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Abstract

Background

Neisseria meningitidis group W (NmW) belonging to the clonal complex ST-11 (NmW/cc11) spread in Europe and in France in 2000 and declined thereafter. In France, invasive meningococcal disease (IMD) due to NmW increased again in 2012 and thereafter since 2015. Several sub-lineages of NmW/cc11 are circulating worldwide with successive epidemic waves. We aimed to describe recent epidemiological trends of NmW in France and to explore the microbiological and epidemiological characteristics associated with different NmW/cc11 sub-lineages.

Methods

The epidemiology of NmW was described based on data collected through mandatory notification of IMD and strain typing data for culture-confirmed and PCR-confirmed cases for the period 2000-2016. All culture-confirmed cases due to NmW from the period 2010-2016 were characterized by whole genome sequencing (WGS). A detailed epidemiological analysis was performed for culture-confirmed cases on the basis of WGS data.

Findings

During the period 2010-2016, genotyping was obtained for 148 cases including all the 132 culture-confirmed cases, among which 127 were matched with epidemiological data, and 16 PCR-confirmed cases (out of a total of 47 PCR-confirmed cases). An increase in IMD was observed in 2012 and was linked to isolates belonging to the "Anglo-French-Hajj" sub-lineage. These isolates have decreased significantly since 2013 and have been replaced by NmW/cc11 isolates related to the "South American – UK" sub-lineage which caused a marked increase in the number of cases of NmW in 2016. In this sub-lineage, the "original UK strain" was first detected in 2012 and increased thereafter, followed by the recently described "UK 2013-strain". Isolates

related to the “South American-UK” sub-lineage represented 45% of all NmW cultured isolates from the whole period 2010-2016 but were the most frequent isolates in 2016, representing 76% of the total NmW typed isolates and 94% of the typed NmW/cc11 isolates. A changing pattern in the epidemiology of NmW has been observed in 2015-2016 in relation to the spread of the “UK 2013-strain” with a sharp increase in the number of cases among persons aged 15 years and over and a high case fatality rate (CFR) . Among cases due to the “UK 2013-strain”, 94% of cases were aged 15 years and over and the CFR was 28%.

Interpretation

Our data suggest a recent clonal replacement among NmW/cc11 isolates with the expansion of the “South American-UK” sub-lineage in France and particularly the “UK 2013-strain” which was predominant in 2015 and 2016. A shift in the age-distribution of IMD due to NmW to older ages and the high CFR are consistent with the expansion of a new virulent clone in a naive population. These data may have an impact on tailoring vaccination strategies against NmW.

Highlights

- *Neisseria meningitidis* group W is currently increasing in Europe and in France.
- Previous increases in France were linked to the Hajj and to travel to Africa.
- Whole genome sequencing was used to characterise W isolates in France in 2010-2016.
- The “UK 2013-strain” has recently expanded in adults aged 15 years and over.
- A high case fatality rate was observed among cases caused by the “UK 2013-strain”.

Introduction

Invasive meningococcal disease (IMD) is caused by the Gram negative bacterium *Neisseria meningitidis* (Nm) and mainly manifests as septicaemia and meningitis. This bacterium is frequently hosted in the nasopharynx (1). Invasive isolates (responsible for IMD) are usually capsulated while carriage isolates are frequently non-capsulated (2). The polysaccharide composition of the capsule defines the serogroup. Twelve serogroups are currently described but 6 of them (A, B, C, Y, W and X) are responsible for almost all IMD worldwide with high geographic variation (3, 4). Vaccines based on the capsular polysaccharides are available against serogroups A, C, Y and W meningococci while protein-based vaccines target serogroup B meningococci (5).

In France, routine vaccination with meningococcal C conjugate vaccines (MCCV) was introduced into the immunization schedule in early 2010 for infants at 12 months of age and a catch-up until 24-years-old (one dose of MCCV). Vaccination against meningococci ACWY and against meningococci B are recommended for at risk subjects and outbreaks control (6).

The management of IMD requires both treatment of patients and prophylactic measures among close contacts to avoid secondary spread. Surveillance is also a key element to better tailor vaccination strategies. Indeed, IMD occurs as sporadic cases with occasional outbreaks in Europe and North America while major epidemics periodically occur in Sub-Saharan Africa (7). Meningococcal isolates showed high diversity with occasional global spread of epidemic strains belonging to different serogroups (7). Serogroup W (NmW) isolates have been detected since the late 70s but historically caused a minor proportion of IMD cases globally. In 2000, NmW underwent a first global spread among Hajj pilgrims and their contacts (8). Subsequently NmW spread in the African Sub-Saharan meningitis belt and caused major outbreaks in Sub-Saharan

countries at the beginning of the 2000s and in 2010 (9-11). During the 2000 decade, NmW also emerged in the South America Cone and spread to the United Kingdom (UK) in 2009 (12-14). Tracking these isolates requires powerful typing methods such as multilocus sequence typing (MLST) based originally on the sequence of 7 meningococcal genes. MLST determines sequence types (ST) that can be clustered into clonal complexes (cc) (15). Several clonal complexes have been identified but most NmW outbreak isolates belong to the clonal complex cc11 (cc22 maintains a steady presence in many countries) (7, 12). However, MLST may lack resolution to discriminate clones that can be resolved by whole genome sequencing (WGS) (16). Core genome MLST (cgMLST) has already been used to describe the the global spread of W/cc11 lineage in several countries over decades and allowed the characterisation of distinct sub-lineages within W/cc11 isolates. In particular, it showed the emergence of the "South American – UK" sub-lineage in UK which has been further characterised in two distinct strains: the "original UK strain" which emerged in UK in 2009 and the "UK 2013-strain" which emerged in UK in 2013 (17).

In France, studying the characteristics of NmW/cc11 is particularly interesting as various W/cc11 sub-lineages have been co-circulating in the country in addition to NmW isolates belonging to other clonal complexes (10, 18, 19). A thorough genetic analysis of NmW isolates combined with epidemiological data on cases may unravel differential spread/virulence/replacement abilities. We aimed to explore the characteristics of NmW IMD cases caused by these different lineages and sub-lineages in France since 2010.

