# Limited impact of adolescent meningococcal ACWY vaccination on group W carriage in university students

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## SUMMARY

We investigated the impact of conjugate MenACWY immunization on carriage of *Neisseria meningitidis* in university students. Expansion of capsule-expressing isolates from the 2013-strain of serogroup W:cc11, but not serogroup Y:cc23 isolates, suggests differential susceptibilities to vaccine-induced immunity.

## ABSTRACT

***Background.*** In the UK rising disease levels due to *Neisseria meningitidis* serogroup W clonal complex ST-11 (MenW:cc11) strains led to introduction of conjugate MenACWY vaccination for teenagers. We investigated the impact of immunization on carriage of targeted meningococci by whole genome sequencing of isolates recovered from a cohort of vaccinated university students.

***Methods.*** Strain designation data were extracted from whole genome sequence data. Genomes from carried and invasive MenW:cc11 were compared using a gene-by-gene approach. Serogrouping identified isolates expressing capsule antigens targeted by the vaccine.

***Results.*** Isolates with a W: P1.5,2: F1-1: ST-11 (cc11) designation, and belonging to the emerging '2013-strain' of the South American-UK MenW:cc11 sub-lineage, were responsible for an increase in carried group W. A multifocal expansion was evident with close transmission networks extending beyond individual dormitories. Carried group Y isolates were predominantly from clonal complex 23, but showed significant heterogeneity and individual strain designations were only sporadically recovered. No shifts towards acapsulate phenotypes were detected in targeted meningococcal populations.

***Conclusions.*** In a setting with high levels of conjugate MenACWY vaccination, expansion of capsule-expressing isolates from the 2013-strain of MenW:cc11, but not MenY:cc23 isolates, is indicative of differential susceptibilities to vaccine-induced immunity.

Keywords: *Neisseria meningitidis*; whole genome sequencing; carriage; serogroup W; serogroup Y; epidemiology; vaccine

## BACKGROUND

*Neisseria meningitidis* is a commensal of the human oropharynx which can cause invasive meningococcal disease (IMD), principally characterized by sepsis and/or meningitis [1]. The majority of worldwide IMD is caused by isolates expressing the polysaccharide capsules that define serogroups A, B, C, W, Y, and X, whilst carriage isolates are frequently acapsulate due to the inactivation or absence of genes involved in capsule expression [2-4]. The incidence and contribution of different meningococcal lineages to the overall burden of IMD varies geographically, temporally and by age group, and can be further influenced by vaccination; capsule polysaccharide-based vaccines are available against serogroups A, C, W and Y, whilst protein-based vaccines are available against serogroup B isolates [5]. Monitoring trends in meningococcal populations requires discriminatory typing strategies. Whole genome sequence (WGS) analyses are now routine and enable differentiation of clonal complexes, sequence types and even highly-similar clones thereby facilitating detection of population level trends and individual transmission events [6].

Over the last two decades, multiple countries have experienced increases in the incidence of IMD due to serogroup W (MenW) meningococci belonging to the sequence type 11 clonal complex (cc11) [7-11]. Analysis of WGS data has indicated that most MenW:cc11 isolates belong to cc11 lineage 11.1, with the global increases in MenW:cc11 disease resulting from emergence of two diversifying sub-lineages [12]. The Hajj sub-lineage comprises three main clusters of isolates (strains) corresponding to the Hajj outbreak of 2000 onwards (Anglo-French Hajj strain), expansion of endemic disease in South Africa from 2003 (endemic South African strain) and epidemics in sub-Saharan Africa (Burkina Faso/North African strain) [12]. The South American-UK sub-lineage comprises MenW:cc11 that emerged in South America and subsequently spread to the UK and Europe [12]. This second lineage is associated with atypical clinical presentation, including gastrointestinal

symptoms, and a high case-fatality rate [13, 14]. Ongoing genomic surveillance has revealed further population structure details for the South American-UK MenW:cc11 sub-lineage such as the initial emergence of the 'original UK' strain in 2009, followed by subsequent emergence of the novel '2013-strain' from this original UK strain [15].

The year-on-year increase in MenW:cc11 IMD cases in the UK since 2009 led to an emergency immunization program with meningococcal ACWY conjugate vaccine (MenACWY) being recommended to adolescents. This program included a phased catch-up campaign for individuals 14-18 years of age and began in August 2015 [16]. Older adolescents and young adults were targeted because these age groups exhibit higher oropharyngeal carriage rates than other age groups due to social factors [17, 18]. Particularly high carriage rates are evident in young adult populations residing in semi-closed communities (*e.g.* university students), where the potential for person-to-person transmission is especially high, and can lead to isolated clusters or outbreaks of meningococcal disease [19-22]. Hence, the MenACWY vaccination was also offered to new university entrants <25 years of age. Furthermore, from previous experience with meningococcal group C conjugate vaccines, targeting adolescents and young adults could result in sustained decreases in disease incidence in all age groups by reducing the acquisition of meningococcal carriage (herd protection) [23].

