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Molecular insights into meningococcal carriage isolates from Burkina Faso 7 years after introduction of a serogroup A meningococcal conjugate vaccine

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Abstract

In 2010, Burkina Faso completed the first nationwide mass-vaccination campaign of a meningococcal A conjugate vaccine, drastically reducing the incidence of disease caused by serogroup A meningococci. Since then, other strains, such as those belonging to serogroups W, X and C, have continued to cause outbreaks within the region. A carriage study was conducted in 2016 and 2017 in the country to characterize the meningococcal strains circulating among healthy individuals following the mass-vaccination campaign. Four cross-sectional carriage evaluation rounds were conducted in two districts of Burkina Faso, Kaya and Ouahigouya. Oropharyngeal swabs were collected for the detection of *Neisseria meningitidis* by culture. Confirmed *N. meningitidis* isolates underwent whole-genome sequencing for molecular characterization. Among 13758 participants, 1035 (7.5%) *N. meningitidis* isolates were recovered. Most isolates (934/1035; 90.2%) were non-groupable and primarily belonged to clonal complex (CC) 192 (822/934; 88%). Groupable isolates (101/1035; 9.8%) primarily belonged to CCs associated with recent outbreaks in the region, such as CC11 (serogroup W) and CC10217 (serogroup C); carried serogroup A isolates were not detected. Phylogenetic analysis revealed several CC11 strains circulating within the country, several of which were closely related to invasive isolates. Three sequence types (STs) were identified among eleven CC10217 carriage isolates, two of which have caused recent outbreaks in the region (ST-10217 and ST-12446). Our results show the importance of carriage studies to track the outbreak-associated strains circulating within the population in order to inform future vaccination strategies and molecular surveillance programmes.

DATA SUMMARY

The genome sequences for the carriage isolates used in this study are publicly available from the National Center for Biotechnology Information under BioProject PRJNA635376.

INTRODUCTION

The meningitis belt is a region of sub-Saharan Africa with annual outbreaks of meningococcal meningitis [1]. *Neisseria meningitidis* serogroup A has historically been responsible for most epidemics in the region [2], but other serogroups such as X [3], W [2, 4] and more recently C [5, 6] have all caused major outbreaks. Burkina Faso is a landlocked country that completely resides in the meningitis belt, and has been one of the most affected by meningococcal epidemics, experiencing hyperendemic rates of meningitis [7]. Several carriage studies have been conducted within Burkina Faso to assess the meningococcal carriage and strain diversity circulating in the country. In 2003, a carriage study in Bobo-Dioulasso consisting of 152 isolates identified the sequence type (ST) 192 with the non-groupable capsule serogroup as

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Keywords: Burkina Faso; meningitis; meningitis belt; meningococcal carriage; *Neisseria meningitidis*; whole-genome sequencing.

Abbreviations: CC, clonal complex; CDC, Centers for Disease Control and Prevention; MACV, meningococcal serogroup A vaccine; ST, sequence type.

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Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. A supplementary table is available with the online version of this article.

the predominantly carried strain [8]. In 2010, Burkina Faso completed the first nationwide mass-vaccination campaign of the meningococcal serogroup A vaccine (MACV, MenAfriVac) [9] among 1–29 year-olds. A carriage study was performed prior to and up to 2 years following vaccination (2009–2012) to determine the impact of vaccination on meningococcal carriage in the country [10]. This study found that 70% of the carriage isolates collected prior to vaccination expressed a capsule detected using slide agglutination, with 56% of the isolates belonging to serogroup Y, the majority of which belonged to ST-23. Additionally, 9.8% of the carriage isolates belonged to ST-2859, a strain that is primarily associated with *N. meningitidis* serogroup A [11]. In the carriage evaluation rounds that followed vaccination, *N. meningitidis* serogroup X belonging to ST-181 dominated the collection up to 1 year after vaccination [12, 13], whereas *N. meningitidis* serogroup W dominated the second year afterwards [14]. No *N. meningitidis* serogroup A nor *N. meningitidis* serogroup A-associated STs were detected in vaccinated regions.

In the years post-vaccination, incidence of disease caused by *N. meningitidis* serogroup A in Burkina Faso was dramatically reduced [15]; however, other meningococcal strains continued to cause disease [16]. In 2011, strains belonging to *N. meningitidis* serogroup X were the predominant cause of meningococcal disease in the country [17]. In 2012, Burkina Faso experienced meningococcal epidemics caused by strains of *N. meningitidis* serogroup W belonging to the hyper invasive clonal complex 11 (CC11) [18, 19]. In neighbouring country Niger, epidemics of *N. meningitidis* serogroup C belonging to CC10217 were reported in 2015 [5], following outbreaks in Nigeria in 2013–2014 [6].

