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Geographically widespread invasive meningococcal disease caused by a ciprofloxacin resistant non-groupable strain of the ST-175 clonal complex

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SUMMARY

Introduction: Invasive meningococcal disease (IMD) caused by non-serogroupable (NG) strains mainly affects immunocompromised individuals. Reduced susceptibility to penicillin in meningococci is increasing in Europe but ciprofloxacin resistance remains rare. In 2019, three travel-related meningococcal disease cases caused by a ciprofloxacin-resistant NG strain were identified in England, leading Germany to report four additional IMD cases (2016 to 2019). We describe these and newly identified cases and characterise the strain responsible.

Methods: Cases were identified as part of national surveillance and by analysing available genomes using PubMLST tools.

Results: Of the cases identified in England in 2019, two geographically distinct cases developed conjunctivitis after returning from Mecca (Kingdom of Saudi Arabia) and a third linked case presented with IMD. Of the four cases from Germany, three occurred in asylum seekers – two familial and a further geographically distinct case. Further IMD cases were identified in Italy ($n = 2$; 2017–2018), Sweden ($n = 1$; 2016) and England ($n = 1$; 2015). A single ST-175 clonal complex (cc175) strain with genosubtype P1.22–11,15–25 was responsible. Decreased susceptibility to penicillin was widespread with three ciprofloxacin resistant subclusters. Constituent isolates were potentially covered by subcapsular vaccines.

Conclusion: This disease associated NG cc175 strain exhibits resistance to antibiotics commonly used to prevent IMD but is potentially covered by subcapsular (meningococcal B) vaccines.

Introduction

Neisseria meningitidis is an obligate commensal of the human nasopharynx and is carried asymptomatically in ~10% of the general population.[1] Occasionally, invasive strains may penetrate the mucous membrane and enter the bloodstream to cause life-threatening diseases such as meningitis and/or septicaemia.[2] In addition to invasive meningococcal disease (IMD), which is associated with a case fatality rate of 5–10%,[3,4] *N. meningitidis* is also an uncommon cause of bacterial conjunctivitis.[5,6]

The meningococcal polysaccharide capsule is essential for survival in the blood of healthy individuals and allows evasion of complement-mediated killing and phagocytosis by the host's immune system.[7] Meningococcal strains are classified into serogroups based on the composition of their polysaccharide capsule, with most invasive disease-causing strains belonging to serogroups A,

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B, C, W, X and Y. Unencapsulated or non-serogroupable (NG) meningococci are mainly associated with a carrier state and very rarely cause invasive disease in healthy individuals.[8,9] Most IMD due to NG meningococci has been reported among individuals with hereditary deficiencies of the terminal complement pathway, who have a 1000 to 10,000-fold higher risk of IMD than the general population.[10] Additionally, treatment of immune-mediated conditions such as atypical hemolytic-uremic syndrome (aHUS) and paroxysmal nocturnal haemoglobinuria (PHN) with terminal complement inhibitors including eculizumab can lead to acquired complement deficiencies, rendering individuals much more susceptible to IMD. Such individuals may receive meningococcal vaccination and long-term penicillin prophylaxis.

In the United Kingdom, third generation cephalosporins are recommended for treating suspected IMD cases, while ciprofloxacin is the first-line chemoprophylaxis for preventing secondary cases among IMD patients and their close contacts.[11] Due to the associated high immediate risk of IMD to cases and their close contacts, meningococcal conjunctivitis is also considered an indication for public health action, including systemic antibiotic treatment for cases and antibiotic chemoprophylaxis for their close contacts.[12]

Polysaccharide-conjugate vaccines are licensed for serogroups A, C, W, and Y and subcapsular protein-based vaccines are licensed for serogroup B disease (4CMenB and MenB-fHbp). The antigens of 4CMenB include *Neisseria* adhesin A (NadA) peptide 8 (variant NadA-2/3; cross-reactive with NadA-1 and NadA-2/3 peptides), factor H-binding protein (fHbp) peptide 1 (variant 1; cross-reactive with variant 1 peptides), neisserial heparin-binding antigen (NHBA) peptide 2 (NHBA does not form discrete variant groups), and outer membrane vesicles containing Porin A (PorA) subtype P1.4.[13] MenB-fHbp contains fHbp peptides 55 (variant 1; cross-reactive with variant 1 peptides) and 45 (variant 3; cross-reactive with variant 2 and 3 peptides).[14] These sub-capsular antigens elicit immune responses independently of the capsular polysaccharide and, therefore, may protect against unencapsulated meningococci.

In June 2019, three cases of meningococcal disease due to a NG ciprofloxacin-resistant strain (P1.22–11,15–25: ST-175 (cc175) associated with recent travel to Mecca, Kingdom of Saudi Arabia (KSA), were identified through national surveillance in England.[15] Following a public health announcement, the Public Health England Meningococcal Reference Unit was contacted by the Institute for Hygiene and Microbiology, University of Würzburg in Germany about four cases of invasive disease yielding similar isolates during 2016 to 2019, three of which were also ciprofloxacin-resistant.

This study aimed to describe these and newly identified cases identified through genomic analysis of available cc175 genomes. We also aimed to characterise the strain responsible in terms of geo-temporal distribution, antibiotic resistance determinants and potential vaccine coverage.

