Evolution of Sequence Type 4821 Clonal Complex Hyperinvasive and Quinolone-Resistant Meningococci

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Expansion of quinolone-resistant Neisseria meningitidis clone ChinaCC4821-R1-C/B from sequence type (ST) 4821 clonal complex (CC4821) caused a serogroup shift from serogroup A to serogroup C invasive meningococcal disease (IMD) in China. To determine the relationship among globally distributed CC4821 meningococci, we analyzed whole-genome sequence data from 173 CC4821 meningococci isolated from 4 continents during 1972–2019. These meningococci clustered into 4 sublineages (1–4); sublineage 1 primarily comprised of IMD isolates (41/50, 82%). Most isolates from outside China (40/49, 81.6%) formed a distinct sublineage, the Europe–USA cluster, with the typical strain designation B:P1.17-6,23:F3-36:ST3200(CC4821), harboring mutations in penicillin-binding protein 2. These data show that the quinolone-resistant clone ChinaCC4821-R1-C/B has expanded to other countries. The increasing distribution worldwide of serogroup B CC4821 raises the concern that CC4821 has the potential to cause a pandemic that would be challenging to control, despite indirect evidence that the Trumenba vaccine might afford some protection.

Neisseria meningitidis, a leading cause of bacterial meningitis and sepsis globally, causes ≈1.2 million invasive meningococcal disease (IMD) cases annually and a case-fatality rate of 11% (1). Meningococci are classified into 12 serogroups based on capsular polysaccharides (1); genetic relationships among isolates are defined by clonal complexes (CCs) identified by multilocus sequence typing (MLST), which are surrogates for lineages (2). The relationship among serogroups, CCs (lineages), and IMD fluctuates over time and by location, but IMD isolates are dominated by CCs known as hyperinvasive lineages, usually associated with one of the 6 disease-causing serogroups (MenA, MenB, MenC, MenW, MenX, and MenY).

In China, the national dissemination of hyperinvasive sequence type (ST) 4821 clonal complex (CC4821) meningococci led to a shift in IMD epidemiology from mostly MenA to predominantly MenC (3,4). Although no quinolone resistance was identified in CC4821 in China during 1965–1985, high-frequency resistance (79%) occurred from 2005 onward due to expansion of the quinolone-resistant clone ChinaCC4821-R1-C/B (5). Previous studies discovered that CC4821 can be divided into 2 groups, with group 1 associated with IMD (6,7). Peng et al. identified 6 strain-specific genome regions resulting from horizontal gene transfer (HGT) in isolate 053442 (8); this finding was consistent with the emergence of the ChinaCC4821-R1-C/B clone associated with multiple HGT events within genes encoding surface antigens (6), although the donors of these events were not identified.

Globally, the number of CC4821 IMD isolates has increased. At the time CC4821 was identified, isolates were confined to China (4,9); however, by June 2020, a total of 59 CC4821 isolates had been identified in 19 countries worldwide (Figure 1). Moreover, 3 IMD cases caused by quinolone-resistant CC4821 isolates were reported in Canada (n = 2) and Japan (n = 1) after 2013 (10,11); 3 other CC4821 isolates were found to colonize the anorectal tract of men who have sex with men (MSM) (12). We investigated the genomic events leading to the emergence and expansion of hyperinvasive CC4821 meningococci by describing the phylogenetic relationships among meningococci with different serogroups (MenC, MenB, MenW, and nongroupable), sources (IMD, carriage, and MSM), locations (China or other countries),

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and dates of isolation (1972–1978 vs. 2004–2019). We assessed genes encoding key antigens and antimicrobial resistance phenotypes, identified putative donors of HGT events unique to the epidemic and quinolone-resistant clone China CC4821-R1-C/B, and characterized isolates outside of China.

**Materials and Methods**

**Isolate Collection and Whole-Genome Sequencing**
A total of 173 CC4821 genomes were collected dating from 1972–1978 (n = 19) and 2004–2019 (n = 154), including isolates from IMD (66/173, 38.2%), genitourinary sites (6/173, 3.5%), asymptomatic carriage (86/173, 49.7%), and unknown sources (15/173, 8.7%) (Appendix 1 Table 1, https://wwwnc.cdc.gov/EID/article/27/4/20-3612-App1.xlsx). Shanghai CDC sequenced 76 CC4821 isolates with Illumina HiSeq (Illumina, https://www.illumina.com) using paired-end 150 base reads as previously described (13). An additional 97 publicly available CC4821 genomes consisted of 48 genomes from 14 provinces of China, including the reference strain 053442 (6–8) and 49 genomes from countries outside of China, including the United Kingdom (n = 20), United States (n = 8), and 11 other countries (n = 21) (Figure 1; Appendix 1 Table 1) (10,12,14–17). The completeness and contamination of the genomes were evaluated using CheckM (18).