In response to the changing molecular landscape of invasive meningococcal disease in the meningitis belt in the post-MACV era, an additional carriage study was conducted in Burkina Faso across four rounds between 2016 and 2017. This carriage study aimed to assess the continued impact of MACV, and to analyse the diversity of the meningococcal strains being carried in a population that is prone to meningococcal epidemics.

Impact Statement

Burkina Faso is a landlocked country within the meningitis belt that has been one of the most affected by meningococcal epidemics. Following a nationwide mass-vaccination campaign of a meningococcal A conjugate vaccine, Burkina Faso saw a drastic reduction in the incidence of serogroup A meningococcal disease; yet, other serogroups have continued to cause outbreaks in the country. Carriage studies provide insights into the strains circulating within the population, enabling us to assess the impact of vaccination on carriage and plan for future surveillance and country preparedness efforts for epidemics. We identified a larger proportion of non-groupable isolates being carried within the country as compared to prior carriage studies, and the presence of strains matching genotypes associated with recent outbreaks in the region. These results reflect the changing landscape of meningococcal disease in the region and highlight the importance of molecular surveillance for detecting outbreak-associated strains circulating among the population.

METHODS

Carriage study design and isolate collection

The carriage study consisted of four cross-sectional evaluation rounds across 10 villages in the Kaya district and 10 villages in the Ouahigouya district. Rounds one and three took place during the dry season (April/May in 2016 and 2017) and rounds two and four took place during the rainy season (October/November in 2016 and 2017). Compounds (group of people who have the same head of household and share meal preparation in the same area) were selected by random sampling in each round and all persons in the compound aged 9 months to 36 years were eligible; compounds randomly selected to participate in more than one round were recorded, but not individual participants within the compound. The complete details on the carriage study design, isolate preparation and laboratory methods have been described by Mbaeyi *et al.* [20]. Briefly, oropharyngeal swabs were collected from consenting participants, and were subsequently inoculated and streaked on modified Thayer–Martin media and stored in CO_2-rich anaerobic jars. Suspected *N. meningitidis* isolates with *Neisseria*-like colony morphology were tested by biochemical tests and slide agglutination serogrouping (SASG) at the regional laboratories, and sent to either the Centers for Disease Control and Prevention (CDC) or the Norwegian Institute of Public Health (NIPH) for confirmation and additional characterization using whole-genome sequencing and molecular testing. Any serogroup discrepancies between the laboratories were resolved using SASG.

Whole-genome sequencing and analysis

Whole-genome sequencing was performed on isolates using either the MiSeq system or the HiSeq 2500 system (Illumina). The resulting paired-end 250 bp sequence reads were trimmed using Cutadapt [21] and underwent *de novo* assembly using SPAdes 3.7 [22]. The genome assembly was used to confirm the isolate species [23], to obtain multilocus sequence typing (MLST) information from PubMLST [24], and to characterize the capsule locus, as described by Marjuki *et al.* [25]. This capsule analysis includes identifying the capsule genes present, checking each capsule gene for mutations and reporting the capsule genotype. Isolate genomes were labelled as capsule null if they lacked the capsule biosynthesis genes and harboured the capsule null locus (*cnl*). Each genome was annotated through BLAST [26] comparisons against the PubMLST *Neisseria* allele collection. The resulting annotation files were used as input for Roary [27] to generate a core-genome alignment, which was then used as input for RAxML [28] to generate a maximum-likelihood phylogenetic tree

including all meningococcal carriage isolates from the study. The CC-specific phylogenetic trees were generated by first creating a reference-based core-SNP alignment using Snippy (https://github.com/tseemann/snippy) using a reference genome belonging to the same CC. The resulting SNP alignment was used as input for Gubbins [29] to mask predicted recombination sites. The output alignment from Gubbins was used to calculate SNP differences between genomes and as input for RAxML to produce the final, maximum-likelihood phylogenies. The SNP differences between genomes were used for pairwise comparisons between isolates from the same compound. The significance of the median SNP differences between isolates from within-compound and across-compound participants was determined using the Wilcoxon rank sum test.

RESULTS

A total of 1035 *N. meningitidis* isolates were recovered among 13758 participants from whom a specimen was collected, representing a carriage rate of 7.5% (Table S1, available with the online version of this article). Among the *N. meningitidis* isolates, the majority (90.2%, 934/1035) were non-groupable, of which 89% (832/934) were capsule null; 9.3% (87/934) had one or more capsule genes, but did not have any serogroup-defining capsule gene identified and were labelled as having an 'unclear genetic capsular backbone.' The remaining 15 non-groupable isolates had a serogroup-defining capsule gene identified but had a disrupted capsule locus either due to premature internal stops or truncated capsule genes. The genetic backbones of the capsule for these 15 non-groupable isolates were as follows: 9 serogroup W, 3 serogroup E and 3 serogroup C. The remaining encapsulated *N. meningitidis* isolates (9.8%, 101/1035) consisted of 83 serogroup W, 13 serogroup C and 5 serogroup E. No serogroup A isolates were collected during the carriage study.