Materials and methods

Case identification

IMD cases were identified as part of national surveillance in the respective countries, where isolates are submitted to the national reference laboratory for confirmation and characterisation.[16,17]

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using antibiotic gradient strip diffusion methods (Etest, bioMérieux UK Limited, Basingstoke or MIC test strips, Liofilchem, Italy). The resulting minimum inhibitory concentration (MIC) values were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST; v9.0; 2019–01–01).

Identification of cc175 and potentially closely-related genomes

Genomes were obtained from the PubMLST *Neisseria* database (pubmlst.org/neisseria) and two currently unpublished UK carriage collections[18,19] In the first instance we aimed to capture all cc175 and potentially closely-related genomes, including those with incomplete Multilocus Sequence Type (MLST) profiles or clonal complex-unassigned sequence types containing at least three ST-175 MLST alleles. MLST data were exported from the PubMLST *Neisseria* database for all available genomes >2 Mb ($n = 19{,}802$, accessed 22 October 2019). These included $n = 62$ cc175 genomes. Genomes assigned to non-cc175 clonal complexes were removed from the dataset ($n = 14{,}181$). The remaining genomes comprised those with sequence types unassigned to a clonal complex ($n = 5240$) and those with incomplete profiles ($n = 381$). Among these, genomes which had three ST-175 alleles ($n = 11$) were retained and the rest were discarded. Genome sequence data of additional cc175 isolates from UK carriage studies were also included in the analysis ($n = 6$), resulting in a total of 79 genomes for phylogenetic analysis (Supplementary table).

Genotypic data

Genotypic data for antibiotic resistance determinants and MenB vaccine antigens were exported from the PubMLST *Neisseria* database using the 'export dataset' function.

Phylogenetic analyses

Core genome (1605 loci) comparisons (*N. meningitidis* cgMLST v1.0) were performed using the PubMLST genome comparator tool.[20] The resulting distance matrices were visualised as Neighbor-Net networks using SplitsTree4 (version 4.13.1).[21]

The sequences of all known *gyrA* alleles were downloaded from the PubMLST database ($n = 379$, accessed 13 May 2020). All *gyrA* alleles were imported into BioEdit,[22] where a ClustalW alignment was performed. A Neighbor-Joining tree was created using MEGA4.[23] Clades were annotated by species according to the distribution of the respective alleles on the PubMLST *Neisseria* database (accessed 13 May 2020).

Results

Phylogenetic analysis of cc175

Of the 79 genomes that underwent phylogenetic analyses (supplementary Table 1), 68 were assigned to cc175 while the remaining 11 were either unassigned to a cc (ST-4051 [$n = 6$], ST-6525 [$n = 2$], ST-5540 [$n = 1$]), or had incomplete MLST profiles ($n = 2$). On a phylogenetic network (Fig. 1), the ST-4051 and ST-5540 isolates and the two isolates with incomplete MLST profiles were distinct from the cc175 isolates and were disregarded for the remainder of the analyses. The remaining genomes ($n = 70$), including the cc-unassigned ST-6525 genomes, formed six distinct sublineages (arbitrarily named sublineage 1 to sublineage 6) (Fig. 1; supplementary Table 1) and included carriage and disease isolates belonging to multiple serogroups and collected in 15 countries on four continents (Africa, North America, South America and Europe) between 2000 and 2019 (Fig. 2, supplementary Table 1).

Distribution and characteristics of English, German and related case isolates: sublineage 1 – the NG cc175 sublineage

Isolates from the known English and German cases belonged to sublineage 1 (referred to here as the NG cc175 sublineage) which comprised $n = 31$ NG isolates from Europe and Africa