Methods

Surveillance of IMD in France

The French surveillance system of IMD is based on the mandatory notification of IMD cases on the basis of a standardized case definition. Cases are notified to the Regional Health Agencies which implement local control measures. The notification forms are then sent to Santé publique France, the French national public health agency, for data entry and analysis for the purpose of epidemiological surveillance. IMD cases are notified on the basis of at least one of the following criteria: isolation of *N. meningitidis* or detection of *N. meningitidis* nucleic acid by PCR (PCR was introduced in 2002 in the case definition) from a normally sterile site (blood, cerebrospinal fluid (CSF), other sterile sites or purpuric skin lesions) ; detection of Gram-negative stained diplococci in CSF; purulent CSF associated with purpuric skin lesions or with the detection of *N. meningitidis* antigens in blood, urine or CSF (antigen detection was not used anymore after 2014); *purpura fulminans* (severe sepsis with extensive hemorrhagic and necrotic skin lesions) (19). In addition, *N. meningitidis* isolates, as well as PCR positive samples are sent to the National Reference Centre for Meningococci (NRCM) for confirmation and full strain typing. Meningococcal groups are determined by agglutination of cultured isolates using specific "in house" rabbit sera and by PCR for non-cultured confirmed cases (20). Throughout the manuscript "group" will be used to refer to both serogroup and genogroup to characterise cultured-confirmed and PCR-confirmed cases respectively. PCR confirmation and typing techniques performed at the NRCM include PCR targeting *N. meningitidis*-specific genes as well as capsular genes for serogroup determination (*ctrA, sodC, csaB, csB, csC, csW, csY* and *csxA*).

Genomic analysis

The NRCM performs systematically MLST analysis on all IMD cases (cultured and PCR-confirmed cases) since 2010 as previously described (18, 20, 21). Typing data are expressed as a genetic formula: G :P1.PorA-VR1,PorA-VR2:FetA :cc that define the group (G), the two variable regions (VR1 and VR2) of the outer membrane protein PorA, and of one VR of the protein FetA, as well as the cc.

Moreover, WGS is also performed systematically on all invasive cultured isolates since late 2015. For this work we retrospectively performed WGS on all cultured NmW isolates since 2010. Genomic DNA was extracted by using a Genomic-tip 20/G kit (QIAGEN, Valencia CA, USA). Sequencing was performed by Illumina HiSeq 2000 sequencer (Illumina, San Diego, CA, USA) and assembled as previously described (21). Sequences are available through the PubMLST database which runs on the Bacterial Isolate Genome Sequence Database (BIGSdb) platform (22). WGS data was analyzed using a "gene-by-gene" approach available through the PubMLST Genome Comparator tool using *N. meningitidis* core genome v1.0 that includes 1605 core loci (22). SplitsTree4 (version 4.13.1) was used to visualise the resulting distance matrices as Neighbour-net networks (23). For the genomic analysis we included three reference strains according to Lucidarme et al (24): one French isolate related to the "Anglo-French Hajj" sub-lineage (ID31165), one isolate related to the "original UK strain" (ID20196) and one isolate related to the "UK 2013-strain" (ID30167). The IDs of all these isolates are given in the supplementary Table to allow retrieving of WGS sequence in FASTA formats.

Study period

Two periods were considered: (i) 2000-2016 for the description of the epidemiological trends of NmW IMD and MLST and (ii) 2010-2016 for the whole genome analysis and the characteristics of cases according to the different sub-lineages.

Data analysis

Two databases were used in this study. The first one relates to IMD cases reported through mandatory notification and includes clinical, epidemiological data as well as microbiological data (technique for diagnosis and group identified by the local hospital or by the NRCM). The second database includes data on all NmW invasive strains characterized by the NRCM. These two databases are highly dependent but do not completely overlap. Cases in the database of the NRCM included PCR since 1998 while PCR was added on the criteria of the mandatory reporting in 2002. Data analysis was performed distinctly using these two databases except for the analysis of characteristics of cases by lineages/sub-lineages for which the databases were merged.

Epidemiological and clinical characteristics of cases were studied by groups (NmW versus other groups) and by NmW lineages and sub-lineages identified by WGS. Proportions were compared using Chi-square test or Fisher exact test using the threshold of p-value < 0.05 as statistically significant. Poisson regression was used to estimate the relative risk (RR) for death by group after adjusting for age. Statistical analysis was performed using Stata version 12.1 software.

Results

Epidemiology of NmW IMD

Between 2000 and 2016, the number of cases fluctuated in relation with the global spread of NmW and multinational outbreaks (Fig.1). In 2000-2003, a first increase was described in relation to the global Hajj-associated outbreak (8, 18). Then, from 2003 to 2011, there was a marked decreasing trend and at the lowest level in 2009-2011, NmW caused around 10-15 cases per year representing 2% of all IMD cases (Fig. 1). In 2012, there was a transient increase in the number of cases in relation to outbreaks in African Sub-Saharan countries. In 2015 and 2016, a new upsurge was observed. In 2016, 45 cases were reported (incidence rate of 0.07 / 100 000 inhabitants). NmW remained the least frequent group identified but the proportion of cases due to NmW increased compared to previous years. In 2016, of 506 IMD cases with known group, 52% of IMD belonged to group B, 26% to group C, 12% to group Y and 9% to group W.

In 2015-2016, there were significant differences in characteristics of IMD cases according to the strain group (Table 1). The proportion of cases in age groups above 15 years old was higher for group W compared to group B and C cases. NmW IMD was associated with a higher CFR (22.1% vs 10.4% for other groups, p=0.002). After adjusting for age, the CFR was still greater among group W cases than other groups (RR for death = 1.2 [95% IC 1.01-1.42]). In comparison with group B and C cases, group W cases presented more frequently with septicemia and less frequently with meningitis (Table 1). The proportion of cases with Nm isolated from synovial fluid was greater among NmW cases.

Molecular characteristics of NmW invasive meningococcal isolates

During the period 2000-2016 the NRCM received samples for 527 cases of NmW IMD : 480 (91%) cases were cultured-confirmed and 47 (9%) were only PCR-confirmed (MLST performed on PCR-confirmed cases since 2010). The annual distribution of cases is shown in Fig. 2. MLST data were available for 366 cases (69%) including all the cultured-confirmed cases since 2010 (n=132) and 16 of the 47 cases that were only PCR-confirmed.

Among the 366 cases with MLST data, cc11 was the most frequent and caused 195 cases (53%) followed by cc22 with 128 cases (35%) while other cc were responsible for 43 cases (12%). The cc11 isolates were responsible for the increase of NmW IMD observed at the beginning of the 2000s, in 2012 and in 2016 (Fig. 1 and Fig. 2). The number of NmW/cc11 cases was particularly high in the years 2000, 2001 and 2016. Over all the study period, NmW/cc11 isolates showed the same genotypic formula (W:P1.5,2:F1-1:cc11). NmW/cc22 isolates were more heterogeneous.