At the University of Nottingham (UoN), UK, a campus-based vaccination campaign targeting freshers in September 2015 increased MenACWY vaccination coverage in this specific student population from 31% to 71% [24]. To determine the effect of this vaccination campaign on meningococcal carriage, we conducted a cross-sectional study at the UoN, from September 2015 through to March 2016 [25]. The overall meningococcal carriage rate increased throughout the study in line with previous university-based carriage studies [26-28]. No significant change in carriage of MenY organisms occurred, but we detected a rapid

and significant rise in carriage of MenW strains with PorB serotypes and *porA* and *fHbp* sequence types that matched alleles harbored by endemic UK MenW:cc11 invasive isolates [25]. Here we analyze whole genome data to define the specific MenW, MenY and non-groupable lineages present in this student cohort, investigate the genetic relatedness of carried MenW:cc11 to contemporary invasive isolates, and consider the potential mechanisms by which vaccine-targeted isolates may escape immune responses elicited by vaccination with the MenACWY capsule-based conjugate vaccine.

## METHODS

### Carriage Isolates

A total of 174 meningococcal isolates, all obtained from oropharyngeal carriers in 2015-16 at the UoN (East Midlands), United Kingdom [25] were included in the WGS analysis (Supplementary Table 1). Of these, 49 were MenW and 32 were MenY, together accounting for *ca.* 95% of MenW and MenY isolated during the carriage study [25]. A further 93 isolates were non-groupable (*i.e.* lacked *ctrA* or carried the capsule null locus), corresponding to *ca.* 70% of non-groupable isolates obtained in the 2015-16 UoN study [25]. All isolates were chosen as known MenW, MenY or non-groupable organisms, based on PCR typing methods, without prior knowledge of their clonal complex. The Meningococcal Reference Unit, Public Health England, Manchester, UK performed serogrouping of MenY carriage isolates using dot-blot ELISA. Serogrouping of MenW carriage isolates was reported previously [25]. Chi-square tests for significance were performed by using STATCALC (Epi Info version 7.2.0.1; Centers for Disease Control and Prevention, Atlanta, GA, USA).

### Genomic DNA Extraction, Sequencing, Assembly and Deposition

Meningococci were grown overnight on Columbia agar with chocolated horse blood (Thermo Fisher Scientific) at 37°C in an atmosphere of air plus 5% $CO_2$. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega) according to manufacturer instructions. Index-tagged Illumina sequencing libraries were generated according to the manufacturer instructions, with an average insert size of 420 bp. These were multiplexed and sequenced on Illumina HiSeq 2500 machines to generate 125 bp paired-end sequences. An average of 2,773,968 reads per sample was generated, giving an average 125-fold coverage of the *N. meningitidis* genome. Short read sequences were trimmed with Trimmomatic v0.32 [29] and assembled with SPAdes v3.9.0 [30] using the recommended parameters. Assemblies were deposited in, and subsequently automatically annotated by, the PubMLST.org/neisseria database which implements the Bacterial Isolate Genome Sequence (BIGSdb) platform [31]. Short-read sequences were also deposited in the European Nucleotide Archive (ENA) (Supplementary Table 1).

**Genomic Analyses**

Isolate capsular groups, multilocus sequence types, and porin A (PorA) and ferric enterochelin receptor (FetA) types were identified from whole genome data. For MenW:cc11 carriage isolates, population-wide genomic analyses were undertaken using the BIGSdb Genome Comparator tool implemented within the PubMLST.org/neisseria database using the *N. meningitidis* cgMLST v1.0 core genome scheme (1605 loci) and default settings [31]. Output distance matrices (Nexus format) were used to generate NeighborNet networks using SplitsTree4 (v4.14.5). WGS data from MenW:cc11 carriage isolates were analyzed in conjunction with two other WGS data sets: (1) all UK MenW:cc11 invasive isolates for the epidemiological year 2015-16 ($n$=190) available via the Meningitis Research Foundation (MRF) Meningococcus Genome Library database

(http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_neisseria_mrfgenomes; accessed July 2017), and (2) a sub-set of the isolates previously used to define the sub-lineages/strains of lineage 11.1 by core genome analysis [12, 15] (*n*=60; Supplementary Table 2 and available via at https://pubmlst.org/bigsdb?db=pubmlst_neisseria_isolates; accessed July 2017).

## RESULTS

### Features of Sequenced Carriage Genomes

After de novo assembly, the 125 bp paired Illumina reads from carriage isolates produced contiguous sequences between 2,018,737 to 2,155,185 bp in size, consistent with expectations for meningococcal genomes (Supplementary Table 1). Genome assemblies were automatically annotated in a 'gene-by-gene' approach using the BIGSdb platform and strain designation data extracted (Supplementary Table 1). For comparison, identical typing information was extracted from the WGS data of all invasive UK MenW (*n*=200) and MenY (*n*=104) isolates recovered during the same epidemiological year (2015-2016), and available via the MRF Meningococcus Genome Library database (Supplementary Tables 3 and 4, respectively).