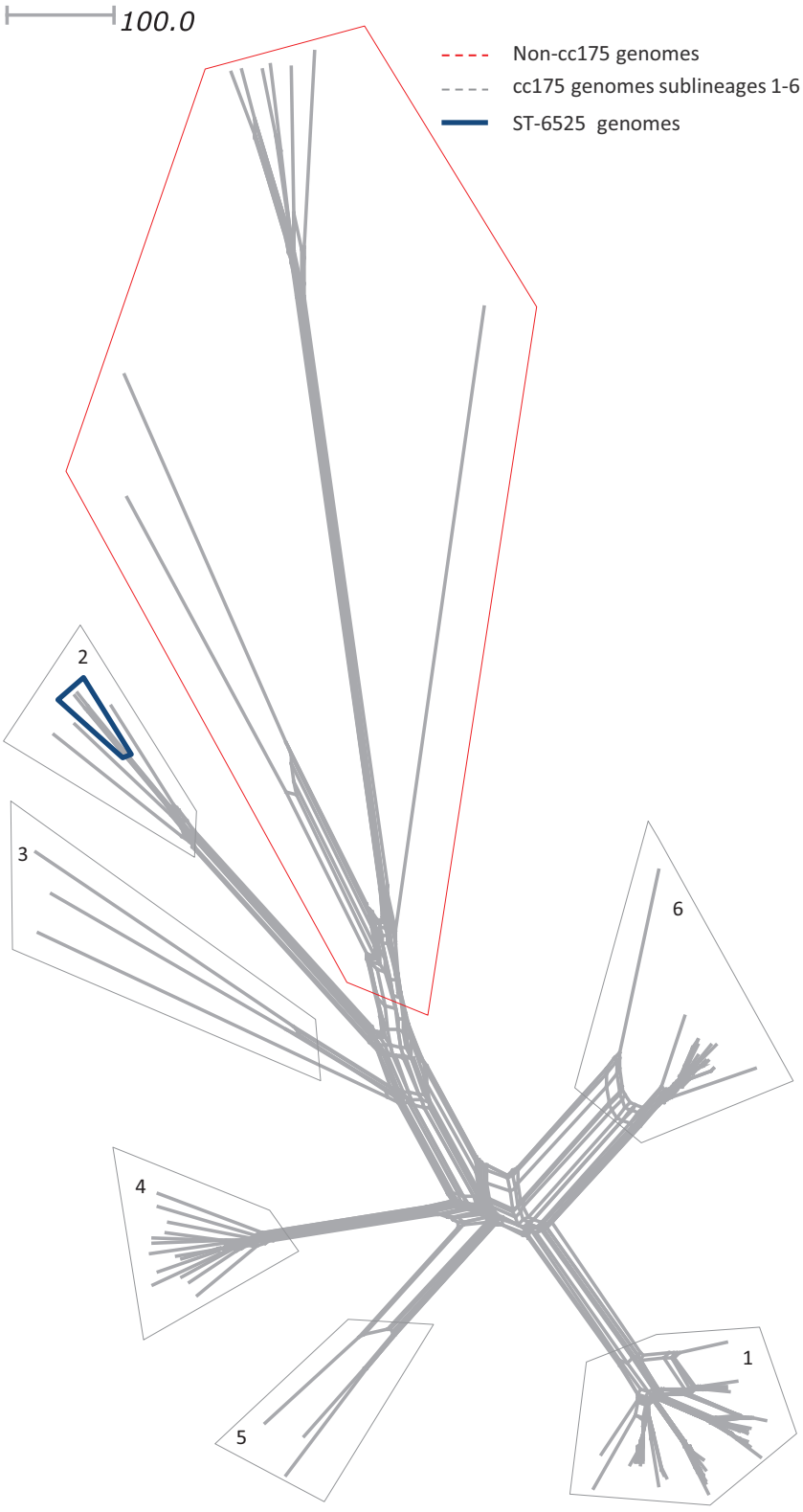

Fig. 1. Population structure of ST-175 complex and closely related genomes.
Neighbor-Net phylogenetic network based on a comparison of 1605 core genome loci among cc175 ($n = 68$), ST-6525 (cc-unassigned; $n = 2$) and closely related non-cc175 isolates ($n = 9$). Several sublineages were identified among cc175 genomes, labelled 1–6. The scale bar indicates the number of different loci among the 1605 that were compared.

