much wider genetic diversity amongst carried compared to invasive meningococcal strains, suggests that the Nm life cycle is dependent on transmissibility and carriage, rather than disease.[3] IMD incidence in Malta varied between 0.4-3/100,000 individuals per year from 2013-2017, compared to an overall European incidence of 0.5-0.7/100,000 individuals/year over the same period.[4] Meningococcal vaccination was only introduced on the national immunisation schedule in June, 2020.

We conducted a meningococcal carriage study at the only university in Malta. The aim of the study was to establish carriage rates amongst university students aged between 18 and 24 years, and describe the microbiological and molecular diagnostic profile of both OMC and IMD in Malta. This study is the first to present whole genome sequencing (WGS) data of carried meningococcal isolates in Malta. By characterising circulating carried meningococci in terms of their sequence type (ST), clonal complex (CC), and vaccine antigen sequence types, WGS will provide a detailed analysis of the potential for carried meningococci to cause IMD locally, as well as identify opportunities for vaccination.

## MATERIALS AND METHODS

The objective of this study was to assess the point prevalence and describe the epidemiology and genetic characteristics of OMC amongst university students in Malta. The inclusion criteria were: students at the University of Malta (UoM), aged 18-24 years of age. Criteria for exclusion from the study were meningococcal vaccination in the prior 5 years, previous meningococcal disease, active oro/nasopharyngeal infection, and recent antibiotic use (within the previous 2 weeks). A sample size of at least 400 subjects was chosen to allow confidence intervals of 95%, based on an estimated prevalence of meningococcal carriage of 5-10%, an α of 0.05 and a β of 0.80, and a population aged 18-24 years reported in Malta in 2017 of 40,840.[5]

Students were recruited after university lectures. Consenting participants were asked to complete a questionnaire assessing risk factors for OMC. Two cotton-tipped swabs (Cliniswab Sterile Transport Swab, Nuova Aptaca, Canelli, AT, Italy, and ∑-VCM, MWE Medical Wire, Wiltshire, U.K) were then taken simultaneously from the posterior pharynx on each participant. One swab was placed in Amie's transport medium, and the second in Universal Transport Culture Medium (UTCM). Both swabs were transported to the Mater Dei Hospital microbiology laboratory within 4 hours, where the charcoal swab was immediately plated onto Thayer-Martin medium with antimicrobial supplement (vancomycin, colistin, nystatin, trimethroprim lactate) and incubated at 37°C in 5% carbon dioxide. The plates were inspected at 24, 48 and 72 hours: plates with a positive growth were subcultured onto chocolate agar, and underwent a Gram stain to confirm the presence of Gram-negative diplococci. Nm identification was done using Matrix-assisted laser desorption/ionisation-Time-of-Flight mass spectrometry (MALDI-TOF MS) (VITEK MS, bioMerieux, Marcy L'Etoile, France). Identification of capsular serogroup was done by means of slide agglutination with commercial antisera based on whole cell preparations for capsular groups A, B, C, D, W, X, Y, and Z (Remel Agglutinating Sera, *Neissera meningitidis*, ThermoFisher Scientific, Waltham, MA, USA). DNA was extracted from the carried meningococcal isolates using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) and the QIAcube machine (Qiagen, Hilden, Germany). Genogrouping of the isolates was done by rt-PCR (M-DiaSero, Diagenode diagnostics, Belgium) targeting the *sacB*, $siaD_B$, $siaD_C$, *synG* and *synF* genes for detection of genogroups A, B, C, W, and Y respectively, and performed using the Qiagen Rotor-Gene Q (Qiagen, Hilden, Germany). The DNA extracts were transferred to the Oxford Genomics Centre (OGC) at -70°C, where samples were quantified using the Quant-iT™ PicoGreen ® dsDNA Kits (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA) and a FLUOstar OPTIMA plate scanner (BMG Labtech, Ortenburg, Germany), and libraries constructed using

the NEBNext Ultra DNA Sample Prep Master Mix Kit (NEB, Ipswich, MA, USA). Each library was PCR-enriched using custom dual index primers.[6] After amplification the reactions were purified with Agencourt AMPure beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA). Sequencing was done using an Illumina NovaSeq6000 (Illumina NovaSeq, Illumina Inc., San Diego, CA, USA) as 150bp paired end.