For MenW, isolates from cc11 predominated (95%), with the strain designation W: P1.5,2: F1-1: ST-11 (cc11) accounting for 88% of all MenW carriage, and 72% of all MenW invasive isolates, respectively (Table 1). Breakdown by isolation time-point confirmed that isolates with the W: P1.5,2: F1-1: ST-11 (cc11) designation were responsible for the increase in MenW carriage detected during the course of the 2015-16 carriage study (Table 1).

For MenY, isolates from cc23 predominated (87%) (Table 2). Despite the overall number of MenY isolates being smaller than for MenW, a greater diversity was evident, with MenY populations encompassing a larger number of unique strain designations than MenW (39 and 26, respectively). Breakdown by isolation time-point revealed the sporadic recovery

of isolates from different MenY designations during the carriage study with only one designation, Y: P1.5-1,10-1: F4-1: ST-12176 (cc23), detected at all isolation time-points.

**WGS Analysis Resolves MenW:cc11 Carriage Isolates to the 2013-strain of the South American-UK Sub-lineage**

Higher resolution genealogical analysis of the MenW:cc11 isolates was realized by comparing the core genome sequences of carriage and invasive MenW:cc11 isolates (*n*=236). Identification of lineage 11.1 sub-lineages and strains was facilitated by inclusion of sixty additional isolates previously assigned by core genome analysis [12, 15]. Carriage and invasive MenW:cc11 isolates predominantly resolved to the 2013-strain cluster within the South American-UK sub-lineage (83% and 62%, respectively) (Figure 1 and Table 1). Core WGS analysis resolved isolates sharing the predominant W: P1.5,2: F1-1: ST-11 (cc11) designation to different 11.1 sub-lineages (*i.e.* South American-UK and Hajj, respectively), and within the South American-UK sub-lineage, into different strain types (Table 1). Isolation time-point analysis revealed that although isolates with the W: P1.5,2: F1-1: ST-11 (cc11) designation from the original and South American strains were recovered at multiple time-points, the increase in MenW carriage was almost entirely due to W: P1.5,2: F1-1: ST-11 (cc11) isolates from the 2013-strain (2, 16 and 19 isolates in September, November and March, respectively) (Table 1).

To visualize the relationships among isolates from the 2013-strain type more clearly, a NeighborNet network was generated from a separate core genome comparison of the carried (*n*=38) and invasive (*n*=117) 2013-strain isolates, with color-coding of nodes detailing provenance. This revealed a multifocal expansion of carriage isolates, with 97% of the November 2015 and March 2016 isolates resolved to five clusters (Figure 2). Isolates within each cluster differed at only a small number of core genome loci suggesting multiple

close transmission networks. The MenACWY coverage rate for clusters A-E ranged from 57%-83% confirming that transmission within all five networks was not restricted to unvaccinated individuals (Figure 2). As sampling of students in November 2015 and March 2016 occurred in five dormitories [25], we examined whether 2013-strain isolate clusters correlated with dormitory of isolation. Each cluster contained isolates recovered from at least three different sampling sites suggesting that transmission networks extended beyond individual dormitories (Supplementary Figure 1).

**Effect of MenACWY Vaccination on Group W and Y Capsule Expression**

Mucosal immune responses elicited by MenACWY vaccination have the potential to influence capsule expression in target serogroups. We determined whether circulating MenW and MenY shifted towards a non-serogroupable (*i.e.* acapsulate) phenotype, however no significant changes were detected in the proportions of MenW:cc11 original strain, MenW:cc11 2013-strain, other MenW, MenY:cc23 or other MenY isolates expressing capsule between time-points (Table 3). Likewise the specific incidences of encapsulated or acapsulate MenY:cc23 (or other MenY) in the population showed no significant changes during the study. For MenW, the increasing incidence of carriage was driven by significant increases in both encapsulated and non-capsulated MenW:cc11 2013-strain isolates (Table 3). Notably 85% of MenW:cc11 2013-strain isolates recovered in March 2016 expressed capsule, with 82% of individuals carrying the encapsulated 2013-strain isolates at this time-point having received MenACWY vaccine before or during September 2015 (Table 3).

**Absence of Isolates From cc11 and cc23 Amongst Non-groupable Carriage Isolates**

We obtained WGS data for an additional 93 non-groupable (*i.e.* isolates lacking *ctrA* or carrying the capsule null locus) carriage isolates. Extracted strain designations showed that

isolates from cc198 were most prevalent (35%), followed by cc53 (26%) and cc865 (9%). Importantly, no isolates from the relevant IMD-associated MenW and MenY clonal complexes (cc11 and cc23, respectively) were detected, suggesting that deletion of part, or all, of the capsule locus by isolates of these lineages to avoid vaccine-induced immune responses had not occurred.