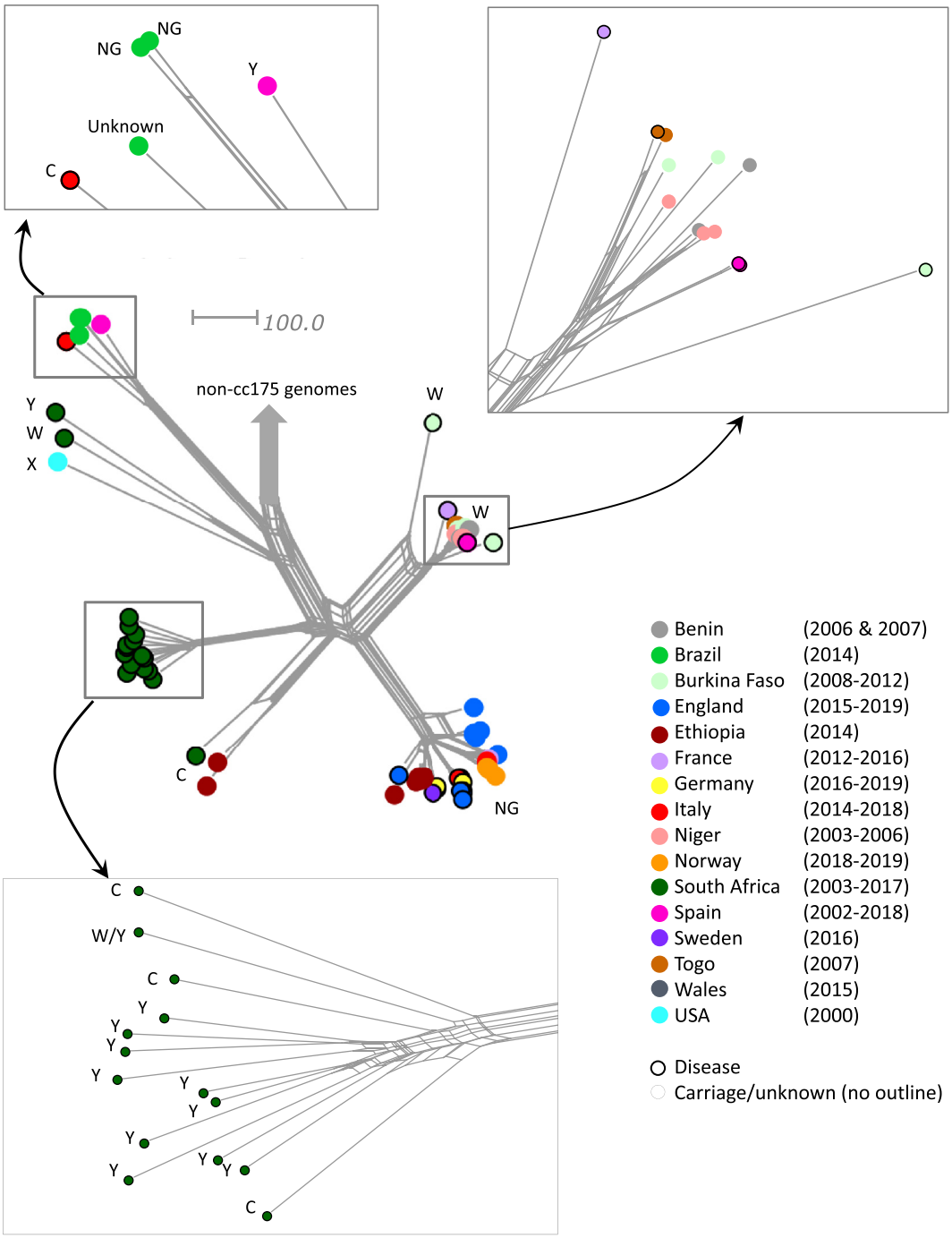

Fig. 2. Geo-temporal, serogroup and disease/carrier status distribution of ST-175 complex and ST-6525 isolates identified from 2000 to 2019.
Neighbor-Net phylogenetic network based on a comparison 1605 core genome loci among cc175 ($n = 68$), ST-6525 ($n = 2$) and distinct non-cc175 isolates ($n = 9$, not shown individually). The scale bar indicates the number of different loci among the 1605 that were compared.

(2014–2019) arranged in several clusters (clusters A to E and a singleton; Table 1; Fig. 3). These included four newly identified invasive isolates from England ($n = 1$; 2015), Italy ($n = 2$; 2017 and 2018) and Sweden ($n = 1$; 2016).

All of the disease isolates in the NG cc175 sublineage (invasive and conjunctivitis) possessed PorA genosubtype P1.22–11,15–25. Some carrier isolates differed in their PorA VR2 (P1.15–56 or P1.15–75). All of the NG cc175 sublineage isolates possessed *penA* alleles associated with reduced susceptibility to penicillin and, where known, this was reflected in their penicillin MICs (Table 1). Several clusters/subclusters possessed *gyrA* alleles with mutations associated with ciprofloxacin resistance and, where known, this was reflected in their MICs (Table 1). All Sublineage 1 isolates possessed allele 583 for DNA topoisomerase IV subunit A (*parC;* NEIS1525).

NG cc175 sublineage case descriptions

The English cases from 2019 (cluster A) included two geographically distinct cases presenting with conjunctivitis upon returning from Mecca (for Umrah) whilst the third case, who did not travel but belonged to the same mosque community as one of the other cases, went on to develop IMD. The latter case was

Table 1
Characteristics of cc175 sublineage 1 isolates: the non-groupable cc175 sublineage isolates and additional details surrounding those identified in immunocompromised patients.

Cluster	PubMLST ID	Year	Country	Disease	Immunocomprimised? (if invasive)	*gyrA* allele	Ciprofloxacin MIC (mg/L)	*penA allele*	Penicillin MIC (mg/L)	*PorA* VR2	fHbp variant	fHbp peptide	NHBA peptide
A	93631	2017	Germany	Invasive	NK	12	0.003	662	0.25	15–25	1	321	9
	84075	2017	Italy	Invasive	NK	12	0.004	662	0.125	15–25	1	321	9
	91539	2018	Italy	Invasive	NK	12	0.006	662	0.25	15–25	1	321	9
	89565	2019	England	Conjunctivitis	n/a	313[e]	0.12	662	0.25	15–25	3	111	9
	89712	2019	England	Conjunctivitis	n/a	313[e]	0.12	662	0.25	15–25	3	111	9
	89713	2019	England	Invasive	Complement deficiency	313[e]	0.12	662	0.25	15–25	3	111	9
	93679	2019	Germany	Invasive	Plasmacytoma	313[e]	0.064	662	0.19	15–25	3	111	9
B	93629	2016	Germany	Invasive	Terminal complement deficiency	187[f]	0.094	909	0.25	15–25	2	151	9
	93630	2016	Germany	Invasive	Terminal complement deficiency	187[f]	0.064	909	0.5	15–25	2	151	9
	42784	2016	Sweden	Invasive	Pregnant	187[f]	0.094	662	0.094	15–25	2	151	9
C	41896[g]	2014	Ethiopia	Carrier	n/a	12	0.004	662	0.25	15–56	2	151	1578[b]
	41897[g]	2014	Ethiopia	Carrier	n/a	12	0.004	662	0.25	15–56	2	151	1578[b]
	42666[h]	2014	Ethiopia	Carrier	n/a	12	0.004	662	0.25	15–56	2	151	9
	42668[h]	2014	Ethiopia	Carrier	n/a	12	0.002	662	0.25	15–56	2	151	9
	60134	2014	Ethiopia	Carrier	n/a	12	0.002	662	0.25	15–25	2	151	9
	60143	2014	Ethiopia	Carrier	n/a	12	0.004	662	0.25	15–56	2	151	9
	60308	2014	Ethiopia	Carrier	n/a	12	0.002	662	0.125	15–56	2	151	9
	61207	2014	Ethiopia	Carrier	n/a	12	NK	662	NK	15–56	2	151	9
D	49960	2015	England	Carrier	n/a	12	NK	662	NK	15–25	2	151	9
	50082	2015	England	Carrier	n/a	12	NK	662	NK	15–25	2	151	9
	52614	2015	England	Carrier	n/a	12	NK	662	NK	15–25	2	151	9
	52715	2015	England	Carrier	n/a	12	NK	662	NK	15–25	2	151	9
	88632	2018	England	Carrier	n/a	12	NK	662	NK	15–25	2	151	9
E	52572	2015	Wales	Carrier	n/a	152[c]	NK	662	NK	15–25	1	321	9
	41727	2016	France	Carrier	n/a	152[c]	0.125	662	NK	15–25	1	321	9
	47101	2016	Italy	Carrier	n/a	152[c]	NK	662	NK	15–25	1	321	9
	47115	2016	Italy	Carrier	n/a	152[c]	NK	662	NK	15–25	1	321	9
	84968	2018	Norway	Carrier	n/a	12	NK	662	NK	15–25	1	321	9
	85033	2018	Norway	Carrier	n/a	12	NK	662	NK	15–25	1	321	9
	92641	2019	Norway	Carrier	n/a	12	NK	662	NK	15–75	1	321	9
S[a]	41526	2015	England	Invasive	No[i]	12	0.004	662	0.19	15–25	2	151	9