The second oropharyngeal swab from each participant was stored in UTCM (∑-VCM, MWE Medical Wire, Wiltshire, U.K.) at 4°C. The AutoMate Express Instrument (Applied Biosystems, ThermoFisher Scientific, MA, USA) was used to extract 50µL of DNA from 400µL of the UTCM. The DNA extracts were stored at -20°C, and subsequently underwent rt-PCR targeting the *porA* gene for meningococcal detection (BactoReal kit Nm porA gene, Ingenetix, Bioactiva, Austria). Those extracts that were positive on PCR alone underwent subsequent genogrouping by rt-PCR as above (M-DiaSero, Diagenode diagnostics, Belgium).

Data analysis was done using Statistical Package for the Social Sciences® (SPSS). Confidence intervals were calculated using Binomial exact calculation.[7] Simpson's Index (D) was used as indicators of genetic diversity amongst CC and vaccine antigen sequence types, and calculated using the Columbia University Biodiversity Calculator.[8] A D closer to 0 was taken as indicative of increasing diversity, indicating a larger number of unique vaccine antigen sequence types or CC amongst the sequenced meningococcal isolates. Odds ratios for assessing risk factors for OMC were calculated according to Altman, 1991.[9] Post-sequencing analysis was performed using the Bacterial Isolate Genome Sequence Database (BIGSdb), including MLST and vaccine antigen sequence typing for the four-component MenB (Bexsero®, MenB-4C) and Bivalent rLP2086 (Trumenba®, MenB-fHbp) vaccines using BAST.[10] BAST analysis with the BIGSdb was used to estimate vaccine coverage with MenB-4C and MenB-fHbp.[10] Comparisons between the findings of this study and pooled meningococcal isolates from across Europe from a comparable population aged 18-24 years were made by accessing the Bacterial Isolate Genome Sequence Database (BIGSdb).[10]

## RESULTS

There were 404 participants in total, aged between 18 and 24 years, with a mean age of 20 years (95% CI 19.9 - 20.2). The M:F ratio was 1:1.2, with 223 (55.2%) female participants. Three hundred and eighty-nine participants were of Maltese nationality (96.3%). Forty-five participants described themselves as smokers (11.1%; 95% CI: 8.2-14.6%). Most participants shared their household with 3 (n=168; 41.6%; 95% CI: 36.7-46.6%) or 4 (n=109; 27%; 95% CI 22.7-31.6%) other people. Assessing the risk factors for meningococcal carriage was not one of the objectives of this study, though a significant association was noted between OMC and attendance to public houses or discotheques at least twice a week (OR=4.62, 95% CI: 1.41-15.15; p=0.01).

Twenty-five (6.2%; 95% CI: 4-9%) participants were found positive for OMC on either *porA* PCR or culture (**Figure 1**). Twenty-one (5.2%; 95% CI: 3.2-7.8%)