Genomic analysis of NmW cultured isolates

To describe the evolutionary relationships of the isolates and particularly those of the cc11, all 132 cultured isolates (from the period 2010-2016) were subjected to WGS and the data were uploaded to the PubMLST database and analysed using the BIGSdb tools in this database. Isolates grouped into two major clusters corresponding to cc11 and cc22. Isolates belonging to other cc were distinct from these two clusters (Fig. 3A). Isolates belonging to “cc22” and to “other cc” showed a higher diversity than “cc11” as suggested by their genetic distances (Fig.3 B and C). Eighty-four isolates belonged to cc11 (of which 70 were identical by MLST) and showed differentiation into two groups that corresponded to the “Anglo-French-Hajj” sub-lineage and to the “South American-UK” sub-lineage (Fig 3A). Few cc11 isolates were separated from the above mentioned two lineages and may correspond to other local cc11 isolates that were related to the “Anglo-French-Hajj” sub-lineage (Fig. 3D dashed-line circle). It is noteworthy that isolates

belonging to the "Anglo-French-Hajj" sub-lineage were distributed throughout 2011-2016 (no cc11 was detected in 2010 among all typed cases). In particular, the numbers of isolates related to the "Anglo-French Hajj" strain increased in 2012-2013 and decreased thereafter (only two isolates in 2016) (Fig. 3D). On the other hand, the "South American-UK" isolates were first detected in 2012 and increased thereafter (Fig. 3E). The first case was detected in a patient from United Kingdom who was in holiday in France in June 2012. Isolates belonging to the "South American-UK" sub-lineage represented 45% of all NmW cultured isolates from the whole period 2010-2016. They increased after 2012 and were the most frequent NmW/cc11 isolates in France in 2016, representing 34 isolates (76%) of the 45 NmW typed isolates and 94% of the typed NmW/cc11 isolates.

Moreover, the "South American-UK" sub-lineage can be further clustered at the genetic level into two distinct strains that corresponded to two temporal periods: "the original UK strain" with isolates from the period 2012-2016 and the "UK 2013-strain" (24) that only included isolates from the period 2015-2016 and increased significantly in 2016. Non-cc11 isolates (cc22 and other cc) did not showed significant variations during the period 2010-2016 (Fig. 4).

Epidemiological characteristics of NmW cases according to WGS data

Of 132 isolates typed by WGS, 127 could be matched to clinical and epidemiological data collected through mandatory notification. We compared the characteristics of cases according to four groups defined using WGS data: the "original UK strain", the "UK 2013-strain", the "Anglo-French Hajj" sub-lineage and non-cc11 lineages (Table 2). There was no overall statistical difference in the distribution of cases by age group between these four groups. However a sharp increase in the number of cases due to the 'UK 2013-strain" was observed in 2015 and 2016 in individuals aged 15 years old and over (Fig. 5). In total, there were 36 IMD

cases due to the "UK 2013-strain" among which 34 (94%) occurred in adults aged 15 years old and over. In the other WGS group, the number of cases in each age group was stable overall the study period, excepting the "Anglo-French Hajj" strain mainly affecting children in 2012.

The CFR was 25.9% among cases within the "South American-UK" sub-lineage and 11.6% in the other groups (4% for the "Anglo-French Hajj" and 15.9% for non cc11) (p=0.04). This difference was still observed but not significant when analysing the four lineages / sub-lineages distinctly. Moreover, the CFR was not significantly different between the "original UK strain" and the "UK 2013-strain" (p=0.67). Over the period 2010-2016, 15 deaths occurred among cases due the "South American – UK" sub-lineage: 1 death among children aged 0-14 years old (CFR 12%), 3 deaths among the 15-24 years old (CFR 20%), 9 deaths among the 25-59 years old (CFR 43%) and 2 deaths among individuals 60 years and over (CFR 14%).

There was no significant association between the infecting strain and the clinical presentation (Table 2).

The geographical distribution of IMD cases according to the four WGS groups is shown in Fig. 6 suggesting a country-wide expansion of the strains of "South American – UK" sub-lineage in 2015 and 2016. It is noteworthy that 5 cases due to these strains occurred in persons who reported a city of residence outside France: 2 cases in 2012 who lived in UK and Australia (both "original UK strain"), 2 cases in 2014 who lived in UK (1 case due to the "original UK strain" and 1 case due to the "UK 2013-strain") and 1 case in 2015 who lived in UK ("UK 2013-strain). However, without comprehensive information about their history of travel prior to IMD, it is not possible to determine if they acquired the infection in France or in their country of residence.

Discussion

Three periods with high number of NmW IMD cases were observed in France in the last 17 years, considering both number of cases and their proportion relatively to all IMD cases: 2000-2003, 2012 and 2016. All of them were caused by NmW/cc11 isolates. Typing analysis showed that most isolates in 2012 clustered within the "Anglo-French Hajj" sub-lineage. This is consistent with a previous report on cases imported from Sub-Saharan countries to France in 2012 (25). WGS provided additional discrimination compared to the classical MLST approach and showed the emergence of the Anglo-French Hajj strain in 2012 (10). Few other isolates represented local cc11 isolates that were still clustered within the "Anglo-French Hajj" sub-lineage. These isolates may have diverged from a common ancestor introduced in France since at least the year 2000 or may have been imported later on. The last increase in the number of NmW IMD cases was observed in 2016 and was mostly due to the "UK 2013-strain", which emerged in the UK from the "original UK strain", a descendant from the South American strains (13, 14, 17). The strain may now to have expanded even more widely and other countries have reported increase incidence of NmW (Australia, The Netherlands and Spain,) (26-28). The "South American-UK" sub-lineage is distinct from the "Anglo-French Hajj" sub-lineage although all isolates shared the same genotypic formula (W:P1.5,2:F1-1:cc11) suggesting an important role for variation of other bacterial components (17).

Surveillance of IMD in France is associated with high exhaustiveness and completeness of data. In a study conducted in 2011, the exhaustiveness was estimated at 91% for mandatory notification and 84% for NRCM (data not published). Completeness of data is also very high and has improved in recent years. Since 2010, there has been less than 7% missing information for the strain group identification and less than 3% missing information on patients' vital status resulting from IMD.