All isolates in table 1 were ST-175 with *parC* allele 583 and *nadA* allele 311 (NadA peptide 0).
[a] Singleton.
[b] 1 bp different from *nhba* allele 7.
[c] Mutation D95N.
[e] Mutations A69S, T91I, N103D, I111V, V120I, D210E.
[f] Mutations T91I, N103D, I111V, V120I, V199I, D210E
[g/h] One of two isolates from a single person 8 weeks apart.
[i] the following were ruled out - inherited complement deficiencies, complement deficiencies due to glomerulonephritis, vasculitis or Eculizumab therapy, asplenia, splenic dysfunction and HIV. NK = not known.

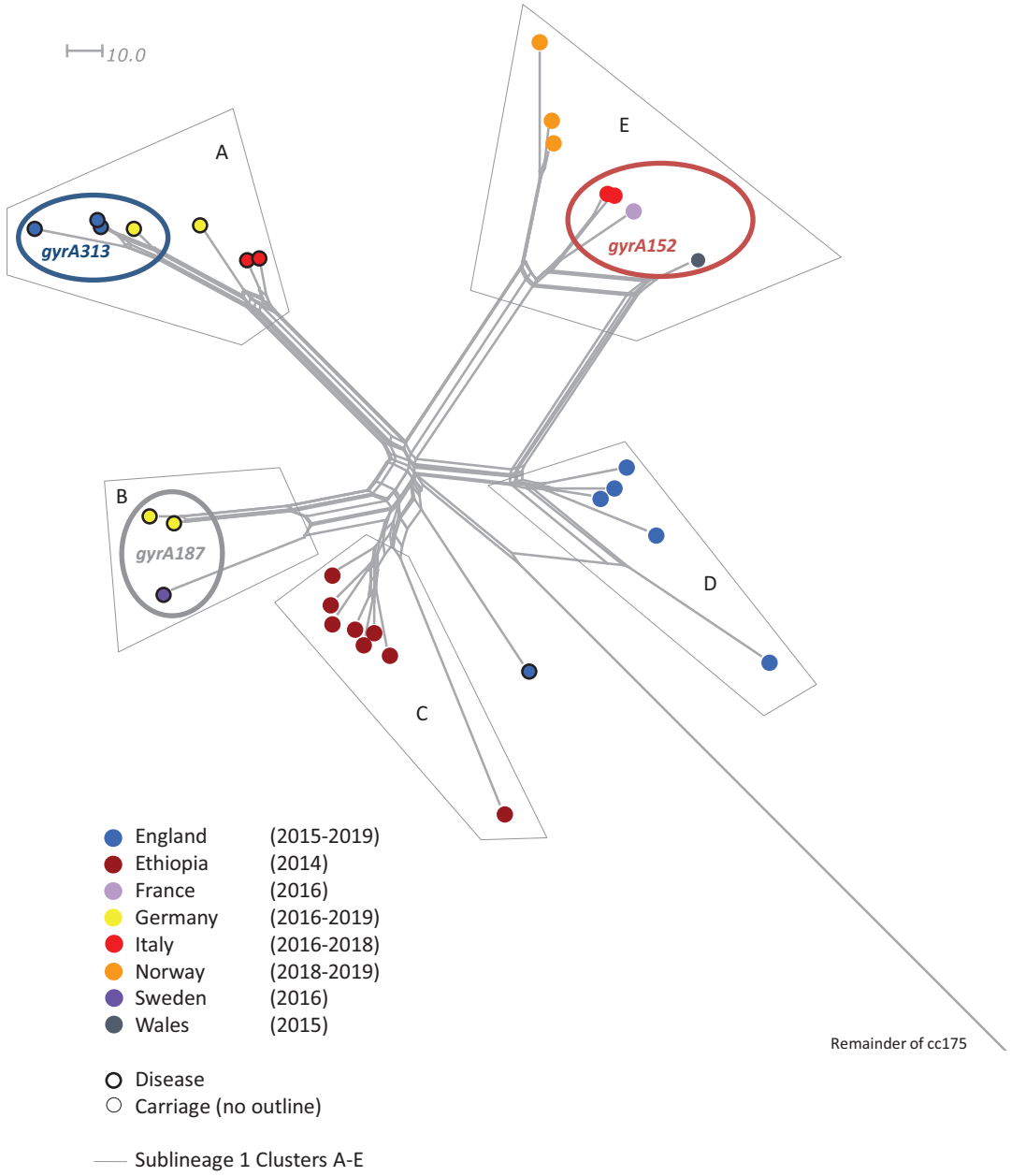

Fig. 3. Population structure and geo-temporal, disease/carrier status and ciprofloxacin resistance-associated *gyrA* allele distribution of isolates belonging to the non-groupable cc175 sublineage.
Neighbor-Net phylogenetic network based on a comparison 1605 core genome loci among cc175 sublineage 1 (the non-groupable cc175 sublineage), isolates ($n = 31$). The sublineage contained several clusters labelled A-E and a singleton. The scale bar indicates the number of different loci among the 1605 that were compared. The reference strain (remainder of cc175) was 40,593.