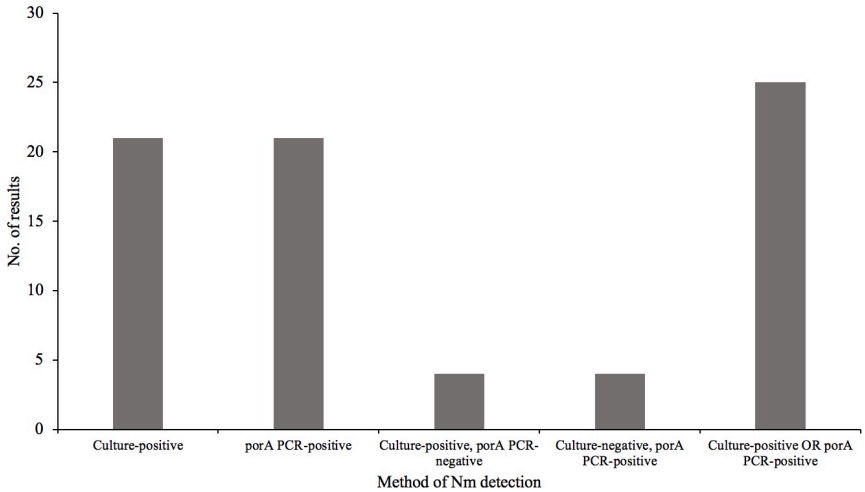


**Figure 1** *Number of positive results with each method of Nm detection performed on throat swabs. (PCR: Polymerase Chain Reaction)*

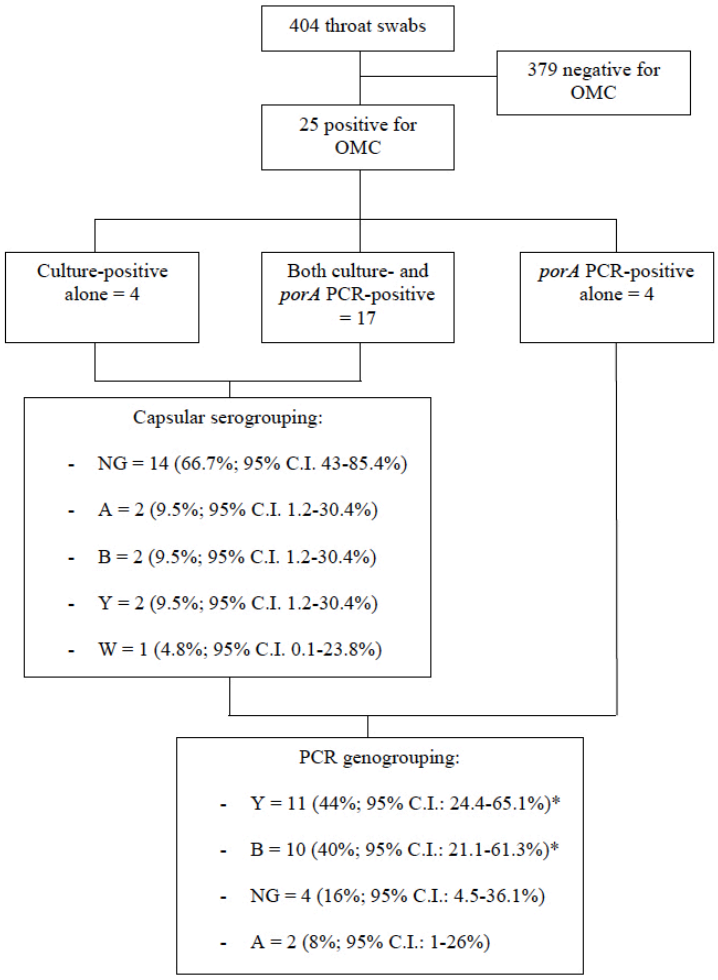


**Figure 2** *Capsular serogrouping and PCR genogrouping of carried meningococcal isolates. (OMC = oropharyngeal meningococcal carriage; PCR = polymerase chain reaction; NG = non-groupable) *Two samples were positive for both genogroups B and Y on genogrouping PCR.*

charcoal throat swabs were positive for Nm on culture and MALDI-TOF. Seventeen of these were also positive for Nm on *porA* PCR (81%; 95% C.I.: 58.1-94.6%). Twenty-one samples were positive for Nm on *porA* PCR (5.2%; 95% C.I.: 3.2-7.8%). Therefore, culture successfully identified 4 cases of meningococcal carriage missed on *porA* PCR, while *porA* PCR identified 4 cases of carriage not identified by culture and MALDI-TOF. Of the cultured meningococcal isolates (n=21), most were found to be non-groupable on capsular serogrouping (n=14, 66.7%; 95% CI: 43-85.4%), with genogroups Y (n=11; 44%; 95% CI: 24.4-65.1%) and B (10; 40%; 95% CI: 21.1-61.3%) being the most prevalent of all isolates (culture or PCR) (Figure 2). Two samples were identified as both genogroup B and Y.