The CFR was higher for the "South American-UK" sub-lineage compared to the other lineages and sub-lineages which highlights the virulence of this strain as also observed in Chile and the UK (14, 29). This strain appears highly transmissible and virulent. Since its emergence in France, the "South American-UK" sub-lineage caused two clusters of cases among university students in France. In both clusters, the "UK 2013-strain" was identified. The first cluster occurred in 2016 with three cases among university students in Dijon (Burgundy region). Two deaths were reported. A mass vaccination campaign was organised targeting all students and staff in the campus and no further cases have been reported. Another cluster of 2 cases (1 death) occurred in 2017 among students in a small university in Paris and lead again to the organization of a vaccination campaign (30).

A shift in the age of NmW IMD cases has been observed since the introduction of the strains derived from "South American / UK" sub-lineage" in the population in France. In 2015 and 2016, the "UK 2013-strain" affected mainly individuals aged 15 years and over and was rare in children. This shift towards older age groups was not observed for other NmW lineages/sub-lineages during the period 2010-2016, nor in other studies on NmW previously conducted in France (31) (10). Meningococcal isolates carriage and circulation occurs most frequently among adolescents and young adults (32). The shift of IMD to older age groups was suggested as a predictor for epidemic periods of IMD (33). This may reflect the expansion of a new clone in a naïve population and/or higher transmission and virulence properties of the new clone. Similar observations were made in the UK where the increase of NmW/cc11 cases was first reported in older adults during the epidemic year 2012-2013 and then extended downwards, especially to adolescents and infants, by the epidemic year 2014/2015 (14, 34). However, in Chile where the "South American-UK" sub-lineage emerged, in 2011-2012, 47% of cases were reported in children <5 years (29). Accordingly, vaccination against NmW was initiated in October 2012 in

children aged 9 months to 5 years using the tetravalent conjugate vaccine. A direct effect was observed in this age group. However, this strategy did not show any impact in other age groups and it was argued that targeting adolescents and young adults may be needed to improve the vaccination strategy against NmW in Chile (12). In the UK, an adolescent program using MenACWY conjugate vaccine was recommended in 2015 targeting 13-14 years old subjects and university entrants in addition to a catch up campaign for 14-18 year olds during 2015 to 2017 (35). A major decrease in the number of NmW cases was observed in the first vaccine-targeted cohort who entered university (34). The epidemiology of NmW IMD should be followed up carefully in order to guide vaccination strategies. If a vaccination was to be recommended in France, epidemiological data will be instrumental in identifying the optimal target population. Epidemiological data available for the first semester of 2017 are consistent with those presented here and show the persisting increase in the number of NmW IMD cases (32 cases in the first half of 2017 versus 24 cases in the first half of 2016), a high proportion of cases aged 15 years and over (75%) and a high CFR (31%). Finally, WGS provided new inputs in the analysis to describe the epidemiological and clinical profile of this emerging strain. We found some specific characteristics linked to the "UK 2013-strain" although comparison between lineages / sub-lineages was probably hampered by the small number of cases in each group. Our work in addition to other studies (17, 24, 36) provides evidence on the usefulness of combining WGS and epidemiological data to better describe the population expansion and spread of *N. meningitidis* and provides good basis for further work on the routine use of WGS in the surveillance of IMD in France.

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Table 1: Characteristics of invasive meningococcal disease (IMD) cases according to strain groups, France, 2015-2016 (source: mandatory notification and National reference centre for meningococci)

NB: data are presented for the most frequent groups

	For each group					**For group W versus other groups (BCY)**		
	Group B (n=503) n (%)	**Group C (n=253) n (%)**	**Group W (n=77) n (%)**	**Group Y (n=116) n (%)**	**p-value**	**Group W (n=77) n (%)**	**Group BCY (n=872) n (%)**	**p-value (W vs BWY)**
Age group (years)					**$<10^{-3}$**			**0.003**
0-14	243 (48.3)	89 (35.2)	15 (19.5)	23 (19.8)		15 (19.5)	355 (40.7)	
15-24	100 (19.9)	54 (21.3)	21 (27.3)	20 (17.2)		21 (27.3)	174 (20.0)	
25-59	104 (20.7)	60 (23.7)	21 (27.3)	24 (20.7)		21 (27.3)	188 (21.6)	
≥ 60	56 (11.1)	50 (19.8)	20 (26.0)	49 (42.2)		20 (26.0)	155 (17.8)	
No. of deaths	39 (7.8)	31 (12.3)	17 (22.1)	30 (17.2)	**$<10^{-3}$**	90 (10.4)	17 (22.1)	**0.002**
Clinical presentation (several sites of infection are possible)								
Meningitis	408 (81.1)	166 (65.6)	44 (57.1)	71 (61.2)	**$<10^{-3}$**	44 (57.1)	645 (74.0)	**0.002**
Septicemia	254 (50.5)	152 (60.1)	60 (77.9)	90 (77.6)	**$<10^{-3}$**	60 (77.9)	496 (56.9)	**$<10^{-3}$**
Arthritis	7 (1.3)	19 (7.5)	7 (9.1)	5 (4.3)	**$<10^{-3}$**	7 (9.1)	31 (3.6)	**0.01**
Purpura fulminans	113 (24.8)	56 (24.1)	8 (13.1)	9 (9.1)	**0.002**	8 (13.1)	178 (22.7)	0.08

Table 2: Characteristics of NmW invasive meningococcal disease (IMD) cases according to thefour lineages/sub-lineages identified by Whole-Genome Sequencing (WGS), France, 2010-2016 (source: mandatory notification and National reference centre for meningococci)

	Original UK strain	**UK 2013-strain**	**Anglo-French Hajj**	**Not cc11 lineages**	**p-value**
	N=22	**N=36**	**N=25**	**N=44**	
	n (%)	**n (%)**	**n (%)**		
Age (years)					0.09
0-14	6 (27.3)	2 (5.6)	9 (36.0)	10 (22.7)	
15-24	3 (13.7)	12 (33.3)	4 (16.0)	9 (20.4)	
25-59	8 (36.4)	13 (36.1)	7 (28.0)	9 (20.4)	
≥ 60	5 (22.7)	9 (25.0)	5 (20.0)	16 (36.4)	
Case fatality rate	5 (22.7)	10 (27.8)	1 (4.0)	7 (15.9)	0.08
Clinical presentation (several sites of infection are possible)					
Meningitis	12 (54.5)	20 (55.6)	16 (64.0)	20 (45.4)	0.51
Septicemia	17 (77.3)	30 (83.3)	18 (72.0)	31 (70.4)	0.57
Arthritis	1 (4.5)	4 (11.1)	3 (12.0)	4 (9.1)	0.87
Purpura fulminans	3 (17.6)	4 (13.3)	2 (8.3)	5 (13.9)	0.85