confirmed as being complement deficient. All three case isolates were ciprofloxacin resistant (*gyrA* allele 313). A fourth, newly identified, English IMD case from 2015 did not belong to any cluster (singleton). The isolate was susceptible to ciprofloxacin (*gyrA* allele 12). The patient had no inherited complement deficiencies or complement deficiencies due to glomerulonephritis, vasculitis or Eculizumab therapy and did not have asplenia, splenic dysfunction or HIV.

The four German IMD cases occurred in 2016 ($n = 2$), 2017 ($n = 1$) and 2019 ($n = 1$). The 2016 cases (cluster B) occurred in Afghan asylum seekers who were siblings with confirmed terminal complement deficiency. Both yielded ciprofloxacin resistant isolates (*gyrA* allele 187). The 2017 case (cluster A) was geographically distinct and occurred in an asylum seeker from Nigeria. The immune status of this patient was not known. The case isolate was ciprofloxacin sensitive (*gyrA* allele 12). The 2019 case (cluster A) was a cancer patient and the corresponding isolate was ciprofloxacin resistant (*gyrA* allele 313).

Among the other IMD cases, details of the Italian cases (2017 and 2018; both cluster A) including immune status and background were unknown. Both isolates were ciprofloxacin sensitive (*gyrA* allele 12). The Swedish case (2016; cluster B) was pregnant and the corresponding isolate was ciprofloxacin resistant (*gyrA* allele 187).

The remaining sublineage 1 isolates (clusters C, D and E) were carrier isolates from England ($n = 5$; 2015 to 2018), Ethiopia ($n = 8$; 2014), Italy ($n = 2$; 2016), Norway ($n = 3$; 2018 to 2019), France ($n = 1$; 2016) and Wales ($n = 1$; 2015). All possessed *gyrA* allele

12 with the exception of a cluster E subcluster, comprising isolates from Italy ($n=2$; 2016), France ($n=1$; 2016) and Wales ($n=1$; 2015), with the ciprofloxacin resistance-associated *gyrA* allele 152. This was reflected in MIC values, where known (Table 1).

Other cc175 sublineages - Sublineages 2 to 6

cc175 sublineages 2–6 included geo-temporally diverse invasive disease and carrier isolates collected in Africa, South America, North America and Europe in 2000–2018 (Fig. 2). All isolates in sublineages 2–6 possessed *gyrA* allele 12 associated with ciprofloxacin sensitivity.

Putative origins of ciprofloxacin resistance determinants

The ciprofloxacin resistance-associated alleles observed among NG cc175 sublineage isolates (alleles *gyrA313, gyrA187* and *gyrA152*) fell into two main clades corresponding to *N. meningitidis* and *N. cinerea* (Fig. 4). The *gyrA313* and *gyrA187* alleles, which were present among invasive and conjunctivitis cc175 isolates only, fell into the *N. cinerea* cluster. Allele *gyrA152* which was present among carrier cc175 isolates only, fell into the *N. meningitidis* clade.

Potential subcapsular (MenB) vaccine coverage of the NG cc175 sublineage

The NG cc175 isolates did not possess alleles for PorA P1.4 and were unable to express NadA due to an interruption of the *nadA* allele (allele 311) with insertion sequence IS*1301*. All isolates possessed *nhba* alleles for either peptide 9 ($n=29$) or peptide 1578 ($n=2$). Ten of the 31 NG cc175 isolates, including 3/9 invasive isolates, possessed fHbp variant 1 alleles (peptide 321). The remainder all had fHbp variant 2 alleles ($n=17$; peptide 151) or fHbp variant 3 alleles ($n=4$, peptide 111) (Table 1).

Discussion

Since 2015, a single NG cc175 strain (the NG cc175 sublineage) has caused nine IMD and two conjunctivitis cases in Europe. Seven of these (2x conjunctivitis and 5x IMD) were due to ciprofloxacin resistant isolates with one of two distinct *gyrA* alleles of *N. cinerea* origin, thus highlighting the significance of natural transformation among *Neisseria* species in the continued emergence of antibiotic resistance.[24] Horizontal gene transfer occurs more readily between closely related bacteria.[25] That these resistance-associated *gyrA* alleles from *N. cinerea* have established a stable presence within persistent cc175 sublineages is a concern since they may also now be more readily transferred to other, more virulent, meningococcal strains. A further subpopulation comprising only carriage isolates possessed a distinct ciprofloxacin resistance-associated *gyrA* allele of meningococcal origin. None of the other five diverse cc175 sublineages included any isolates with ciprofloxacin resistance-associated alleles. It not known whether the existence of several distinct resistance-associated alleles in just a single sublineage marks a propensity for acquiring/maintaining antibiotic resistance or just opportunism. The fact that the affected sublineage is non-groupable is probably just a coincidence however, as the corresponding isolates share alterations in over 100 other genes compared to the isolates in the other sublineages. The loss of a capsule is therefore one of over 100 changes that might influence their apparent shared propensity to acquire resistance.