On WGS, 6 carriage isolates were found to belong to genogroup B (28.6%; 95% CI: 11.3-52.2%), 5 to genogroup Y (23.8%; 95% CI: 8.2-47.2%), 2 were genogroup E (9.5%; 95% CI: 1.2-30.4%), 1 was genogroup W (4.8%; 95% CI: 0.1-23.8%), 1 non-genogroupable (4.8%; 95% CI 0.1-23.8%), and the remaining 6 possessed the capsule null locus (cnl) (28.6%; 95% CI: 11.3-52.2%). Fourteen different sequence types (ST) were identified amongst the carried meningococcal isolates, with 2 isolates of unidentified ST (Figure 3). The commonest ST was ST-53 (n=4; 19%; 95% CI: 5.4-41.9%), one of 3 STs found to have the capsule-null locus (cnl) on genogrouping,

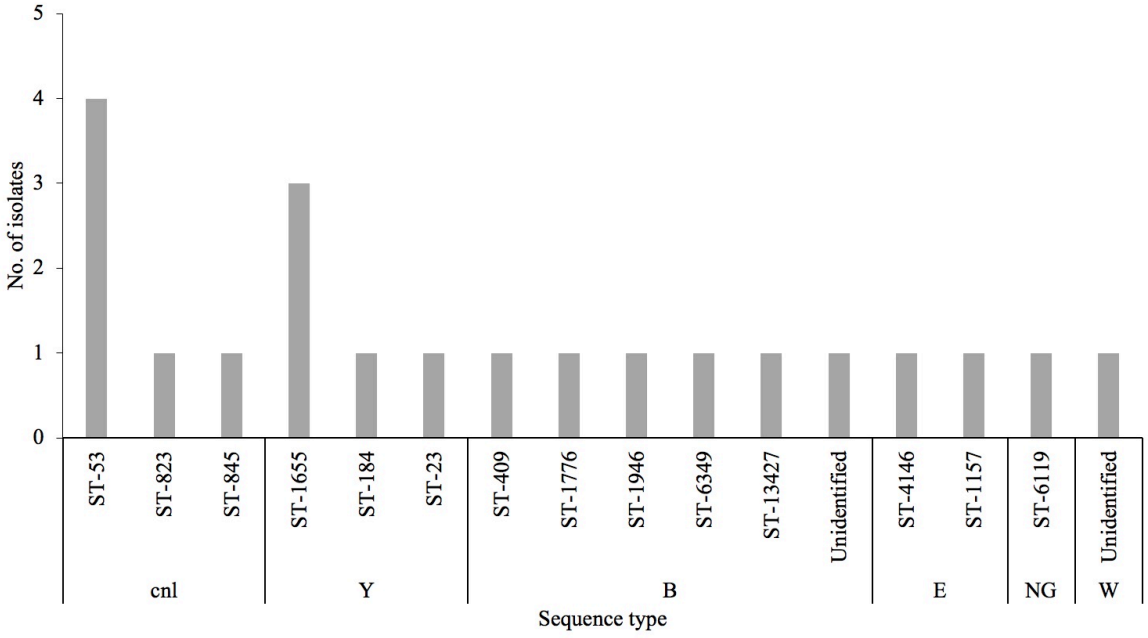


**Figure 3** *Distribution of sequence types amongst carrier meningococcal isolates (cnl: capsule null locus, B: genogroup B on WGS, Y: genogroup Y on WGS, E: genogroup E on WGS, NG: non-groupable on WGS, W: genogroup W on WGS).*