## DISCUSSION

Since its appearance in 2013, cases of IMD in the UK due to the 2013-strain of the South American-UK sub-lineage of MenW:cc11 have approximately doubled year-on-year, while expansion of the original strain has slowed [15]. Here we show that the increase in carried MenW detected at a UK university during 2015-16 [25] was also due to expansion of the 2013-strain, a finding that was reliant on the ability of core genome analysis to resolve apparently indistinguishable isolates sharing the designation W: P1.5,2: F1-1: ST-11 (cc11). Importantly, both the original and the 2013-strain MenW:cc11 strains were carried by in-coming students, yet only the 2013-strain expanded. This suggests differences in strain transmissibility and/or host susceptibility to oropharyngeal carriage in this population. Further studies may indicate whether the differential expansion relates to the previously determined four point mutations and three distinct recombination events which distinguish the strains [15] or other, as yet undetermined genetic differences. As well as being a highly virulent strain with a notable tendency for atypical clinical presentation and a high case-fatality rate [13-15], our findings suggest that the 2013-strain may result in relatively high levels of carriage in semi-closed communities of young adults, a phenomenon not previously detected for the original strain or MenC:cc11 [32]. Such settings may act as a reservoir for the 2013-strain, leading to case-clusters or outbreaks of disease in susceptible students [22] and onward transmission to unvaccinated cohorts in the wider population.

Of concern, the expansion of the 2013-strain occurred in the context of a student population which had, for the most part, received conjugate MenACWY vaccination. This vaccination had been introduced specifically because of the rapid and sustained increase in MenW:cc11 IMD in the UK [16] and led to a reduction in MenW cases in the first vaccine-targeted UK cohort who entered university [33]. Vaccination was targeted at older adolescent and young adults in order to provide direct protection to these age groups but also to generate indirect 'herd' protection as observed for the MenC and MenA monovalent conjugate vaccines where high vaccine coverage in these age groups reduced serogroup-specific carriage [32, 34]. Evidence supporting a comparable impact of quadrivalent MenACWY conjugate vaccines on carriage is currently lacking, albeit two studies in different populations have shown MenACWY vaccination elicited a modest impact on meningococcal carriage in vaccinated individuals [35, 36]. In a study involving UK university students, carriage rates of serogroup Y and combined serogroups CWY were significantly lower two months after vaccination with MenACWY [35], whilst in Polish soldiers meningococcal carriage was 9.6% in unvaccinated individuals and 1.2% in individuals vaccinated 1-3 years previously with MenACWY vaccine [36]. Of note, however, prior to vaccination serogroup Y carriage predominated over serogroup C and W carriage in the former study, and serogroup Y and C carriage were dominant in the latter study, suggesting that the observed effects of MenACWY vaccination on carriage were predominantly due to reductions in carriage of serogroup Y, or Y and C strains, respectively.

Our data suggest that the MenW component of conjugate MenACWY vaccines does not impact significantly on MenW carriage or does so at a lower level as compared to the MenY component. Thus the sporadic and limited recovery of MenY designations, particularly cc23 isolates, during this carriage study, is indicative of an absence of transmission events in this cohort. This is in marked contrast to the findings of previous

studies of meningococcal carriage in university students, where MenY strains of similar clonal complexes expanded significantly and persisted in unvaccinated populations [27, 28, 37, 38]. Furthermore, the finding that the vast majority of the isolates of the 2013-strain were expressing the W capsule at the March time-point is consistent with the hypothesis that the capsular polysaccharide antigen was not under significant selective pressure from the introduction of the MenACWY vaccine in this population. In contrast, in a study examining the impact of MenC monovalent conjugate vaccination on carriage, Maiden and colleagues detected a significant reduction in both the prevalence of MenC:cc11 and in the proportion of recovered MenC:cc11 isolates expressing capsule (81% in 1999 and 43% in 2001, respectively) [32]. A caveat is that the majority of MenW:cc11 transmission events may have occurred in students immunized in September and during the early part of the academic year, a period known to coincide with rapid meningococcal transmission and carriage acquisition in first-year students [19]. Thus, vaccine-elicited immune responses may have developed too slowly to impact on the acquisition of MenW:cc11 but not MenY:cc23 strains. Vaccinating adolescents earlier and achieving higher coverage (*i.e.* the aim of the routine adolescent schools program where preliminary coverage was >77% [39]) may reduce MenW:cc11 acquisition and carriage and eventually lead to population-wide herd immunity. Ongoing surveillance will be needed to establish whether MenW:cc11 carriage declines as these cohorts enter the university population.