**Antigenic and Antimicrobial Resistance Characteristics of CC4821 Genomes**
To describe the antigenic and antimicrobial resistance characteristics of CC4821 genomes, we extracted from genomes nucleotides of 9 antigen coding genes (porA, fHbp, nhba, porB, fetA, opcA, nspA, tbpA, and NMB0315) (19–22) and 5 resistance-associated genes (gyrA, parC, penA, ponA, and rpoB) (23,24) for analysis. We annotated and analyzed deduced
encoding factor H–binding protein (fHbp), Neisseria heparin-binding antigen (NHBA), Neisseria adhesion antigen (NadA), and outer membrane protein (PorA) peptides and deduced meningococcal vaccine antigen reactivity (MenDeVAR) index from the PubMLST Neisseria database (25).

Identifying CC4821 (L44) Sublineages
In the Neisseria PubMLST database, a lineage-specific core genome MLST typing scheme containing loci found in 95% of CC4821 isolates was established and designated L44 cgMLST consistent with the previously described CC4821 lineage 44 (26). We compared the 173 CC4821 genomes using Genome Comparator (27) and the L44 cgMLST scheme, identifying distinct sublineages. To characterize each sublineage, we visualized a FASTA output from the Genome Comparator Tool using all 2,860 defined loci (NEIS0001–NEIS3173, not contiguous) using MEGA version 5 (28). We used Z2491 (GenBank accession no. NC_003116) as outgroup in accordance with previous studies (6,8). Assembled contigs and annotation information of 173 genomes in this study can be accessed at https://pubMLST.org/neisseria (Appendix 1 Table 1).

Identifying and Characterizing Unique Alleles in Sublineages
We determined shared and unique alleles using outputs from Genome Comparator. An allele was defined as unique to a sublineage if it was present in >90% of the genomes in that sublineage but absent in other sublineages. Genes with unique alleles were functionally characterized according to the Kyoto Encyclopedia of Genes and Genomes Orthology groupings of its database (29).

Identifying HGT Events and Putative Donors
Inputting the aligned sequences generated from Parsnp (30), we predicted putative HGT events using Gubbins (31). To search for potential donors, we blasted alleles and sequences of contiguous loci that were predicted to originate from HGT against the PubMLST database. We identified potential donors as previously described (32). We labeled recombination areas with unique loci on the circular genome map of genome 053442 by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) comparisons to strains of other sublineages, as generated using BRIG (33).

Screening Molecular Markers of MSM Infection Strains from Europe
In addition to the lineage of 11.2 possessing PorA P1.5–1,10–8, 3 other molecular features have been identified in meningococci causing infections among MSM in Europe during 2012–2014; these features were functional nitrite reductase (AniA); frameshifted fHbp allele found mostly in urethritis and proctitis isolates; and penA327 that had reduced susceptibility to penicillin and third-generation cephalosporins (34). These 3 molecular markers were screened among all the 173 CC4821 genomes.

Results
Isolate Characterization
The 173 CC4821 isolates represented 46 different STs; ST4821 (n = 41, 23.7%) and ST3200 (n = 30, 17.3%) were the most prevalent. We identified 43 PorA subtypes, of which P1.7-2,14 (n = 25, 14.5%) and P1.17-6,23 (n = 18, 10.4%) were the most frequent. We identified 27 FetA variants; F3-3 (n = 47, 27.2%) and F3-36 (n = 37, 21.4%) were the most prevalent (Appendix 1 Table 1).