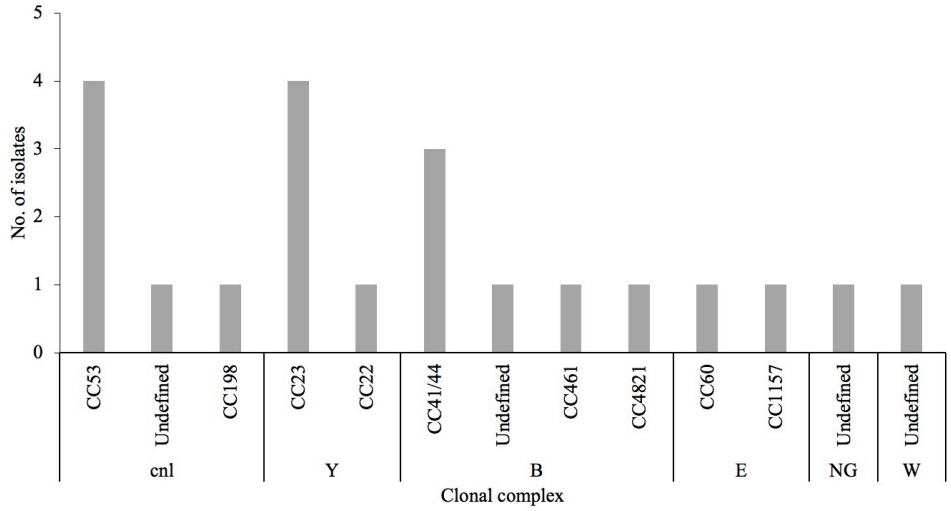


**Figure 4** *Distribution of clonal complexes amongst carrier meningococcal isolates (cnl: capsule null locus, B: genogroup B, Y: genogroup Y, E: genogroup E, NG: non-groupable, W: genogroup W; all on WGS).*

followed by the Y genogroup ST-1655 (n=3; 14.3%; 95% CI: 3 – 36.3%). D for the carried isolate ST was found to be 0.053. Four isolates each (19%; 95% CI: 5.4-41.9%) belonged to the ST-23 and ST-53 CCs (Figure 4). Three isolates (14.3%; 95% CI: 3-36.3%) belonged to the hyperinvasive ST-41/44 CC. D for the carried isolate CC was 0.11.

Seventeen different BAST were recorded amongst 20 carried isolates (BAST for 1 isolate remained undefined), with 3 isolates of BAST-22 (15%; 95% CI: 3.2-37.9%), and the remaining isolates each of a different BAST. The ratio of unique BAST to isolates was 1:1.12, and there was a high degree of BAST diversity, with a D of 0.02. Of the 6 genogroup B carried isolates, 2 isolates (33.3%; 95% CI: 4.3-77.7%) showed exact or cross-reactive matches for MenB-4C antigens. On the other hand, all 6 of the genogroup B carried isolates (100%; 95% CI:54.1-100%) showed exact or cross-reactive matches for MenB-fHbp antigens.

Comparing our findings to pooled European isolates available on BIGSdb, serogroup A and non-serogroupable meningococci accounted for significantly greater proportions of carried meningococci locally compared to pooled European isolates (MenA: 9.5% vs 0.5%, p<0.0001; NG: 66.7% vs 42.7%, p=0.03), while serogroup B was significantly underrepresented locally (9.5% vs 34.1%, p=0.018) (Table 1). Furthermore, CC23, CC53, and CC4821 accounted for a significantly higher proportion of Maltese than European carried isolates (5.7% vs 19% [p=0.012], 6.5% vs 19% [p=0.025], 0% vs 4.8% [p<0.0001] respectively) (Table 2).

## DISCUSSION

OMC is essential for Nm transmission, influencing IMD epidemiology and the effectiveness of vaccination.[3] We showed Maltese university students aged 18-24 years, an age-group at high risk for OMC, to have a meningococcal carriage rate of 6.2%. The meningococcal carriage rate in the Mediterranean region in a comparable young adult population