Our cross-sectional study precluded comment on the duration of carriage of the 2013-MenW:cc11 strains in individuals. However prolonged carriage of these strains in MenACWY-vaccinated individuals could be critically important due to the potential for spread to non-vaccinated individuals in the population. A longitudinal study is required to determine whether there are differences in MenW/Y carriage duration in vaccinated individuals as implied by our current study. Additionally, an examination of the impact of

MenACWY vaccination on the density of meningococcal carriage is required. In a recent study, Finn and colleagues utilized quantitative PCR to assess the density of meningococcal carriage and observed temporal and individual variation of several orders of magnitude [40]. Our study cannot exclude the possibility of an effect of MenACWY immunization on carriage density in vaccinated as compared to unvaccinated individuals.

In conclusion, we show that the hyper-virulent 2013-strain of the South American-UK MenW:cc11 sub-lineage was responsible for an increase in group W carriage reported at a UK university. Analysis of WGS data revealed close transmission networks that extended beyond individual dormitories. Furthermore, on-campus MenACWY vaccination did not prevent expansion of capsule-expressing isolates from the 2013-strain of MenW:cc11. These findings are important for predicting the rate of development of population-wide herd immunity in the UK and for protecting older unvaccinated cohorts. In the period January through March 2017, there were 51 cases of MenW IMD in individuals aged >45 years in England [39]. Finally, further studies are required to determine whether carriage of the 2013-strain is increasing in the wider population of older adolescents and young adults in UK, and in other countries where there are similar increases in IMD due to this emerging strain.

## POTENTIAL CONFLICTS OF INTEREST

N. J. O. and L. R. G. have no potential conflicts. J. P. has been a consultant to Specific Technologies and has received research funding from Pfizer. C. D. B. collaborates with GlaxoSmithKline. D. P. J. T. has received support from Novartis Vaccines (now a part of GlaxoSmithKline), Sanofi Pasteur and GlaxoSmithKline, including honoraria, grants and travel assistance for conferences.

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## FIGURE LEGENDS

**Figure 1.** NeighborNet network based on the comparison of 1605 core genome loci amongst lineage 11.1 genomes (*n*=296). Three sets of isolates were included: (1) MenW:cc11 UK carriage isolates (*n*=46); (2) MenW:cc11 UK 2015-16 invasive isolates (*n*=190), and (3) previously assigned MenW:cc11 isolates (*n*=60). Thirty eight carriage isolates localized to the 2013-strain of the South American-UK sub-lineage, whilst four carriage isolates resolved to each of the original and South American strain clusters, respectively. Thirty previously assigned isolates and two MenW:cc11 UK 2015-16 invasive isolates resolved to the Hajj sub-lineage ('to Hajj sub-lineage'). Nodes are color coded: carriage isolates in red; invasive isolates in black; previously assigned isolates in blue. Scale bar = number of allelic differences.

**Figure 2.** NeighborNet network based on the comparison of 1605 core genome loci amongst 2013-strain isolates. Two sets of isolates were included: (1) 2013-strain MenW:cc11 UK carriage isolates (*n*=38) and (2) 2013-strain MenW:cc11 UK 2015-16 invasive isolates (*n*=117). Nodes are color coded: invasive isolates in black; September 2015 carriage isolates in red; November 2015 carriage isolates in green; March 2016 carriage isolates in blue. 97% of the November 2015 and March 2016 carriage isolates resolved to five clusters (labelled A-E), with isolates within each cluster being highly similar (cg diff. = core genome differences). 82%, 60%, 83%, 57% and 80% of carriers harboring the isolates in clusters A-E, respectively, had received MenACWY vaccine before or during registration (September 2015). Scale bar = number of allelic differences.

476 **Table 1. Breakdown of MenW Carriage and Invasive Isolates by Strain Designation, 11.1 Sub-lineage and Strain Type, and Isolation**

477 **Time-Point**

| Strain designation[a] | 11.1 sub-lineage[b] | Strain type[b] | Isolation time-point | | | Total carriage (*n*=49) | Invasive 2015-16 (*n*=200) | Total carriage and invasive (*n*=249) |
|---|---|---|---|---|---|---|---|---|
| | | | Sep 2015 (*n*=5) | Nov 2015 (*n*=20) | Mar 2016 (*n*=24) | | | |
| W: P1.5,2: F1-1: ST-11 (cc11) | South American-UK | 2013 | 2 | 16 | 19 | 37 | 101 | 138 |
| | | Original | 2 | 0 | 0 | 2 | 40 | 42 |
| | | South American | 0 | 2 | 2 | 4 | 0 | 4 |
| | Hajj | ND | 0 | 0 | 0 | 0 | 2 | 2 |
| W: P1.5,2: F1-1: ST-ND (cc11) | South American-UK | 2013 | 0 | 0 | 1 | 1 | 15 | 16 |
| | | Original | 0 | 0 | 0 | 0 | 6 | 6 |
| W: P1.5,2: F1-1: ST-10651 (cc11) | South American-UK | Original | 1 | 1 | 0 | 2 | 8 | 10 |
| W: P1.5,2: F1-146: ST-11 (cc11) | South American-UK | Original | 0 | 0 | 0 | 0 | 8 | 8 |
| W: P1.5,2: F1-146: ST-ND (cc11) | South American-UK | Original | 0 | 0 | 0 | 0 | 2 | 2 |
| Other cc11[c] | South American-UK | 2013 | 0 | 0 | 0 | 0 | 1 | 1 |
| | | Original | 0 | 0 | 0 | 0 | 7 | 7 |
| Other non-cc11[d] | NA | NA | 0 | 1 | 2 | 3 | 10 | 13 |