A total of 21 STs belonging to 7 CCs were identified. The phylogeny in Fig. 1(a) shows the relationship of all CCs collected in the study. In general, isolates within the same CC clustered together by district rather than round. CC192 was the predominant CC identified, constituting 79.4% (822/1035) of the isolates, followed by CC175 and CC11 with both CCs comprising 8.6% (89/1035) of the isolates each. All of the isolates belonging to CC192 and CC175 were non-groupable with the capsule null genotype. Most CC11 isolates were collected from Kaya (97.7%; 87/89), with 59 and 14 of the isolates being collected from the district in round one and two, respectively (Fig. 1b). Among the CC11 isolates, 93.3% (83/89) were serogroup W, and 6.7% (6/89) were non-groupable with a disrupted serogroup W capsule backbone. Eleven isolates were CC10217 and belonged to three STs: ST-10217 (*n*=5), ST-12446 (*n*=3), ST-13402 (*n*=3). Among the CC10217 isolates, 81.8% (9/11) were serogroup C, and

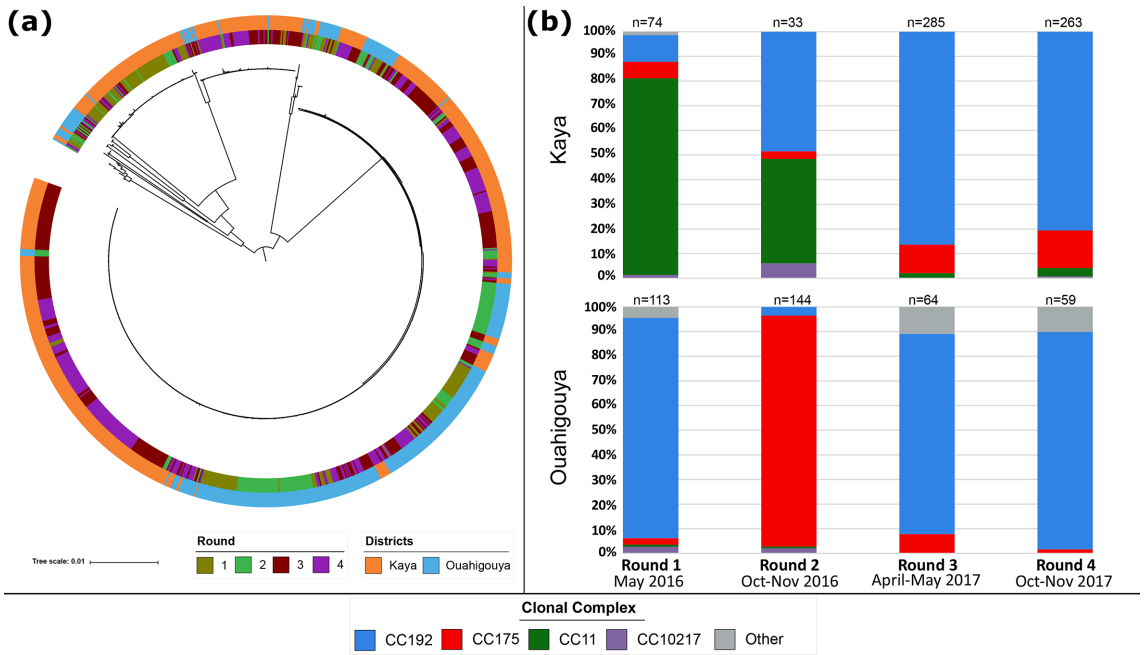

Fig. 1. (a) Phylogeny of meningococcal carriage isolates: maximum-likelihood core-genome multilocus sequence typing phylogeny of all 1035 meningococcal carriage isolates collected during the study. The colouring within the tree indicates the CC, and the outer rings indicate carriage round and district. (b) Composition of meningococcal carriage isolates collected per round: the *y*-axis indicates the percentage of total meningococcal carriage isolates collected in that district and round, and the *x*-axis indicates the specific carriage round. The count of meningococcal carriage isolates collected for each specific round and district is included at the top of each bar. The other category in the CC key includes isolates belonging to CCs or STs (if unassigned to a CC) with less than six isolates collected: CC178 (*n*=6), CC41/44 (*n*=5), ST-6924 (*n*=5), ST-9945 (*n*=3) and CC35 (*n*=1).