**Table 1** *Capsular groups of carried meningococci in European participants aged 18-24 years, compared with Maltese carried isolates in a similar sample population*

| Capsular group | European carriage (N=979) | | Maltese carriage (N=21) | | |
|---|---|---|---|---|---|
| | n | % (95% CI) | n | % (95% CI) | *p*-value |
| **A** | 5 | 0.5 (0.2-1.2) | 2 | 9.5 (1.2-30.4) | ***<0.0001*** |
| **B** | 335 | 34.1 (31.2-37.2) | 2 | 9.5 (1.2-30.4) | ***0.018*** |
| **C** | 75 | 7.6 (6.1-9.5) | 0 | 0 (0-16.1) | 0.19 |
| **E** | 35 | 3.6 (2.5-4.9) | 0 | 0 (0-16.1) | 0.38 |
| **W** | 33 | 3.4 (2.3-4.7) | 1 | 4.8 (0.1-23.8) | 0.73 |
| **X** | 14 | 1.4 (0.8-2.4) | 0 | 0 (0-16.1) | 0.59 |
| **Y** | 57 | 5.8 (4.4-7.5) | 2 | 9.5 (1.2-30.4) | 0.48 |
| **Z** | 6 | 0.6 (0.2-1.3) | 0 | 0 (0-16.1) | 0.72 |
| **NG** | 419 | 42.7 (39.6-45.9) | 14 | 66.7 (43-85.4) | ***0.03*** |

**Table 2** *Distribution of clonal complexes amongst carried meningococci in Malta and Europe, in individuals aged 18-24 years (CC = clonal complex)*

| | European carriage (N=773) | | Maltese carriage (N=21) | | |
|---|---|---|---|---|---|
| **CC** | **n** | **% (95% CI)** | **n** | **% (95% CI)** | ***p*-value** |
| 32 | 33 | 4.3 (3-5.9) | 0 | 0 (0-16.1) | 0.33 |
| 11 | 42 | 5.4 (3.9-7.3) | 0 | 0 (0-16.1) | 0.27 |
| 41/44 | 179 | 23.2 (20.2-26.3) | 3 | 14.3 (3-36.3) | 0.34 |
| 23 | 44 | 5.7 (4.2-7.6) | 4 | 19 (5.4-41.9) | ***0.012*** |
| 53 | 50 | 6.5 (4.8-8.4) | 4 | 19 (5.4-41.9) | ***0.025*** |
| 461 | 6 | 0.9 (0.4-1.8) | 1 | 4.8 (0.1-23.8) | 0.08 |
| 60 | 23 | 3 (1.9-4.4) | 1 | 4.8 (0.1-23.8) | 0.64 |
| 4821 | 0 | 0 (0-0.5) | 1 | 4.8 (0.1-23.8) | ***<0.0001*** |
| 22 | 43 | 5.6 (4.1-7.4) | 1 | 4.8 (0.1-23.8) | 0.87 |
| 198 | 39 | 5 (3.6-6.8) | 1 | 4.8 (0.1-23.8) | 0.97 |
| 1157 | 7 | 0.9 (0.4-1.9) | 1 | 4.8 (0.1-23.8) | 0.08 |
| 35 | 41 | 5.3 (3.8-7.1) | 0 | 0 (0-16.1) | 0.28 |
| 269 | 20 | 2.6 (1.6-4) | 0 | 0 (0-16.1) | 0.45 |
| 162 | 20 | 2.6 (1.6-4) | 0 | 0 (0-16.1) | 0.45 |
| 92 | 29 | 3.8 (2.5-5.3) | 0 | 0 (0-16.1) | 0.36 |
| 106 | 22 | 2.8 (1.8-4.3) | 0 | 0 (0-16.1) | 0.44 |
| 254 | 21 | 2.7 (1.7-4.1) | 0 | 0 (0-16.1) | 0.45 |

ranged from 2% to 18%.[11-18] However, meningococcal carriage is dynamic, changing over time and in different regions, with a systematic review showing meningococcal carriage in university students to range from 1.5% to 71.1%.[19] Therefore, the Maltese carriage rate appears comparable with that in other regions, and most likely subject to similar temporal fluctuations. Frequent attendance to discotheques or public houses was found to be a significant risk factor for carriage (OR: 4.62, 95% CI: 1.41-15.15; p=0.01) similar to what has been reported in other studies.[20]