Figure 1: Number of NmW invasive meningococcal disease (IMD) cases and proportion of NmW over all IMD cases, France, 2000-2016 (source: mandatory notification and National reference centre for meningococci)

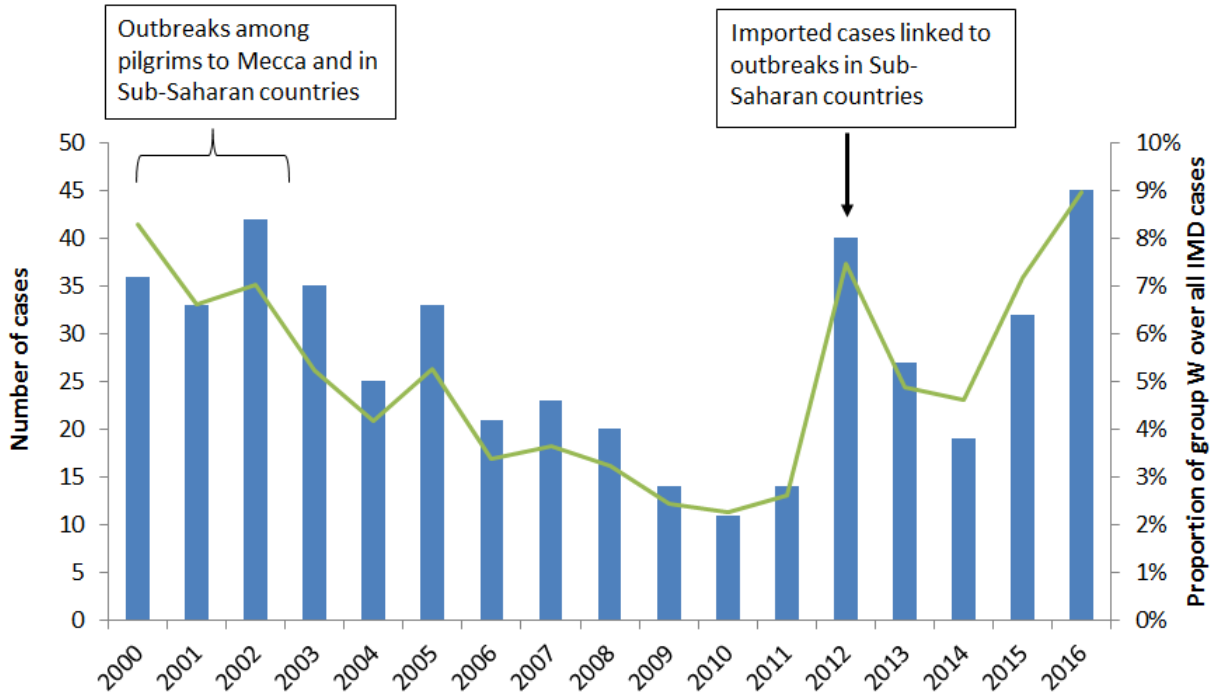


Figure 2: Annual distribution of NmW invasive meningococcal disease (IMD) corresponding to both culture- and non-culture-confirmed cases by major clonal complexes characterised by the National reference centre for meningococci (NRCM), France, 2000-2016. For the period 2000-2009, MLST typing was not systematically performed and MLST data were obtained for 52% to 100% of cases. Missing data in the period 2010-2016 correspond to non-culture cases. PCR was added in the mandatory reporting criteria in 2002, explaining the discrepancy in number of cases between figure 1 and 2 for the period 2000-2002