Identifying 4 Sublineages
We identified 2,161 loci in reference genome 053442, including 1,699 core genes. Most (1,527/1,699, 89.9%) of the core loci had p-distance values of 0–0.1; 0.8% (14/1,699) showed high p-distance values of 0.50–0.68. On the basis of the L44 cgMLST scheme, we divided the CC4821 isolates into 4 sublineages (Figure 2): L44.1, identical to the China CC4821-R1-C/B clone (n = 50, 28.9%), composed of isolates from China (n = 44) and other countries (n = 6) during 2004–2019 that were very closely related (Figure 3); L44.2 (n = 29, 16.8%), composed of isolates from China (n = 28) and the United Kingdom (n = 1) during 2005–2019; L44.3 (n = 58, 33.5%), composed of isolates from China (n = 18) and countries outside China (n = 40) during 1977–2019; and L44.4 (n = 32, 18.5%), composed of isolates from China (n = 30) and India (n = 2) during 1972–2017. Four additional isolates from China were not assigned to any sublineages.

Features of the 4 Sublineages
The percentage of IMD isolates was significantly higher in L44.1 (41/50, 82%) than the other 3 sublineages (17.2%–22.4%; p<0.001) (Figure 2). L44.1, containing the reference strain 053442, was mainly composed of MenC isolates (44/50, 88%) and had ST4821 as its central ST. L44.2, was mainly composed of MenB isolates (27/29, 93.1%) and its central ST was ST5664. L44.3 was mainly composed of MenB isolates (27/29, 93.1%) and its central ST was ST5664. L44.4 was mainly composed of MenC (14/32, 43.8%) and MenW (11/32, 34.4%) with its central ST3436.

Analysis of the 5 antimicrobial resistance genes revealed that both gyrA-71 (with T91I) and parC-12 were specific to L44.1; parC-275 and penA-9 (with 5 mutations) were both specific to L44.3, and gyrA-294 (with T91I) was discovered only in L44.4 (Table 1; Appendix 2 Figures 2–6). In L44.1, all of the isolates possessed the quinolone resistance–associated mutation T91I in GyrA (Figure 4). In L44.3, 40/58 (69.0%) harbored PBP2 mutations, almost always from countries outside of China (38/40, 95%) (Figures 5, 6).

Vaccine Antigens among the 4 Sublineages

Analysis of 9 antigenic genes identified several alleles unique to a certain sublineage (Table 2; Appendix 2 Figures 7–17). For example, FetA-VR F3-3 was found in L44.1, F1-91 in L44.2, F3-36 and F3-9 in L44.3, and F1-7 in L44.4 isolates (Appendix 2 Figure 11). In L44.1, most isolates had the same antigenic gene profile (nhba-124, porB-29, fetA-64, opcA-4, nspA-4, ibpA-7, and NMB0315-21) (Figure 4), and 25/50 (50%) had the PorA subtype of P1.7-2,14 (Figure 3). In L44.3, most had the same gene profile (fHbp-16, nhba-553, porB-265, fetA-1069, opcA-100, nspA-26, and NMB0315-194), with porA and ibpA showing high genetic diversity (Figure 5).

We analyzed deduced peptide sequences for vaccine antigen constituents among MenB isolates (n = 97). We identified 16 fHbp peptides, of which peptide 16 (variant 2/subfamily A) was present in 70/97 (72.2%) isolates, including 31/70 isolates from China. There were 20 NHBA peptides, of which alleles 669 (46/95, 48.4%), 901 (11/95, 11.6%), and 668 (10/95, 10.5%) occurred most frequently. The nadA gene was absent in all isolates (including other serogroups). Of 31 PorA VR1/VR2 combinations, the most frequently occurring was P1.20,23 (11/97, 11.3%).

MenDeVAR Index values were assigned for MenB disease isolates (n = 29, including the 6 isolates from genitourinary sites), but 27/29 (93.1%) isolates had insufficient data from experimental studies to estimate the coverage of the MenB vaccine Bexsero (Appendix 2 Table 2). We predicted cross-reactivity to the MenB vaccine Trumenba for 18/29 (62.1%) isolates. For the MenB disease isolates from China, 7/17 (41.2%) were deemed cross-reactive with Trumenba;
however, we had insufficient data for the remaining 10/17 (58.8%) to determine reactivity.

**Molecular Markers of Strains from Europe Infecting MSM**

None of the CC4821 isolates harbored frameshifted \( fHbp \) allele or \( penA_{327} \), but the distribution of putatively functional \( AniA \) proteins was diverse. The \( aniA \) gene was absent in all L44.1 isolates (Figure 3) but was present in all of the other 123 CC4821 isolates, of which 96.7% (119/123) isolates harbored putatively functional \( AniA \) proteins (Figure 5; Appendix 2 Figures 16–17).