The Nm polysaccharide capsule protects meningococci from phagocytosis, opsonisation and complement-dependent bacteriolysis, and is the major antigen to generate bactericidal antibodies after infection or vaccination (with the exception of serogroup B vaccines).[21] The role of the capsule in invasion explains why invasive isolates are rarely acapsular, and therefore non-serogroupable, compared with carriage isolates, of which 66.7% were non-serogroupable in this study.[22] There are various mechanisms behind failure of capsule expression: six isolates in this study carried the *cnl* locus, without potential for capsule expression, while 9 isolates (64.3%) were non-serogroupable despite an intact *cps* locus, suggesting down-regulation of capsule expression during carriage.[23,24]

The proportion of non-serogroupable and serogroup A carriage isolates were significantly higher in Malta compared to pooled European carried isolates from participants 18-24 years (9.5% vs 0.5%, p<0.0001), while that of serogroup B was lower (9.5% vs 34.1%, p=0.018). Aside from geographical variation, these differences may relate to discrepancies in the year of analysis: local isolates were collected in 2018, at a time of low rates of IMD-MenB, while the European isolates date back to the 1970s. The proportion of serogroup B in carriage in more recent Mediterranean studies has varied between 9.4% in Turkey and 43% in Italy.[11,13] MenA is very rarely

identified in Europe, accounting for a limited proportion of IMD and OMC in the Eastern European region and Asia, and is more important in Africa: its presence in our sample may therefore relate to Malta's proximity to the African continent, and thus the influence of migrants and tourists from this and other regions where MenA carriage has been documented.[25-28] It is notable, however, that both MenA isolates detected in this study contained the cnl locus, raising doubts about the reliability of their assigned serogroup.

The carriage isolates were mostly genogroup B (36%) and Y (24%), with 6 isolates bearing the cnl locus (24%). These results align themselves well with similar recent findings from countries in the Mediterranean region, where non-genogroupable and genogroup B isolates were found to predominate, followed by genogroup Y.[14,15,18,29] Genogroup E, while rarely responsible for IMD in Europe or Malta, accounted for a similar proportion of local carriage to that in Spain, Greece and Italy.[14,18,29,30] The absence of genogroup C in carriage locally contrasts with the proportion of Maltese IMD attributed to this serogroup.[30] While the low rate of MenC carriage in other European studies suggests that the sample size of our study might not have been large enough to detect MenC carriage, the prevalence of MenC amongst invasive isolates in Malta possibly implies a preference for invasion rather than carriage, which may be short-lived and thus missed by a point prevalence study.[30] Since conjugate meningococcal C vaccination was not part of a national schedule locally at the time of this research, this finding is unlikely to be a result of herd immunity, which has reduced MenC carriage overseas. [11-13,15,18,29,31]

While carried isolates tend to be clonally heterogeneous, most IMD is caused by a limited number of hyperinvasive CCs, including CC5, CC41/44, CC32, CC11, CC269, and CC8.[32,33] This is reflected in this study: CC distribution was wide amongst carried isolates, with no single predominant CC or ST. Hyperinvasive CCs are rarely found in carriage, exhibiting evolutionary advantages in transmission and disease, but not in carriage, where non-invasive meningococci predominate.[34] Only 3 carriage isolates belonged to a hyperinvasive CC (CC41/44). CC23 and CC53 were important in carriage, accounting for 19% of isolates each, and accounted for a significantly higher proportion of Maltese compared with European carriage (CC23: 19% vs 5.7%, p=0.012; CC53: 19% vs 6.5%, p=0.025). CC53 is also prevalent in carriage in Italy, a country which neighbours Malta and is both a source of tourists and a popular tourist destination.[12,18] CC4821 accounted for one carriage isolate locally, and was not otherwise present amongst European carriage isolates (4.8% vs 0%, p<0.0001). CC4821 first emerged in IMD-MenC outbreaks in China from 2003-2005.[35] It has since been responsible for cases of IMD-MenB and IMD-MenC, as well as carriage, across the world.[36] CC198, accounting for 4.8% of our carriage isolates, is reported amongst cases of IMD with cnl meningococci.[37]