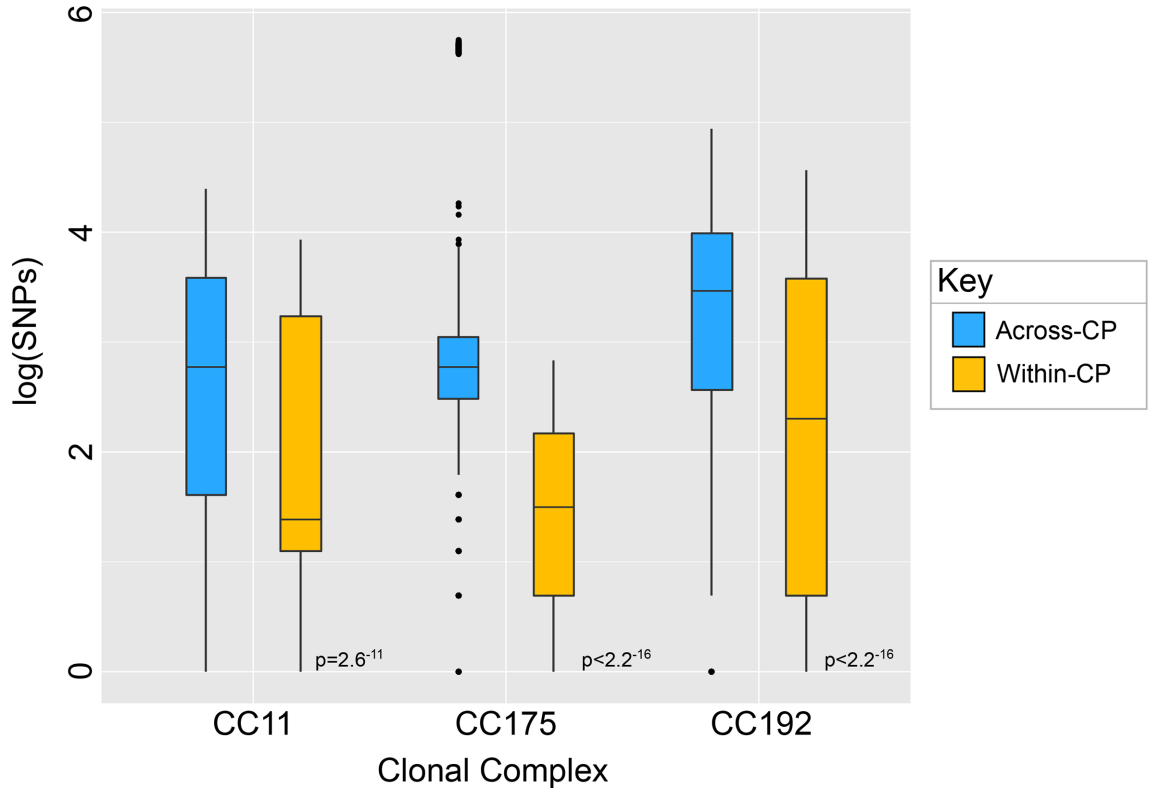

Fig. 2. SNP differences across and within compounds for CC11, CC175 and CC192: the log SNP differences among isolates belonging to these three CCs both within (within-CP) and across (across-CP) compounds are shown in these box plots. The *P* value is the significance of the Wilcoxon rank sum test for difference of medians. The median SNP differences for each CC within and across compounds were as follows: CC175, 4 (within), 16 (across); CC11,4 (within), 16 (across); CC192,10 (within), 32 (across).

2/11 (18.2%) were non-groupable with a disrupted serogroup C capsule backbone. The remaining *N. meningitidis* isolates belonged to one of the following CCs or STs unassigned to a CC with six or less isolates collected: CC178 (*n*=6), CC41/44 (*n*=5), ST-6924/unassigned CC (*n*=5), ST-9945/unassigned CC (*n*=3) and CC35 (*n*=1). No carriage isolates belonging to CCs or STs associated with serogroup A were collected during the carriage study.

Among the 648 unique compounds included in the carriage study, at least one *N. meningitidis* carrier was identified in 52% (338/648) of the compounds (carrier compounds). At least two meningococcal carriage isolates were collected from 65% (219/338) of these carrier compounds irrespective of their genotype; 67.1% (147/219) of these compounds had one CC identified, while 32.9% (72/219) had more than one CC identified – two CCs, 28.8% (63/219); three CCs, 4.1% (9/219) – 54.2% (39/72) of which were identified during a single carriage round.

Among carrier compounds, 96% (326/338) had meningococcal carriage isolates belonging to at least one of the following CCs: CC11, CC175 and CC192. Within each of these three CCs, isolates from participants in the same compound had a significantly lower median SNP difference ($P<0.01$) as compared to isolates collected from participants across compounds (Fig. 2).