**Evolution of Sublineage L44.1 (China CC4821-R1-C/B Clone)**

Five specific loci were present in >90% of L44.1 but in <10% of other sublineages. These loci were involved in signaling and cellular processes (n = 2), metabolism (n = 1), and genetic information processing (n = 1) (Table 3). No loci were specific to any of other 3 sublineages.

Prediction of HGT events contributing to the emergence of L44.1 using Gubbins discovered 126 events involving 686 loci shared by the 50 L44.1 isolates (Appendix 2 Figure 18). These events included 216 loci with alleles specific to L44.1. We discovered an additional 83 unique loci based on analysis of the accessory genome. Therefore, a total of 299 unique loci were identified in L44.1; of those, 139 (46.5%) were involved in metabolic function (Appendix 1 Table 3). These 299 unique loci were distributed across the chromosome; we observed 44 areas (216 loci) harboring contiguous loci with unique alleles (Figure 7), among which the exact donors of 36 areas across 149

![Figure 3. Phylogenetic tree and data of clonal complex 4821 *Neisseria meningitidis* sublineage L44.1 (China CC4821-R1-C/B) isolates. Red text indicates the oldest isolate of the sublineage; blue text, the isolates from countries outside of China; and green text, the dominant type or allele. Scale bar indicates substitutions per site. IMD, invasive meningococcal disease; MSM, men who have sex with men; SG, serogroup; ST, sequence type; VR, variable region.](image-url)
loci were identified in 46 putative HGT events. The total length of these putative recombination fragments was ≈225 kb, including 87 kb (38.7%) originating from the C-ST-9514 cluster isolates in China during 1966–1977, followed by 25 kb (11.1%) from MenA isolates (CC5 and CC1) in China during 1966–1984 (Table 4, https://wwwnc.cdc.gov/EID/article/27/4/20-3612-T4.htm).

**Evolution of CC4821 Isolates from Outside China**

We identified 49 CC4821 isolates from countries outside of China, and most (40/49, 81.6%) were assigned to L44.3, of which there were 39 MenB and 1 MenC, constituting the distinct Europe–USA cluster (Figures 2, 5). The representative molecular characteristics of the Europe–USA cluster was B:P1.17-6,23: F3-36:ST-3200(CC4821); its antigen gene profile was porA-423, fHbp-16, nhba-553, porB-265, fetA-1069, opca-100, nspa-26, tbpA-1333, and NMB0315-194 and antimicrobial resistance profile gyrA-12, parC-275, penA-9 with PBP2 mutations, ponA-7, and rpoB-85 (Figure 6). In Gubbins analysis, 33 events involving 193 loci were shared by all the Europe–USA cluster isolates (Appendix 2 Figure 18); we discovered 60 unique loci for which we could not identify their potential donors. These unique loci were involved in functions mainly associated with metabolism (23/60, 38.3%) and genetic information processing (18/60, 30%) (Appendix 1 Table 4).

In addition to the 40 Europe–USA cluster isolates, there were 6 MenC invasive isolates from India (n = 4, identified 2014–2016), Japan (n = 1, identified in 2017),

![Figure 4](image_url)

**Table 1. Specific alleles of antimicrobial resistance genes in 4 sublineages of clonal complex 4821 of *Neisseria meningitidis***

<table>
<thead>
<tr>
<th>Sublineage</th>
<th>Resistant allele no. (no. isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L44.1, n = 50</td>
<td>gyrA 71 (50) parC 12 (43) penA None ponA None rpoB None</td>
</tr>
<tr>
<td>L44.2, n = 29</td>
<td>None None None None None None</td>
</tr>
<tr>
<td>L44.3, n = 58</td>
<td>None 275 (41) 9 (35) None None None</td>
</tr>
<tr>
<td>L44.4, n = 32</td>
<td>294 (11) None None None None None</td>
</tr>
</tbody>
</table>

![Figure 4](image_url)

**Figure 4. Genomic diversity of clonal complex 4821 *Neisseria meningitidis* sublineage L44.1 (ChinaCC4821-R1.C8) isolates. The numbers underneath the antigen genes and AMR genes are the dominant alleles for that particular gene, and the colored blocks for SNPs/1,000 bp were determined using the allele number labeled above each column as the reference allele. AMR, antimicrobial resistance; SNP, single-nucleotide polymorphism.
and New Zealand (n = 1, identified in 2018). These 6 isolates were clustered together and were closely related with 44 isolates from China within sublineage L44.3 (Figures 2–3). Only the isolate from Japan showed the typical molecular feature of Anhui outbreak strain (C:P1.7-2,14:F3-3:ST-4821[CC4821]).