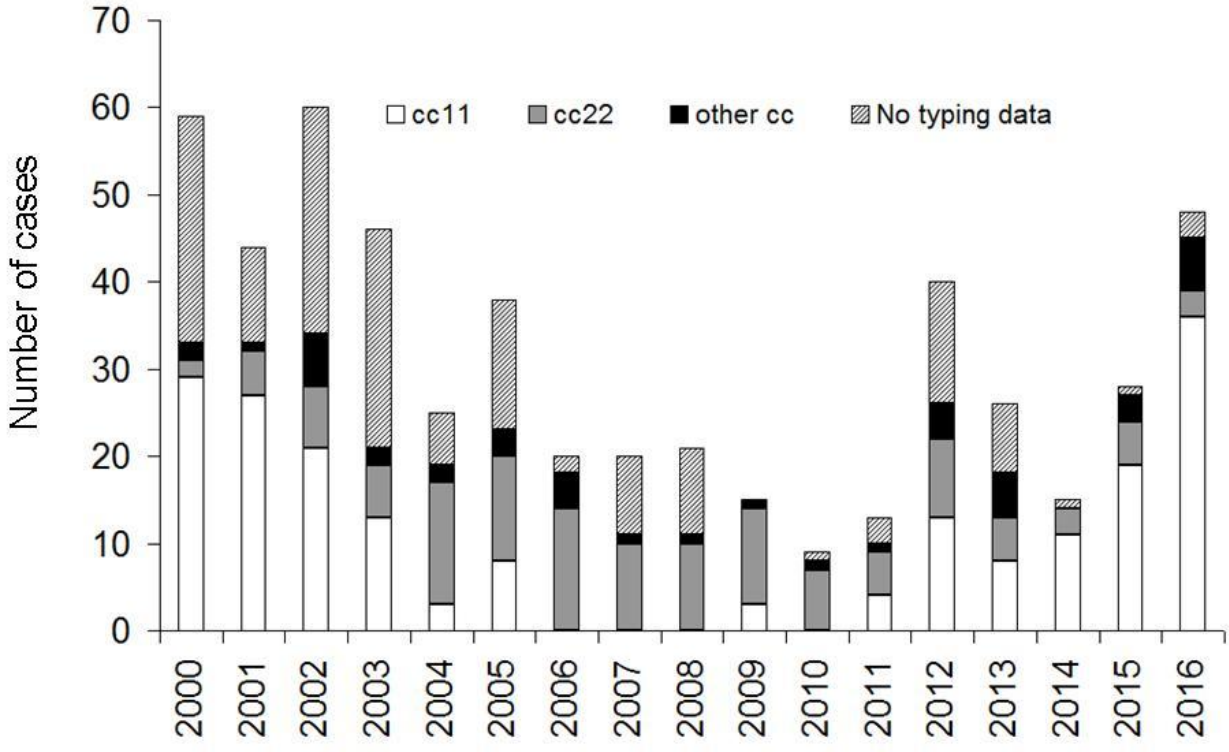


Figure 3 : Neighbour-net phylogenetic network of all invasive NmW cultured isolates received at the national reference centre for meningococci, France, 2010-2016 (A) Global view of the phylogenetic tree showing all the genetic lineages identified. In (B) and (C) a zoom on the tree that includes the non cc11 isolates (cc22 and other cc isolates). In (D) are depicted a zoom on the cc11 isolates that were resolved into the Anglo-French-Hajj sub-lineage. One isolates representative to the Hajj 2000 outbreak was included (ID31165 red arrow). The isolates that were highly related to the Hajj strain were surrounded by a solid line circle and those that were more distantly related to the Hajj strain surrounded by a dashed line circle. (E) The South American-UK sub-lineage that included the two strains: the original UK strain and the UK 2013-strain according to (24). One representative isolate (ID20196) for the "original UK strain" was indicated by a red arrow. One representative isolate (ID30167) for the "UK 2013-strain" was indicated by a blue arrow. The coloured circles represent individual isolates for each year of the period 2010-2016.

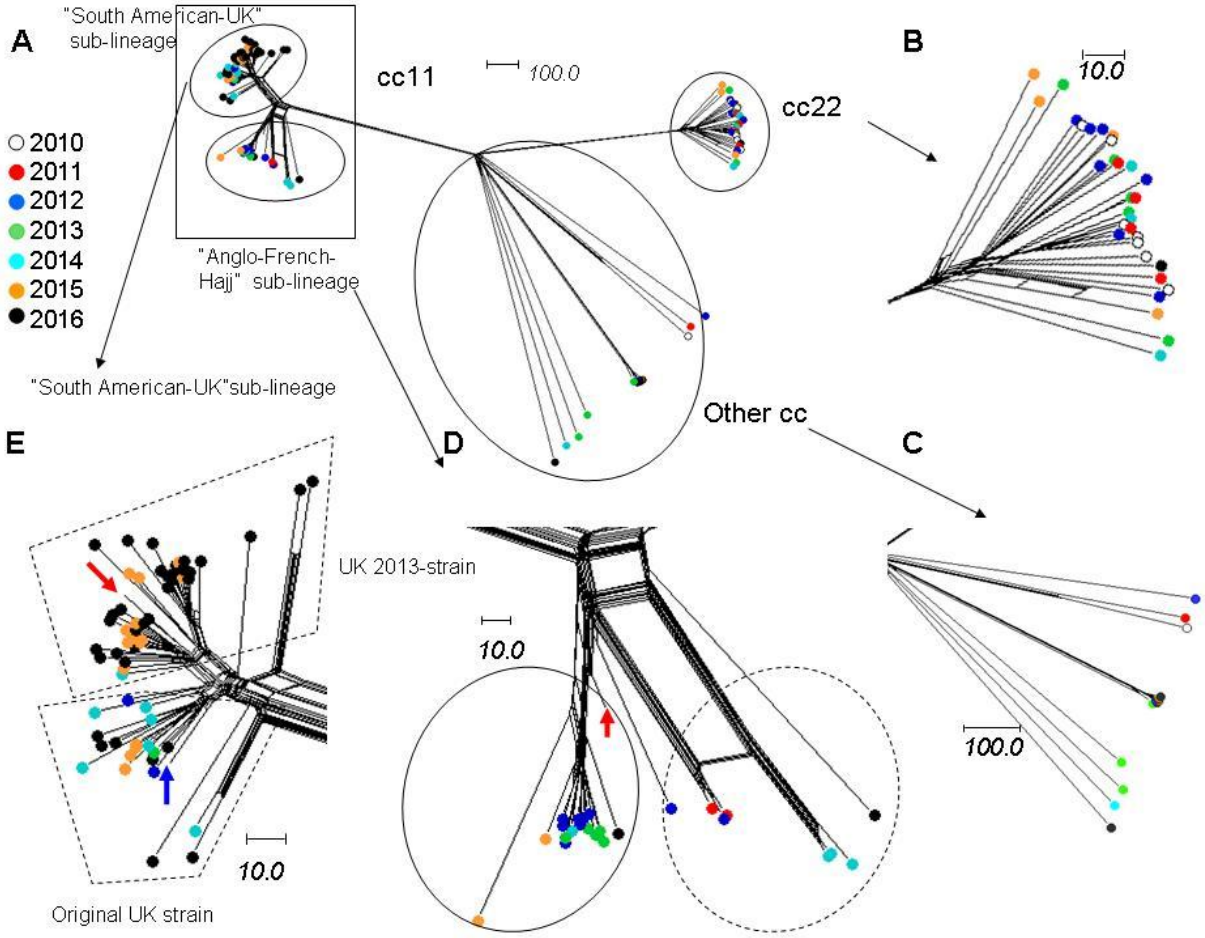


Figure 4: Annual distribution of NmW invasive meningococcal disease (IMD) isolates by WGS sub-lineage characterised by the National reference centre for meningococci, France, 2010-2016. Cases are classified in four groups using WGS data: "original UK strain", "UK 2013-strain", Anglo-French-Hajj and non-cc11.