Two CCs that are associated with recent outbreaks in the region were identified among carriage isolates, consisting of CC11 (*n*=89) and CC10217 (*n*=11). All CC11 isolates identified in the carriage study were ST-11 and were collected from participants spanning 52 unique compounds. The CC11 carriage isolates were compared against 48 invasive CC11 isolates from cerebrospinal fluid collected from meningitis patients in Burkina Faso between 2013 and 2016 (Fig. 3). Two major clades were identified: clade I primarily contained carriage isolates, while clade II primarily contained invasive isolates. The CC11 carriage isolates were largely found in one major clade (clade I), except for two groups found in clade II. Clade I contained one invasive isolate collected from 2016; genomic comparison of this isolate against its two closest carriage isolates revealed frameshift mutations in the genes *pglI* and *pilC2*, which are involved in protein glycosylation and adherence to the host cell, as well as the *hpuA* gene. Two compounds, CP2 and CP6, had CC11 isolates collected from the respective compound during the same carriage round that were found in two separate subclades within clade I. Clade II contained two clusters of carriage isolates, one of which was closely related to invasive isolates collected between 2013 and

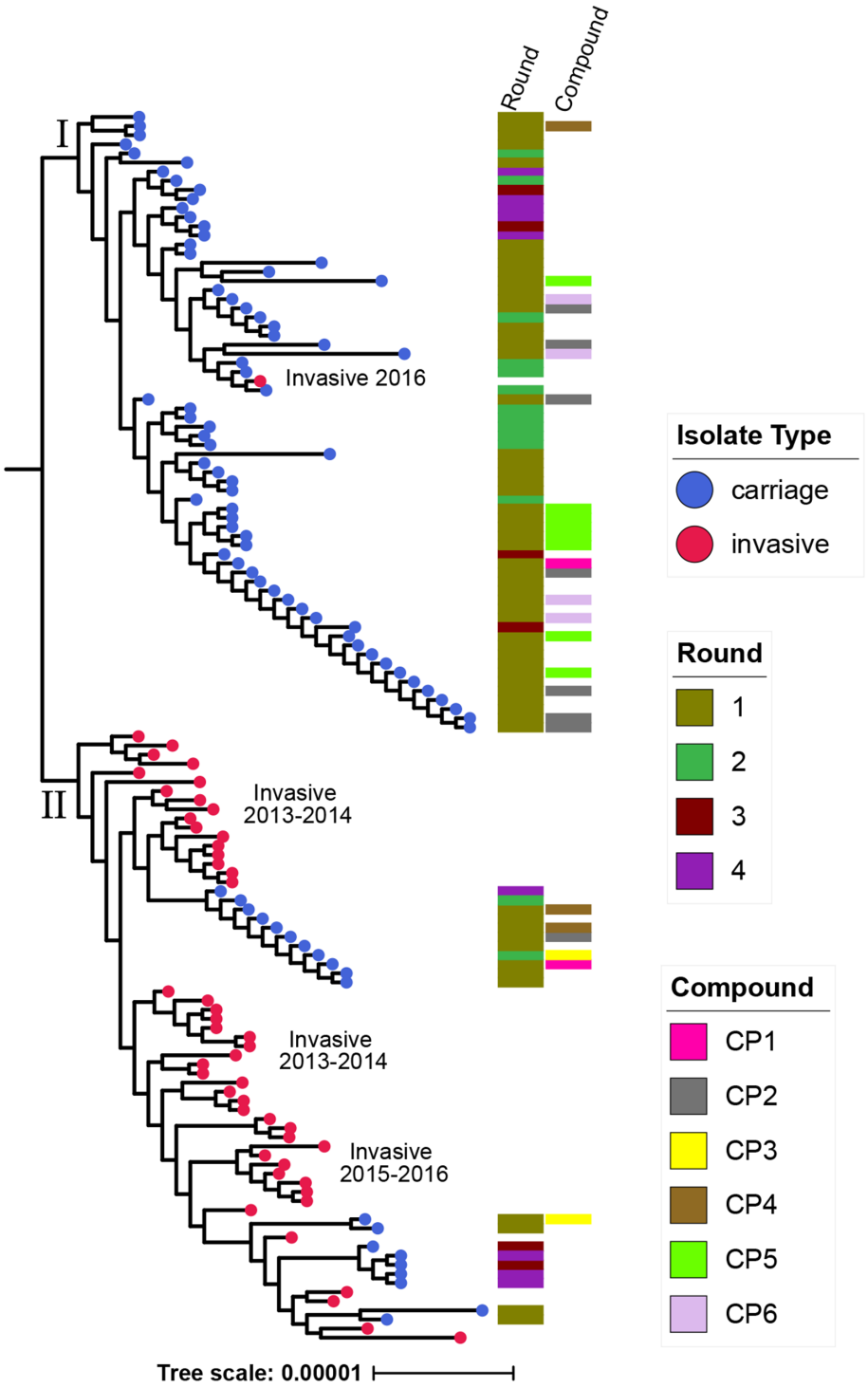

Fig. 3. Phylogenetic tree for CC11: maximum-likelihood phylogeny of all CC11 carriage isolates compared against invasive CC11 isolates collected between 2013 and 2016. Invasive isolates are shown in red, while carriage isolates are shown in blue. The collection round for each carriage isolate is indicated by a colour bar, as well as six compounds of interest. Clade I and Clade II had a mean within-clade divergence of 9.9 and 27 SNPs, respectively, and there was a mean of 34 SNPs difference between the clades.

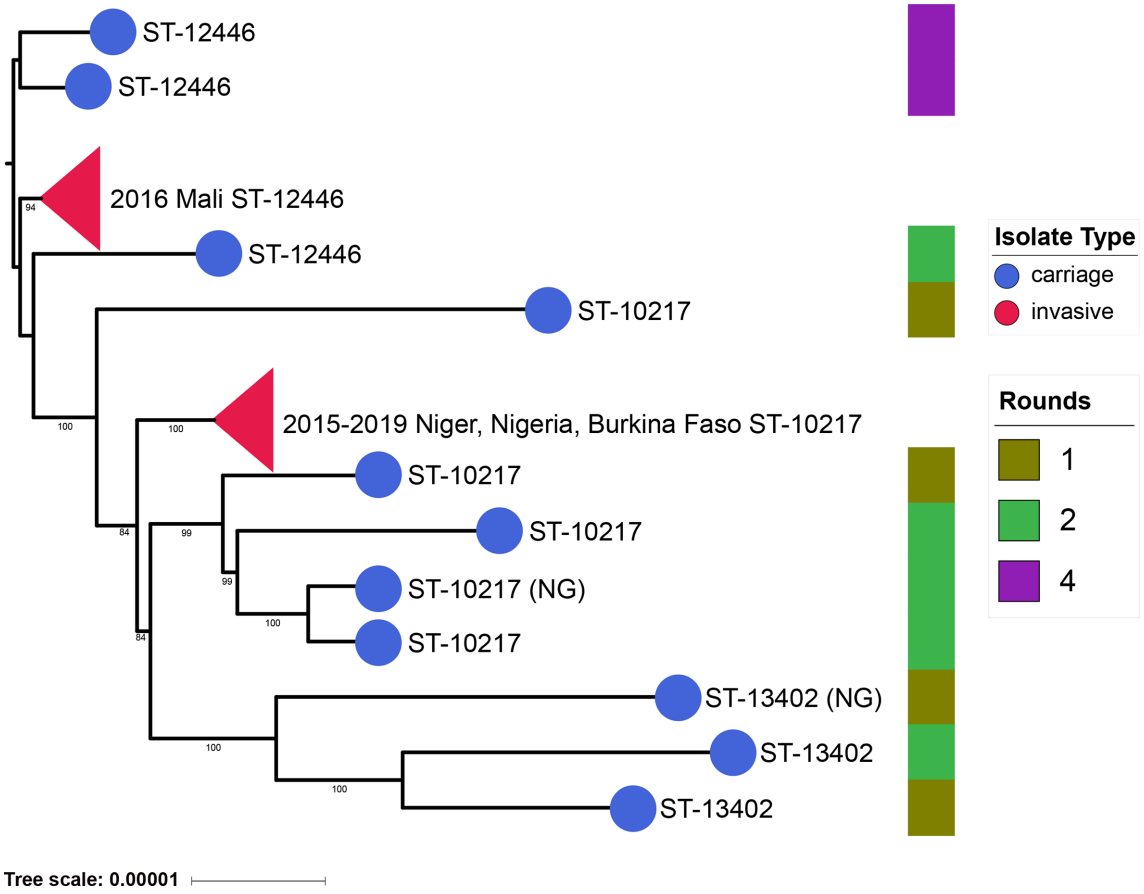

Fig. 4. Phylogenetic tree for CC10217: maximum-likelihood phylogeny of all CC10217 carriage isolates compared against invasive CC10217 isolates collected between 2013 and 2016. Invasive isolates are shown in red, while carriage isolates are shown in blue. The collection round for each carriage isolate is indicated by a colour bar. The ST for each isolate is included as well, along with a 'NG' indication for the two carriage isolates that were non-groupable. ST-10217 carriage isolates had a mean SNP divergence of 76.1 and differed from invasive ST-10217 isolates by a mean of 87.4 SNPs. ST-12446 carriage isolates had a mean SNP divergence of 24 and differed from invasive ST-10217 isolates by a mean of 31 SNPs.