Pharyngeal carriage provides a stable ecological niche for Nm, and a fertile platform for high rates of genetic recombination.[33] This is reflected in the broad diversity of both CC and BAST in carriage isolates in this study: carriage allows Nm to sustain a high rate of horizontal genetic transfer that leads to greater genomic diversity. This could confer advantages vis-a-vis invasiveness and transmissibility, allowing new hyperinvasive clones, with novel antigenic combinations, to emerge, with the potential for

**SUMMARY BOX**

*What is already known about this subject:*

- Oropharyngeal carriage of *N. meningitidis* (Nm), the causative agent of invasive meningococcal disease, occurs frequently in adolescents.
- Meningococcal vaccination programs are most effective when they impact both meningococcal disease and carriage.
- Invasive meningoxcoccal disease occurs with an incidence of 0.4-3/100,000 in Malta.

*What are the new findings:*

- University students in Malta carried oropharyngeal meningococci at a rate of 6.2%.
- The majority of carriage isolates did not express a capsule.
- Genogroups B, Y and W, and CC23, CC41/44 and CC53 predominated in carriage.
- Carried meningococci exhibited wide genomic biodiversity.

epidemic or hyperepidemic spread.[38] Genomic diversity amongst carried meningococci may undermine the potential for newer protein-based vaccines to generate herd protection by reducing meningococcal carriage, a phenomenon seen with conjugate meningococcal vaccines.[39] The high BAST diversity in our carriage sample reinforces such concerns locally. BAST allows a prediction of MenB vaccination efficacy by identifying the genes for the antigens that influence their immunogenicity: a high degree of BAST diversity and a low prevalence of BAST-1, the BAST profile that matches MenB-4C antigens precisely, suggest poor coverage of prevalent meningococcal MenB-4C antigens.[40] A difference in estimated coverage of MenB carriage isolates with MenB-4C and MenB-fHbp was seen in this study, showing potentially superior MenB-fHbp coverage (OR 13.6 [95% CI: 3.1-59.8], p=0.0006). However, no conclusions can be drawn from this, as neither of the currently available MenB vaccine formulations has been shown to impact carriage.[41-43]

Meningococcal conjugate vaccines have been shown to reduce carriage, leading to reduced acquisition and IMD incidence.[44] The presence of capsule-expressing MenY and MenW suggests that MenACWY might have a more significant impact on local meningococcal carriage than MenC vaccination alone. Furthermore, the acapsular MenY isolates found in carriage have the potential to express a capsule through phase variation. With MenC vaccination alone, MenY and MenW may persist in carriage and be transmitted to cause disease in susceptible individuals.[38]

These results will shed no light on vaccine effectiveness or cost-effectiveness in this population without vaccine introduction. The recruitment of subjects excluded those with factors that may have influenced meningococcal carriage, though may be subject to recall bias. The discrepancies between the genogrouping results obtained by rt-PCR compared with WGS suggest a number of inaccuracies in the former, partly explained by the fact that the rt-PCR genogrouping kit used in this research (M-DiaSero, Diagenode diagnostics, Belgium) did not include primers to detect genogroup E (2 isolates). As only 25 participants were found to be positive for meningococcal carriage, sub-analyses of the positive isolates is limited. Lastly, coverage estimates were based entirely on genomic data, while protein expression was not measured: certain isolates may possess antigenic genes without expressing antigenic protein in vivo, potentially leading to overestimates of the predicted Meningococcal B vaccine coverage.


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## CONCLUSION

Students aged 18-24 years at the only university in Malta carried oropharyngeal meningococci at a rate of 6.2%. Although the majority of carried isolates did not express a capsule, genes coding for the MenB, MenY and MenW capsules were identified, proving circulation of these genogroups within the community. The wide genomic biodiversity shown in carriage strengthens its role as a platform for genetic exchange and meningococcal adaptation, with potential to generate new hyperinvasive lineages. The introduction of glycoconjugate MenACWY vaccines on the national immunisation schedule could reduce meningococcal carriage, and potential IMD in Malta.

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