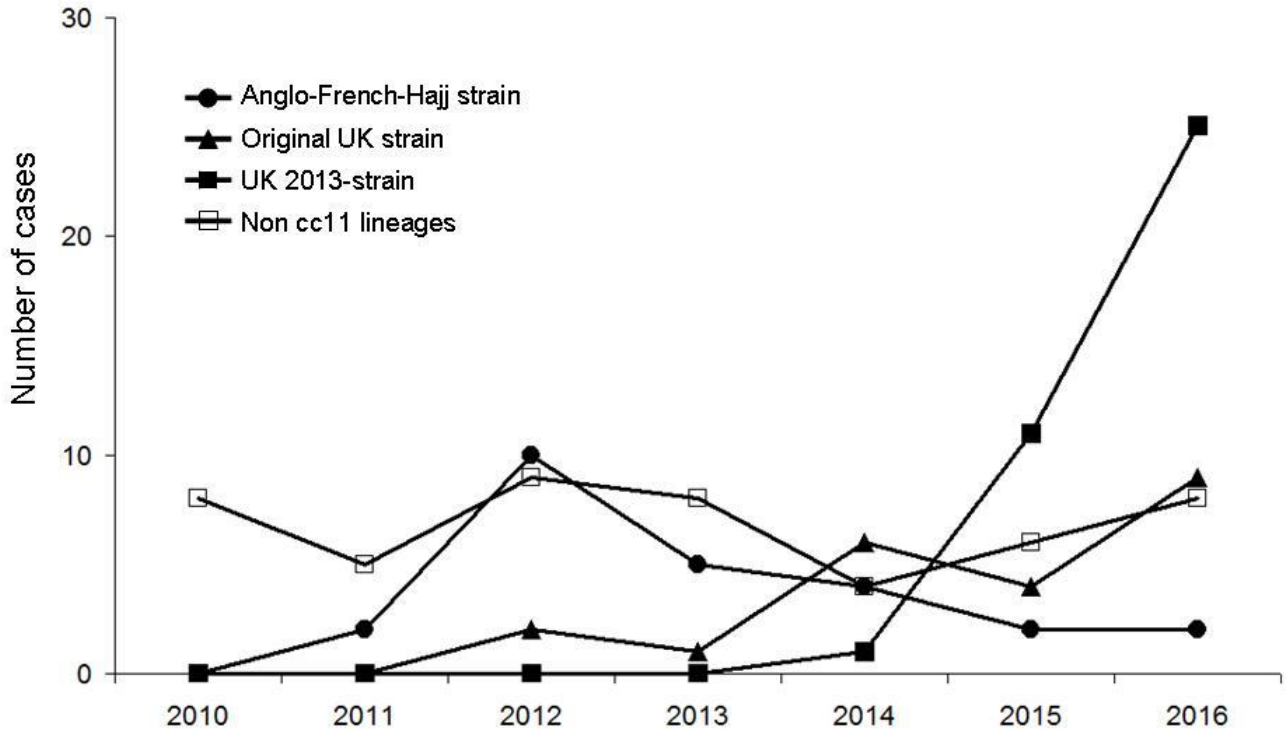


Figure 5: Number of NmW invasive meningococcal disease (IMD) cases by age group and year, according to the four lineages/sub-lineages identified by WGS, France, 2010-2016

Cases are classified in four groups using WGS data: "original UK strain", "UK 2013-strain", Anglo-French-Hajj and non-cc11.

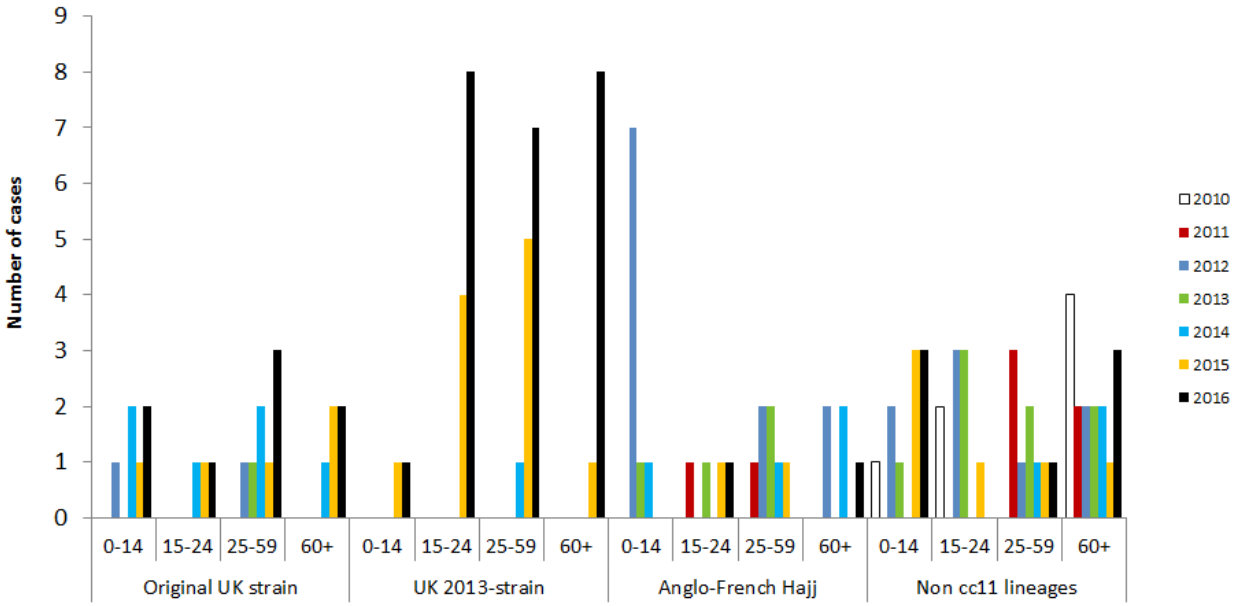


Figure 6

Geographic distribution of NmW invasive meningococcal disease (IMD) cases according to their place of residence, France, 2010-2016.

Cases are classified in four groups using WGS data: "original UK strain", "UK 2013-strain", Anglo-French-Hajj and non-cc11.