2014, and another that was more closely related to invasive isolates collected between 2015 and 2016. One compound, CP3, had two isolates that were collected in rounds one and two that were found in each of these clusters. CC11 isolates from CP1, CP2 and CP4 collected during round one were found across both major clades.

Carriage isolates belonging to CC10217 were compared against invasive isolates from recent outbreaks in nearby countries, such as Mali, Niger, Nigeria and Burkina Faso (2015–2019) (Fig. 4). All CC10217 carriage isolates were collected among participants from unique compounds. Carriage isolates belonging to ST-12446 and ST-10217 were closest to the invasive isolates from the same ST, but still formed their own subclades. Three ST-10217 isolates collected during round two of the carriage study contained the MDA prophage, which has been described elsewhere [30] as a characteristic virulence factor in this ST. ST-13402 isolates were divergent to the other two STs, differing by a mean of 170 core SNPs; carriage isolates belonging to ST-10217 and ST-12446 differ by a mean of 100 core SNPs.

DISCUSSION

A marked finding in our study was the large proportion of non-groupable carriage isolates. It has been well described that the meningococcal capsule is a major determinant of virulence [31, 32], and that non-groupable isolates tend to be associated with carriage [33]. Compared to the carriage study conducted during 2009–2012 in Burkina Faso [10, 14], a much larger proportion of our carriage isolates were non-groupable; most of these isolates belonged to CC192, and this CC was dominant across both the Kaya and Ouahigouya districts. All isolates belonging to this CC were non-groupable and were capsule null. The major ST (ST-192) belonging to this CC was observed as the dominant strain collected in the 2003 Burkina Faso carriage study, but was not observed in the subsequent carriage evaluations prior to (2009) or following vaccination (2012). Our results are similar to that of a 2014 meningococcal carriage study in southern Ethiopia [34] that preceded introduction of MACV in the country, where researchers observed a majority of non-groupable isolates (76.4%), over half of which were ST-192. The data

from these studies suggest that the large proportion of CC192 isolates in our data set is indicative of a clonal wave, rather than selective pressure introduced by the serogroup A conjugate vaccine. This phenomenon has been described previously in an 8 year longitudinal study in Northern Ghana [35], where researchers suggested that the lack of a stable and genetically diverse pharyngeal meningococcal flora in the region may contribute to the rapid spreading of new clones in the meningitis belt. The rampant colonization of CC192 in the meningitis belt may have also been aided by genetic features of the organism, such as the lack of a bacterial capsule resulting in more efficient adhesion, and the deletion of the gene encoding the FetA immunogenic surface protein [36]. Additionally, a MenAfriCar study looking at the diversity of meningococcal carriage across 20 cross-sectional carriage surveys in seven meningitis belt countries found substantial changes in carriage strain prevalence over time in countries that introduced the conjugate A vaccine as well as in those that did not [37], suggesting that dynamic *N. meningitidis* carriage is not driven by vaccination.

We did not identify any isolates belonging to serogroup A or serogroup A-associated STs in our carriage study. However, we did detect isolates belonging to CCs associated with recent outbreaks in the region, such as CC11 and CC10217. Most CC11 carriage isolates were *N. meningitidis* serogroup W, and non-groupable isolates belonging to CC11 contained a disrupted *N. meningitidis* serogroup W capsule locus rather than being capsule null. Among strains that are associated with disease in the region, CC11 *N. meningitidis* serogroup W was the predominant strain collected in our study, which is consistent with *N. meningitidis* serogroup W being the primary strain causing disease in the country during 2016 and 2017 [38, 39]. However, the overall carriage rate of *N. meningitidis* serogroup W in our study was low, which may be explained by the low overall rate of laboratory-confirmed meningitis observed in the country during the study period compared to the previous 5 years [20]. In general, the phylogenetic structure of CC11 carriage isolates suggests the circulation of multiple strains belonging to the CC within the country. While carriage and invasive isolates belonging to CC11 largely formed their own clades, there were several exceptions, including two groups of carriage isolates that were more closely related to invasive isolates collected between 2013–2014 and 2015–2016, as well as one invasive isolate collected in 2016 that was closest to carriage isolates collected in the same year. Comparative genomic analysis of the two most genetically similar carriage isolates to this invasive isolate revealed frameshift mutations in the *pglI, pilC2* and *hpuA* genes. The *pglI* gene has been shown to be involved in pilin glycosylation [40] and plays a role in the invasiveness of the strain [41], while the *pilC2* gene is associated with type IV pilus proteins which are involved in mediating adhesion to human cell receptors [42, 43]. The *hpuA* gene is involved in iron acquisition from haemoglobin, which has been shown to contribute to the virulence of the pathogen [44–46]. These three genes have been shown to be phase variable [40, 44, 47], supporting the hypothesis that genes involved in host–pathogen interaction tend to be more commonly influenced by phase variability mediated by the short sequence repeats introduced by slipped strand mispairing [41], which may explain how this isolate resulted in disease while having a genetic backbone that is more similar to carriage isolates.