Features and Evolution of Serogroup W CC4821 Isolates

A total of 11 MenW isolates from China were identified; the representative strain designation was W:P1.5-3,10-2:F1-7-ST-8491(CC4821), with similar gene profiles of antigen-encoding loci (porA-1804, fHbp-474, nbha-966, fetA-37, opcA-4, nspA-117, and NMB0315-21) and antimicrobial resistance loci (gyrA-294 with T91I, parC-779, porA-7, and rpoB-85). These MenW isolates constituted a distinct cluster in L44.4; they were more closely related to NM193 (C:P1.20-3,23-1:F1-5:ST-3436[CC4821], dating from 1972) than to NM205 (C:P1.20,23-2:F5-135:ST-4821[CC4821], dating from 1973) (Appendix 2 Figure 17).

Discussion

The meningococci can cause IMD, leading to endemic disease in most if not all human populations. Several
Evolution of ST4821 Meningococci

genotypes belonging to hyperinvasive lineages, in combination with the disease-associated capsular serogroups, can cause elevated levels of disease; some of which also possess epidemic and pandemic potential. In the past 100 years, notable epidemics and pandemics have included meningococci such as A:CC1, A:CC5, B:CC41/44, C:CC11, and W:CC11 (35). Here, we employed a genomic analysis of MenB, MenC, and MenW CC4821 isolates dating from 1972–2019 to assess their epidemic and pandemic potential. Of special concern are the expansion of the quinolone-resistant clone China\textsuperscript{CC4821-R1-C/B} from China to other countries; the potential possession of universal resistance to penicillin in Europe–USA cluster isolates; and the uncertainty over the potential efficacy of existing vaccines to prevent B:CC4821 diseases.

CC4821, which corresponds to lineage 44, shares several properties in common with the hyperinvasive CC11 meningococci (lineage 11): its ability to express several serogroups, global distribution, colonization of urogenital and anorectal tracts, and separation into distinct sublineages. CC11 has caused well-documented epidemics and pandemics on several occasions, including US military outbreaks in the 1960s; Hajj-associated outbreaks in 2000s; and the global epidemics from 2010, especially outbreaks among MSM (34–38). These similar characteristics raise the concern that the CC4821 may have the potential to cause similar global pandemics.

Consistent with the presence of the epidemic CC4821 clone in countries outside of China, 6 CC4821 IMD meningococci from India, Japan, and New Zealand, isolated during 2014–2018, clustered with China\textsuperscript{CC4821-R1-C/B} meningococci in L44.1 (Figure 3). IMD cases caused by these 6 isolates were all found in native inhabitants (10,15,39); all 6 isolates shared