Three of the IMD cases were related to travel to Mecca, KSA for the Umrah pilgrimage that takes place throughout the year. A further three were from asylum seekers who may also travel long distances and encounter crowded conditions that are known to propagate the spread of meningococcal carriage.[26–28] Thus, although all nine invasive cases occurred in Europe, this strain is clearly present beyond Europe, where further cases may have occurred. The establishment of a Global Meningitis Genome Partnership[29] as part of the WHO-led 'Global Roadmap to Defeat Meningitis by 2030'[30] is intended to facilitate the participation of all nations in genomic surveillance and thus enhance the scope of similar investigations. The annual Hajj pilgrimage to Mecca has previously been associated with outbreaks of meningococcal disease.[31–33] Since vaccination of all travellers to KSA with the MenACWY polysaccharide vaccine became mandatory in 2002, no pilgrimage-associated IMD outbreaks have been reported.[34] Unconjugated polysaccharides do not prevent the acquisition of carriage,[35–37] however, and would not be expected to prevent disease caused by a NG strain such as the NG cc175 sublineage.[38]

Ciprofloxacin prophylaxis is mandatory for pilgrims travelling to the KSA from high-risk sub-Saharan African meningitis belt countries. This will be ineffective against ciprofloxacin resistant members of the NG cc175 sublineage and may even have had a role in its propagation.[39,40] Approximately 1.5 million doses are estimated to have been given to travellers over the past decade.[41] The benefits of its continued use have, however, been challenged, given the potential contribution in the emergence of ciprofloxacin resistance among meningococci.[42] The findings of a recent study by Coldiron and colleagues showed possible benefits of using ciprofloxacin as prophylaxis in outbreak situations in the meningitis belt.[43] The findings of the present study serve to highlight the importance of antibiotic susceptibility testing at the earliest opportunity to ensure that such interventions are not compromised.

While NG meningococci rarely cause IMD among healthy individuals, they may cause invasive disease in immunocompromised individuals, particularly those with inherited or acquired deficiencies of the terminal complement pathway, some of which have been fatal.[44] Accordingly, among the nine IMD cases caused by the NG cc175 sublineage, at least five had an immune deficiency. Some immunocompromised individuals, e.g. those with terminal complement deficiencies, are recommended to receive MenACWY conjugate and subcapsular (MenB) vaccines. Only the latter may provide protection against the NG cc175 sublineage. Protection by protein-based subcapsular vaccines depends on adequate cross-reactivity and surface expression of at least one vaccine antigen. According to the genetic Meningococcal Antigen Typing System (gMATS),[45] a genetic tool for predicting 4CMenB strain coverage, the coverage afforded by both the predominant NHBA peptide (peptide 9) and, where present, fHbp variant 1 peptide (peptide 321), is unpredictable. In the case of NHBA, this reflects the scarcity of peptide 9 within the original gMATS dataset.[45] Unfortunately, the phenotypic precursor, MATS,[46] is only validated for MenB isolates. Nonetheless, the effectiveness of vaccination in the absence of a working complement system is uncertain and IMD cases have occurred in vaccinated individuals with complement deficiency.[47]

In addition to vaccination, some countries, e.g. the UK and France, recommend antibiotic chemoprophylaxis for individuals with terminal complement deficiencies.[44] The antibiotic of choice is usually penicillin but penicillin-resistant strains have been reported to cause IMD in this group.[47,48] Whilst penicillin resistance amongst *N. meningitidis* is relatively uncommon, isolates displaying reduced susceptibility are becoming increasingly reported worldwide.[49–51] This has been associated with amino acid substitutions in the Penicillin Binding Protein 2 encoded by the *penA* gene[49] all of which were observed among the NG cc175 sublineage isolates. As such, this strain may be particularly dangerous for these individuals. It is noteworthy that the corresponding penicillin MICs were relatively low (mainly 0.25 mg/L). The EUCAST breakpoint for penicillin resistance in meningococci is 0.25 mg/L, however, based on experimental work in mice and penicillin levels reached in the cerebrospinal fluid during treatment, a breakpoint