CC10217 is an emerging invasive CC that has already caused numerous outbreaks in the meningitis belt [5, 6]. In our study, we identified carriage isolates belonging to this CC from three STs, two of which have been the cause of a recent outbreaks in Burkina Faso and in nearby countries such as Mali (ST-12446) [48], Niger and Nigeria (ST-10217) [6, 49]. Our phylogenetic analysis revealed that the carriage and invasive isolates were genetically distinct; while carriage and invasive isolates belonging to the same ST were more closely related than to the other STs, they did not cluster together. A new ST belonging to CC10217, ST-13402, was identified among the carriage isolates and was divergent from the other two STs. In 2018, Brynildsrud *et al*. described a hypothesis regarding the origins of the emerging CC10217 pathogen [30]. Their analysis consisted of genomic comparisons of a carriage CC10217 isolate from Burkina Faso and the Nigeria 2013 outbreak strain; their study revealed the acquisition of virulence factors consisting of the MDA prophage and capsule genes for serogroup C. In our study, we only identified the MDA prophage in three of the four ST-10217 carriage isolates, all of which were collected during round two, as well as the serogroup C capsule in nine of the eleven total CC10217 carriage isolates. The remaining two isolates were non-groupable due to disruptions in the capsule locus, but still contained capsule genes. The emergence of this CC as a major cause of meningococcal outbreaks in the region, along with our results of 3 unique STs being identified among 11 isolates, suggest that this pathogen is rapidly evolving, highlighting the importance of continued molecular surveillance in the region.

A limitation of our study was the inability to examine carriage dynamics longitudinally at the individual level or determine repeat carriers, as this was a cross-sectional study and compounds were randomly selected each round. Regardless, the consideration of the participant's compound enabled us to analyse the strains being carried among people that spend a large portion of their day in relatively close proximity with each other. Our results show that among the compounds that had more than a single meningococcal isolate collected throughout the course of the study, a third of these compounds were carrying isolates belonging to multiple CCs, over half of which were carrying multiple CCs during a single carriage round. A recent publication by the MenAfriCar Consortium [50] tracked household transmission of meningococcal strains in the meningitis belt through cross-sectional carriage surveys. They identified 75 households with an initial carrier that later had carriage acquisitions by other household members. Their findings showed that the initial carriage strain was acquired by another household member in 69% (52/75) of households, and 52% (39/75) of households had a member acquire a different strain. While this study consisted of a

different design than ours, our results were concordant in identifying multiple strains being carried in a portion of our compounds.

Our genomic analysis revealed that same-CC isolates that were carried within the same compound tended to be more genetically closely related when compared against same-CC isolates from participants not sharing a compound; this finding did have some exceptions, notably for CC11, where six compounds had CC11 isolates that were delineated phylogenetically rather than clustering together, and these were largely collected during the same carriage round.

Following the mass-vaccination campaign of the meningococcal serogroup A conjugate vaccine in 2010, Burkina Faso saw a drastic reduction in disease caused by serogroup A meningococci. In the following years, other strains of *N. meningitidis* have continued to cause disease in the country and across the meningitis belt. The results of our carriage study provide important molecular insights into the strains being carried in the Kaya and Ouahigouya districts of Burkina Faso. Our work shows the importance of carriage studies to track the outbreak-associated strains circulating within the population, and to inform future vaccination strategies and molecular surveillance programmes.

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Author contributions
N. T., performed the formal analysis of the genomic data. N. T., P. A. K., S. S., M. C.-O., D. K., S. M., M. P., M. S., were all involved in the data investigation and obtaining resources, such as laboratory samples, which were used in the study. N. T., P. A. K., S. S., S. M., M. P. and X. W., were involved in writing of the original draft. P. A. K., L. S., R. O. and X. W., supervised the study and testing of the isolates. X. W., supervised the writing of the manuscript. All authors critically reviewed the manuscript, provided feedback and gave approval for its publication.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This study was approved by the Ethical Committee for Health Research in Burkina Faso and was determined by CDC human subjects review to be public health non-research; as such, review by the CDC Institutional Review Board was not required. Participation was voluntary and explained to all participants or their guardians in the local language by trained staff facilitating the study. Written informed consent was obtained from all participants, and in the case of participants younger than age 18 consent was obtained from the parent or guardian either through signature or thumbprint.

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