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479 [a] Derived from genome sequence data

480 [b] As assigned by core genome analysis (shown in Figure 1)

[c] Includes all cc11 strain designations occurring only once

[d] Includes all non-cc11 strain designations

ND = not determined; NA = not applicable

**Table 2. Frequency of Strain Designations in the MenY Carriage and Invasive Collections**

| Clonal complex[a] | Strain designation[a] | Isolation time-point | | | Total carriage | Invasive 2015-16 | Total carriage and invasive |
|---|---|---|---|---|---|---|---|
| | | Sep 2015 (*n*=14) | Nov 2015 (*n*=8) | Mar 2016 (*n*=10) | (*n*=32) | (*n*=104) | (*n*=136) |
| cc23 | Y: P1.5-1,10-1: F4-1: ST-1655 (cc23) | 4 | 0 | 1 | 5 | 40 | 45 |
| | Y: P1.5-1,10-4: F4-1: ST-23 (cc23) | 2 | 3 | 0 | 5 | 7 | 12 |
| | Y: P1.5-1,10-1: F4-1: ST-ND (cc23) | 0 | 0 | 0 | 0 | 12 | 12 |
| | Y: P1.5-2,10-1: F4-1: ST-23 (cc23) | 0 | 0 | 0 | 0 | 9 | 9 |
| | Y: P1.5-1,10-4: F4-1: ST-1655 (cc23) | 1 | 0 | 2 | 3 | 4 | 7 |
| | Y: P1.5-1,10-1: F4-1: ST-12176 (cc23) | 1 | 1 | 2 | 4 | 1 | 5 |
| | Y: P1.5-1,10-4: F4-1: ST-ND (cc23) | 0 | 0 | 0 | 0 | 5 | 5 |
| | Y: P1.5-1,10-1: F4-1: ST-11754 (cc23) | 0 | 0 | 0 | 0 | 3 | 3 |
| | Y: P1.5-1,10-10: F4-1: ST-1655 (cc23) | 2 | 0 | 0 | 2 | 0 | 2 |
| | Y: P1.5-1,10-8: F4-1: ST-1655 (cc23) | 1 | 0 | 1 | 2 | 0 | 2 |
| | Other[b] | 1 | 2 | 1 | 4 | 12 | 16 |
| Non-cc23 | Y: P1.21,16: F3-7: ST-1466 (cc174) | 0 | 0 | 0 | 0 | 3 | 3 |
| | Y: P1.18-7,9: F3-9: ST-ND (cc103) | 0 | 1 | 1 | 2 | 0 | 2 |
| | Y: P1.5-1,10-4: F3-4: ST-10730 (cc167) | 0 | 0 | 0 | 0 | 2 | 2 |
| | Y: P1.5-1,10-22: F5-1: ST-ND (cc22) | 0 | 0 | 0 | 0 | 2 | 2 |
| | Other[c] | 2 | 1 | 2 | 5 | 4 | 9 |