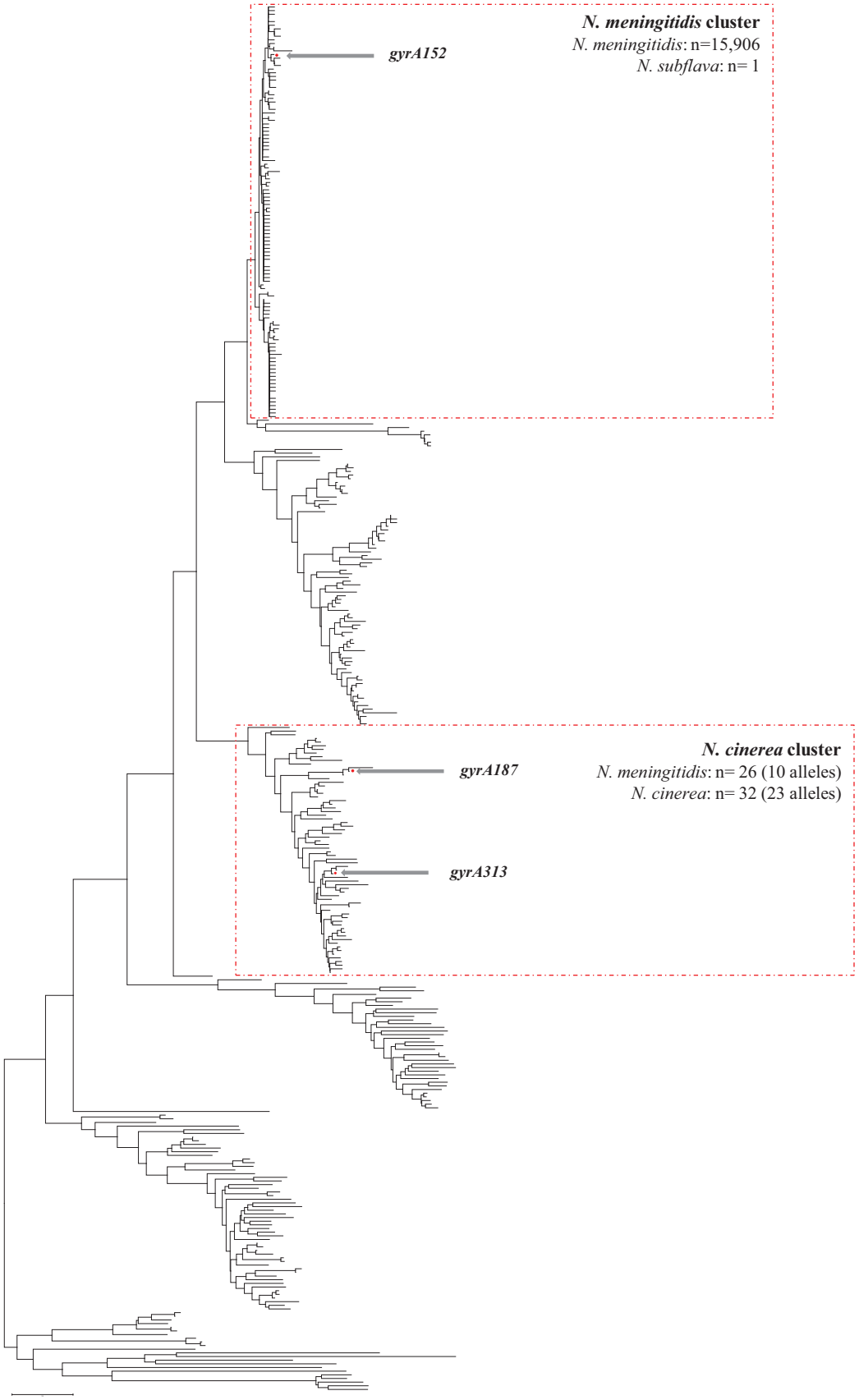

Fig. 4. Phylogenetic analysis of *gyrA* alleles ($n = 379$) highlighting those identified among isolates belonging to the non-groupable cc175 sublineage.
Neighbor-Joining tree of nucleotide sequences of 379 *gyrA* alleles on PubMLST (accessed 13/05/2020). The non-groupable cc175 sublineage alleles (alleles 152, 187 and 313) were distributed between clades predominantly representing *N. cinerea* and *N. meningitis*. The Tree was constructed using MEGA4. The scale bar represents the number of nucleotide differences.

of 1 mg/L has been proposed.[52] Nonetheless, one of two of the invasive cc175 isolates with the rarer *penA* allele (*penA909*) had an MIC of 0.5 mg/L – the same as that of a previous breakthrough strain in a patient on long term penicillin prophylaxis.[48] Thus, it should be considered that low level prophylactic dosing may be less effective than therapeutic doses.

Ciprofloxacin resistance in meningococci is rare in most countries and is mainly associated with sporadic cases, apart from an outbreak that occurred in India in 2005[53] and in China where it is prevalent.[54,55] Ciprofloxacin resistance among *N. meningitidis* is due to altered *gyrA* genes (encoding DNA gyrase),[56–58] as observed among the NG cc175 sublineage. Mutations in the *parC* gene (encoding DNA topoisomerase IV subunit A) have also been associated with increased ciprofloxacin resistance when in combination with *gyrA* mutations, though this was not observed in this study.[24] Meningococcal resistance to ciprofloxacin is concerning because it is currently the chemoprophylaxis of choice for close contacts of IMD cases. It may also be prescribed for prophylaxis[59–61] or rescue therapy for febrile illnesses in patients with complement deficiency,[62] thus adding to concerns surrounding the NG cc175 sublineage with regards to complement deficient patients.

In conclusion, we identified a NG ciprofloxacin-resistant meningococcal strain belonging to cc175 that has recently caused multiple cases of meningococcal disease across Europe. The NG cc175 sublineage is particularly virulent for immunocompromised individuals, including those that may be less well protected by vaccination. Current antibiotic prophylaxis regimes may also be subverted. Clinicians should remain vigilant when presented with meningococcal disease among pilgrims, asylum seekers or those with immunodeficiencies to ensure appropriate treatment of the case and their close contacts. Continued surveillance of antibiotic resistance among meningococci is essential to identify and monitor such threats and to explore the possibility of alternative chemoprophylactic agents such as azithromycin,[63] especially for complement deficient individuals already at increased risk of developing IMD.

Declaration of Competing Interest

LW, JL, and RB perform contract research on behalf of Public Health England for GlaxoSmithKline, Pfizer, and Sanofi Pasteur. The Public Health England Immunisation and Countermeasures Division has provided vaccine manufactures with post-marketing surveillance reports, which the Marketing Authorization Holders are required to submit to the UK Licensing authority in compliance with their Risk Management Strategy. A cost recovery charge is made for these reports. All other authors report no potential conflicts.

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Supplementary materials

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