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6}
\caption{Genomic diversity of clonal complex 4821 \textit{Neisseria meningitidis} sublineage L44.3 isolates. The numbers underneath the antigen genes and AMR genes are the dominant alleles for that particular gene, and the color blocks for SNPs/1,000 bp were determined using the allele number labeled above each column as the reference allele. The Europe–USA cluster can be further divided into subclusters: subcluster L44.3.1, composed of 3 ST6595 isolates from the United States, all of which contained putatively nonfunctional AniA; L44.3.2, composed of 7 ST3200 isolates from the United Kingdom (n = 6) and Brazil (n = 1); and L44.3.3, composed of 30 isolates with multiple geographic locations. All the isolates from urethral (n = 2) and rectal (n = 4) swabs were assigned to L44.3.2 and L44.3.3, both of which comprised isolates with putatively functional AniA. Scale bar indicates substitutions per site. AMR, antimicrobial resistance; SNP, single-nucleotide polymorphism.}
\end{figure}
of the China CC4821-R1-C/B clone isolates from China had to cause IMD worldwide (40). New Zealand isolates, which had different STs, had the typical molecular features of the Anhui outbreak strain (C:PI.7-2,14:F3-3-ST-4821[CC4821]) (4), became the earliest-reported quinolone-resistant meningococcus harboring ParC mutation (S87I, allele L44.3 and constituted a distinct cluster, the Europe–USA cluster, showing the typical strain designation: B:P1.17-6,23-x:F3-36-ST-3200(CC4821), wherein 23-x refers to 23, 23-2, and 23-6. The PorA and FetA types P1.17-6,23-x and F3-36 were only found in this cluster. The Neisseria PubMLST database had no genome data for 24 CC4821 isolates from other countries (United States, Brazil, France, Czech Republic, Spain, Italy, Australia, and Vietnam), but included PorA or FetA variants for the 24 isolates (Appendix 1 Table 5). Of these, 19 (79.2%) exhibited P1.17-6,23-x or F3-36, suggesting they might belong to the Europe–USA cluster. This cluster was distinct from the epidemic clone ChinaCC4821-R1-C/B, and the antigen profile character of the Europe–USA cluster was P1.17-6,23-x, F3-36, PorB-3-229, fHbp -16, nhba-553, opcA-100, nspA-26, and tbpa-1333, compared with P1.7-2,14, F3-3, PorB-3-48, fHbp-498, 22 and 489, nhba-12A, opcA-4, nspA-4, and tbpaA-7 in the ChinaCC4821-R1-C/B clone. In addition, all the ChinaCC4821-R1-C/B isolates harbored the mutation T91I in GyrA, whereas almost all of the Europe–USA cluster isolates possessed mutations in PB2 (E504L, A510V, I515V, H541N, and I566V). This may reflect different antibiotic selective pressures experienced by the Europe–USA and the ChinaCC4821-R1-C/B meningococci. Penicillins were the most-used antimicrobial drugs in outpatients in Europe, whereas China has the second largest global increases of fluoroquinolone consumption (43,44). A high frequency (>70%) of quinolone resistance has been reported in China since 2005 (5), whereas 65% of meningococci

<table>
<thead>
<tr>
<th>Sublineage</th>
<th>PorA</th>
<th>fHbp</th>
<th>nhba</th>
<th>PorB</th>
<th>FetA-VR</th>
<th>opcA</th>
<th>nspA</th>
<th>tbpA</th>
<th>NMB-0315</th>
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</thead>
<tbody>
<tr>
<td>L44.1, n = 50</td>
<td>P1.7–2.14 (25)</td>
<td>22 (12)</td>
<td>124 (48)</td>
<td>3–48 (47)</td>
<td>F3–3 (45)</td>
<td>None</td>
<td>4 (49)</td>
<td>7 (36)</td>
<td>None</td>
</tr>
<tr>
<td>L44.2, n = 29</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>3–81 (15)</td>
<td>F1–91 (20)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>335 (26)</td>
</tr>
<tr>
<td>L44.3, n = 58</td>
<td>P1.17–6,23-x* (23)</td>
<td>None</td>
<td>None</td>
<td>3–229 (35)</td>
<td>F3–9 (8)</td>
<td>100 (40)</td>
<td>26 (39)</td>
<td>1,333 (31)</td>
<td>194 (49)</td>
</tr>
<tr>
<td>L44.4, n = 32</td>
<td>P1.5–3,10–2 (6)</td>
<td>None</td>
<td>None</td>
<td>3–460 (7)</td>
<td>F1–7 (11)</td>
<td>None</td>
<td>117 (20)</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*23-x refers to 23, 23-2, and 23-6.

Table 2. Specific alleles of antigenic genes in 4 sublineages of clonal complex 4821 of Neisseria meningitidis