[a] Derived from genome sequence data

[b] Includes all cc23 strain designations occurring only once

[c] Includes all non-cc23 strain designations occurring only once

**Table 3. Prevalence of Capsule-Expressing and Acapsulate MenW and MenY Genogroups by Isolation Time-Point**

| Isolation time-point (no. of participants) | Capsule expression status | Genogroup | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MenW:cc11 2013-strain only | | MenW:cc11 original strain only | | Other MenW | | MenY:cc23 only | | Other MenY[a] | |
| | | No. (%) of isolates | % of participants (95% CI) | No. (%) of isolates | % of participants (95% CI) | No. (%) of isolates | % of participants (95% CI) | No. (%) of isolates | % of participants (95% CI) | No. (%) of isolates | % of participants (95% CI) |
| September (*n*=769) | On | 0 | 0 | 2 (67) | 0.3 (0.0-0.6) | 0 | 0 | 8 (67) | 1.0 (0.3-1.8) | 1 (50) | 0.1 (0.0-0.4) |
| | Off | 2 (100) | 0.3 (0.0-0.6) | 1 (33) | 0.1 (0.0-0.4) | 0 | 0 | 4 (33) | 0.5 (0.0-0.1) | 1 (50) | 0.1 (0.0-0.4) |
| November (*n*=353) | On | 9[b] (56) | 2.5 (0.9-4.2)*** | 1 (100) | 0.3 (0.0-0.8) | 2 (67) | 0.6 (0.0-1.3) | 1 (17) | 0.3 (0.0-0.8) | 2 (100) | 0.6 (0.0-1.3) |
| | Off | 7[c] (44) | 2.0 (0.5-3.4)** | 0 | 0 | 1 (33) | 0.3 (0.0-0.8) | 5 (83) | 1.4 (0.2-2.6) | 0 | 0 |
| March (*n*=268) | On | 17[d] (85) | 6.3 (3.4-9.3)* | 0 | 0 | 0 | 0 | 4 (57) | 1.5 (0.0-2.9) | 0 | 0 |
| | Off | 3[e] (15) | 1.1 (0.0-2.4) | 0 | 0 | 4 (100) | 1.5 (0.0-2.9) | 3 (43) | 1.1 (0.0-2.4) | 2 (100) | 0.7 (0.0-1.8) |

Asterisks indicate a statistically significant difference compared to prevalence at the preceding time-point (*$p<0.05$; **$p<0.01$, ***$p<0.0001$)

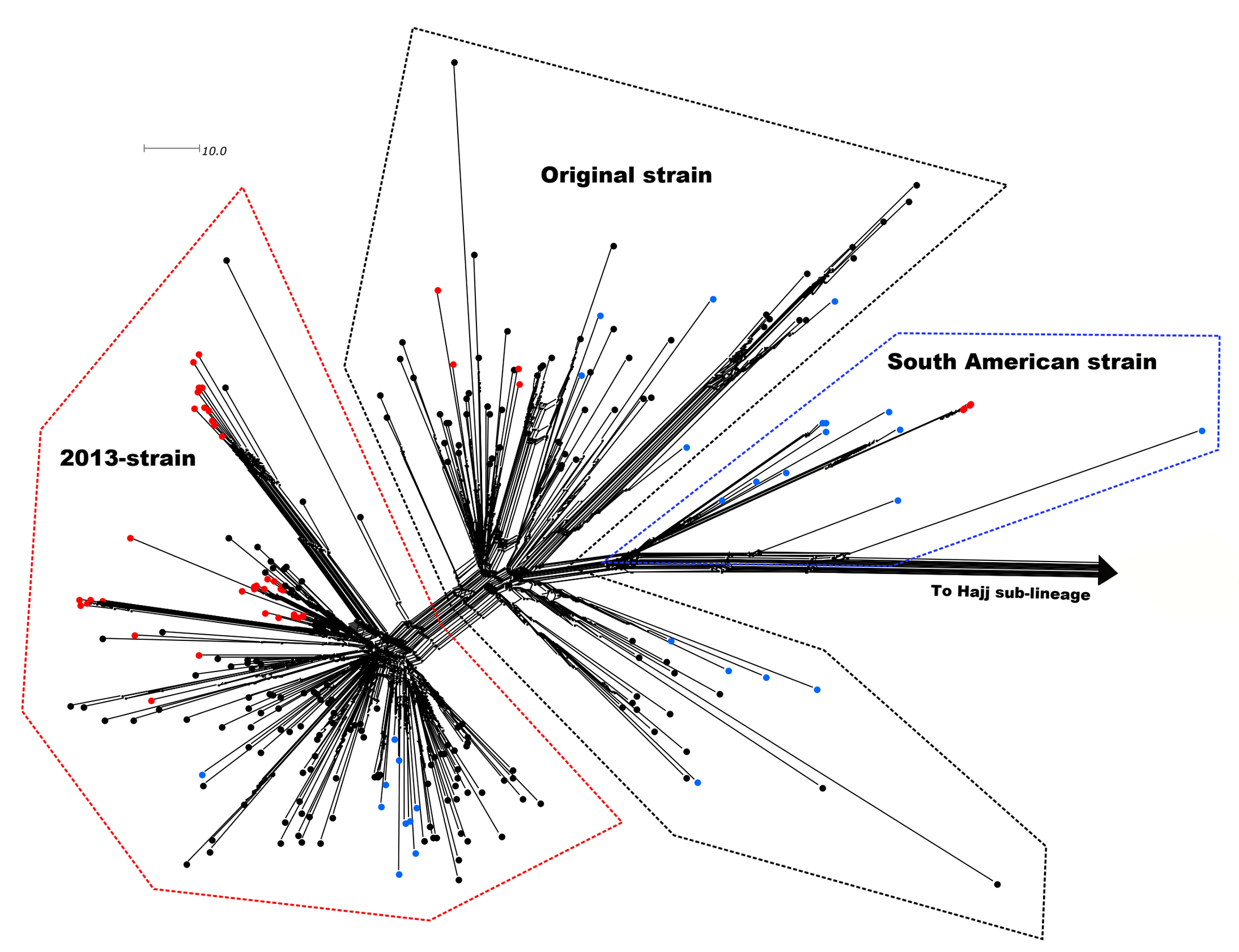
[a] Serogrouping data unavailable for one MenY:cc103 isolate from March 2016