Although we did not identify a putative ancestor of the quinolone-resistant clone ChinaCC4821-R1-C/B in this study, we found 299 loci with alleles unique to this sublineage. Approximately half of these loci were associated with metabolic pathways, suggesting that divergence in metabolic genes may play a role in the emergence of epidemic meningococci. Several studies have indicated that metabolic genes can influence the pathogenesis and virulence of the meningococcus, for example by allowing alternative host resources to be exploited in invasive disseminated infections (40). Changes in the hyperinvasive A:CC5 meningococci circulating in Africa have been associated with HGT of core genes involved in metabolic processes (41). The putative donors of these unique alleles included lineages from different serogroups and dates of isolation, such as C:ST-9514 cluster, 1960s–1970s; A:CC5 and A:CC1, 1960s–1980s; B:CC32, 1960s; B:CC41/44, 1970s; and E:CC178, 1980s (Table 4). The C:ST-9514 cluster, STs that do not presently form part of a clonal complex documented in PubMLST, has ST9514 as the central ST and was predominant in MenC carriage isolates during 1965–1980 in Shanghai, China (42). Therefore, the emergence of ChinaCC4821-R1-C/B clone was perhaps associated with accumulation of these unique alleles, which accounted for the separation from other sublineages in the allele-based phylogeny (Figure 2).
Evolution of ST4821 Meningococci in Europe showed reduced susceptibility to penicillin G during 1945–2006 (45). In the 2 oldest isolates of the sublineage L44.3, Nm282 (B:P1.20,23:F3-36:ST-3200[CC4812]) was much closer to the Europe–USA cluster isolates than Nm323 (B:P1.20,23:F3-36:ST-5798[CC4821]) (Figure 5), and it seemed more likely to be the ancestor of the Europe–USA cluster isolates.

Urogenital and rectal meningococci have raised increasing public health concerns (34). In 2017, CC4821 anorectal isolates were identified in the United Kingdom (12). In this study, we identified CC4821 isolates from urethral and rectal tracts that clustered with isolates from IMD specimens and oropharyngeal carriage (Figure 5). With the exception of L44.1 isolates, most of the CC4821 isolates contained a putatively functional nitrite reductase (AniA), required for growth in anaerobic environments. The CC4821 isolates acquired quinolone resistance alleles from N. lactamica and N. subflava (46); the ability to grow in anaerobic environments will facilitate acquisition of gonococcal alleles, including antimicrobial resistance alleles. Such events seem to have already occurred in a sublineage of CC11, which was responsible for several IMD outbreaks and urethritis among MSM (34). They shared the same penA allele (penA327/penA XXX-IV) with gonococcal bacteria and showed decreased susceptibility to third-generation cephalosporins (47).

Although PubMLST is the largest global repository of meningococcal genomes (>22,000), a paucity of genomic data were available from isolates originating from the genitourinary or respiratory tract, suggesting an underestimation of the global dissemination of CC4821. Therefore, we recommend WGS for urogenital-, rectal-, and respiratory-derived meningococci if they are exhibiting antimicrobial resistance.

CC4821 lineage 44 includes isolates from different serogroups, including MenB, MenC, and MenW. In China, MenC and MenW isolates can be prevented...
by vaccines, such as group A and C meningococcal polysaccharide vaccine (MPV-AC) and MPV-ACYW, but no routinely administered vaccine is available to prevent MenB IMD (48). Two protein-based vaccines, targeting MenB meningococci 4CMenB (Bexsero) and rLP2086 (Trumenba), have been licensed in several countries (49-50; reference 51 in Appendix 2), but limited data are available on the bacterial coverage of these vaccines to CC4821 isolates directly from serum bactericidal activity assays, the Meningococcal Antigen Typing System (MATS) for Bexsero, or meningococcal antigen surface expression for Trumenba. One B:CC4821 isolate (M14-240580, UK) was reported to be tested using the MATS assay and showed no potential protection (reference 52 in Appendix 2). Using systems to index complex genotypic and phenotypic data, such as the MenDeVAR Index, we predicted that ≥60% of B:CC4821 disease-causing isolates might be prevented through vaccination with Trumenba; data are insufficient to infer Bexsero reactivity. Further testing of globally diverse meningococci is needed with these experimental assays to analyze potential vaccine impact in settings outside Europe.

In summary, we have undertaken a comprehensive genomic analysis of a hyperinvasive meningococcal CC4821 expressing MenB, MenC, and MenW with expansion from China to other global geographic locations with currently available genomic data. We identified key genomic factors and putative evolutionary changes that might have led to the emergence and persistence of the epidemic quinolone-resistant clone in China. Vaccine coverage for MenB CC4821 isolates needs further evaluation. Enhanced laboratory surveillance for CC4821 isolates from IMD cases and from oropharyngeal, urethral, and rectal carriage is needed to monitor global trends of expansion, which will be essential for local immunization policies.

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References


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