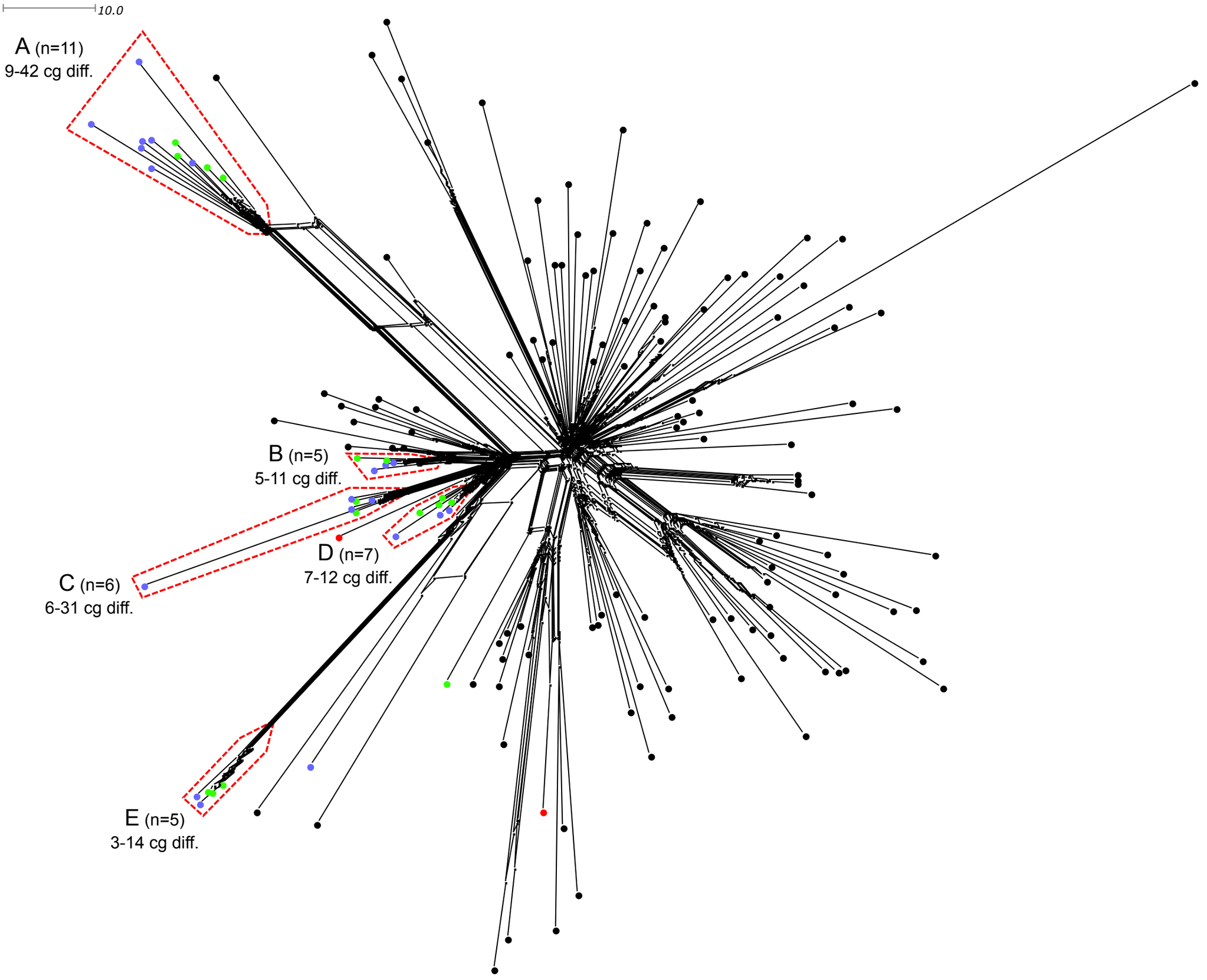
[b] Of these, 5/9 (56%) had received MenACWY vaccine before or during registration (September 2015)

[c] Of these, 6/7 (86%) had received MenACWY vaccine before or during registration (September 2015)

[d] Of these, 14/17 (82%) had received MenACWY vaccine before or during registration (September 2015)

[e] Of these, 3/3 (100%) had received MenACWY vaccine before or during registration (September 2015)


10.0
Original strain
South American strain
2013-strain
To Hajj sub-lineage


10.0
A (n=11)
9-42 cg diff.
B (n=5)
5-11 cg diff.
D (n=7)
7-12 cg diff.
C (n=6)
6-31 cg diff.
E (n=5)
3-14 cg diff.