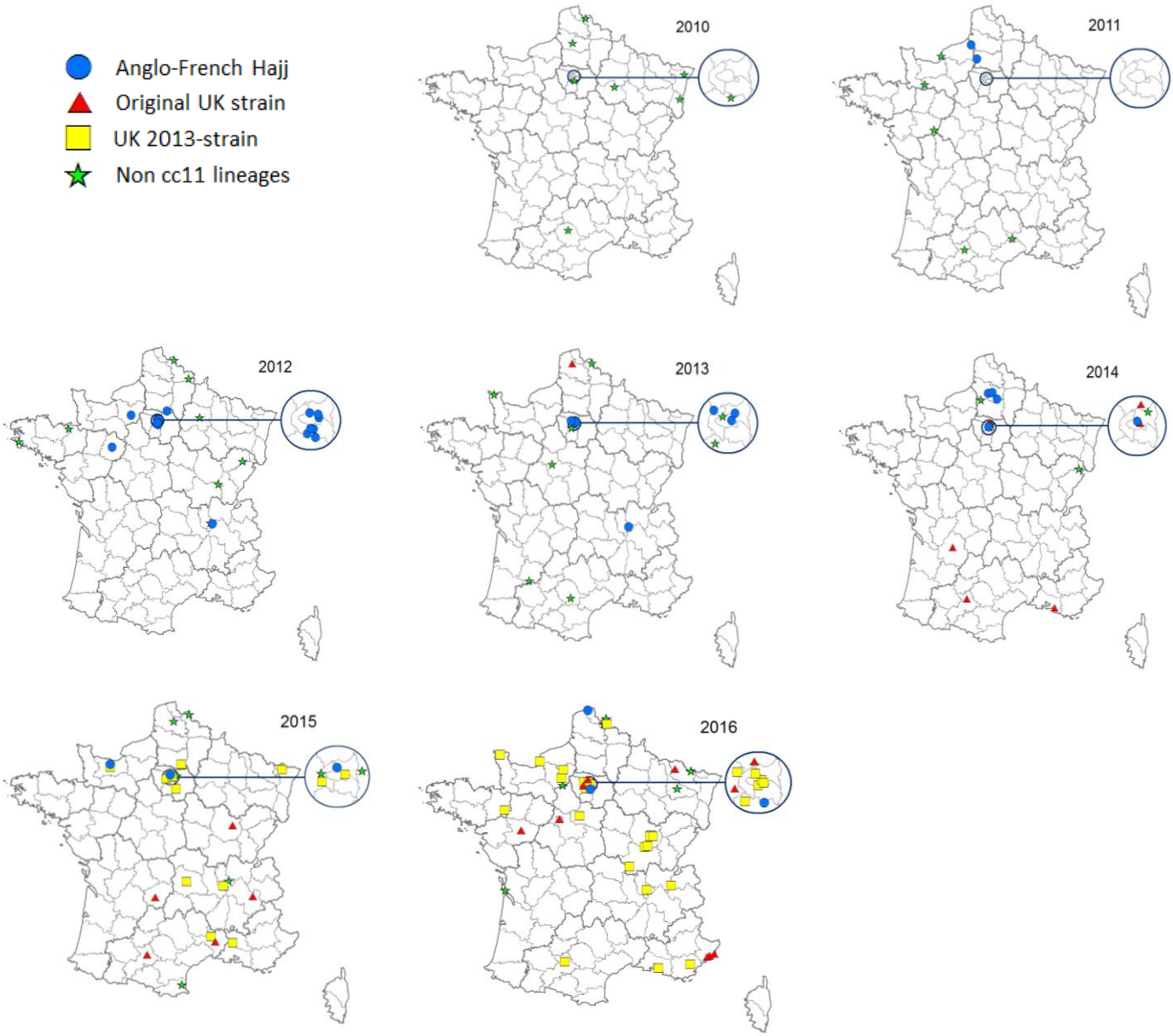


Table1

Table 1: Characteristics of invasive meningococcal disease (IMD) cases according to strain groups, France, 2015-2016 (source: mandatory notification and National reference centre for meningococci)

NB: data are presented for the most frequent groups

	For each group					For group W versus other groups (BCY)		
	Group B (n=503) n (%)	**Group C (n=253) n (%)**	**Group W (n=77) n (%)**	**Group Y (n=116) n (%)**	**p-value**	**Group W (n=77) n (%)**	**Group BCY (n=872) n (%)**	**p-value (W vs BWY)**
Age group (years)					**$<10^{-3}$**			**0.003**
0-14	243 (48.3)	89 (35.2)	15 (19.5)	23 (19.8)		15 (19.5)	355 (40.7)	
15-24	100 (19.9)	54 (21.3)	21 (27.3)	20 (17.2)		21 (27.3)	174 (20.0)	
25-59	104 (20.7)	60 (23.7)	21 (27.3)	24 (20.7)		21 (27.3)	188 (21.6)	
≥ 60	56 (11.1)	50 (19.8)	20 (26.0)	49 (42.2)		20 (26.0)	155 (17.8)	
No. of deaths	39 (7.8)	31 (12.3)	17 (22.1)	30 (17.2)	**$<10^{-3}$**	90 (10.4)	17 (22.1)	**0.002**
Clinical presentation (several sites of infection are possible)								
Meningitis	408 (81.1)	166 (65.6)	44 (57.1)	71 (61.2)	**$<10^{-3}$**	44 (57.1)	645 (74.0)	**0.002**
Septicemia	254 (50.5)	152 (60.1)	60 (77.9)	90 (77.6)	**$<10^{-3}$**	60 (77.9)	496 (56.9)	**$<10^{-3}$**
Arthritis	7 (1.3)	19 (7.5)	7 (9.1)	5 (4.3)	**$<10^{-3}$**	7 (9.1)	31 (3.6)	**0.01**
Purpura fulminans	113 (24.8)	56 (24.1)	8 (13.1)	9 (9.1)	**0.002**	8 (13.1)	178 (22.7)	0.08

Table2

Table 2: Characteristics of NmW invasive meningococcal disease (IMD) cases according to thefour lineages/sub-lineages identified by Whole-Genome Sequencing (WGS), France, 2010-2016 (source: mandatory notification and National reference centre for meningococci)

	Original UK strain	**UK 2013-strain**	**Anglo-French Hajj**	**Not cc11 lineages**	**p-value**
	N=22	**N=36**	**N=25**	**N=44**	
	n (%)	**n (%)**	**n (%)**		
Age (years)					0.09
0-14	6 (27.3)	2 (5.6)	9 (36.0)	10 (22.7)	
15-24	3 (13.7)	12 (33.3)	4 (16.0)	9 (20.4)	
25-59	8 (36.4)	13 (36.1)	7 (28.0)	9 (20.4)	
≥ 60	5 (22.7)	9 (25.0)	5 (20.0)	16 (36.4)	
Case fatality rate	5 (22.7)	10 (27.8)	1 (4.0)	7 (15.9)	0.08
Clinical presentation (several sites of infection are possible)					
Meningitis	12 (54.5)	20 (55.6)	16 (64.0)	20 (45.4)	0.51
Septicemia	17 (77.3)	30 (83.3)	18 (72.0)	31 (70.4)	0.57
Arthritis	1 (4.5)	4 (11.1)	3 (12.0)	4 (9.1)	0.87
Purpura fulminans	3 (17.6)	4 (13.3)	2 (8.3)	5 (13.9)	0.85

Figure1

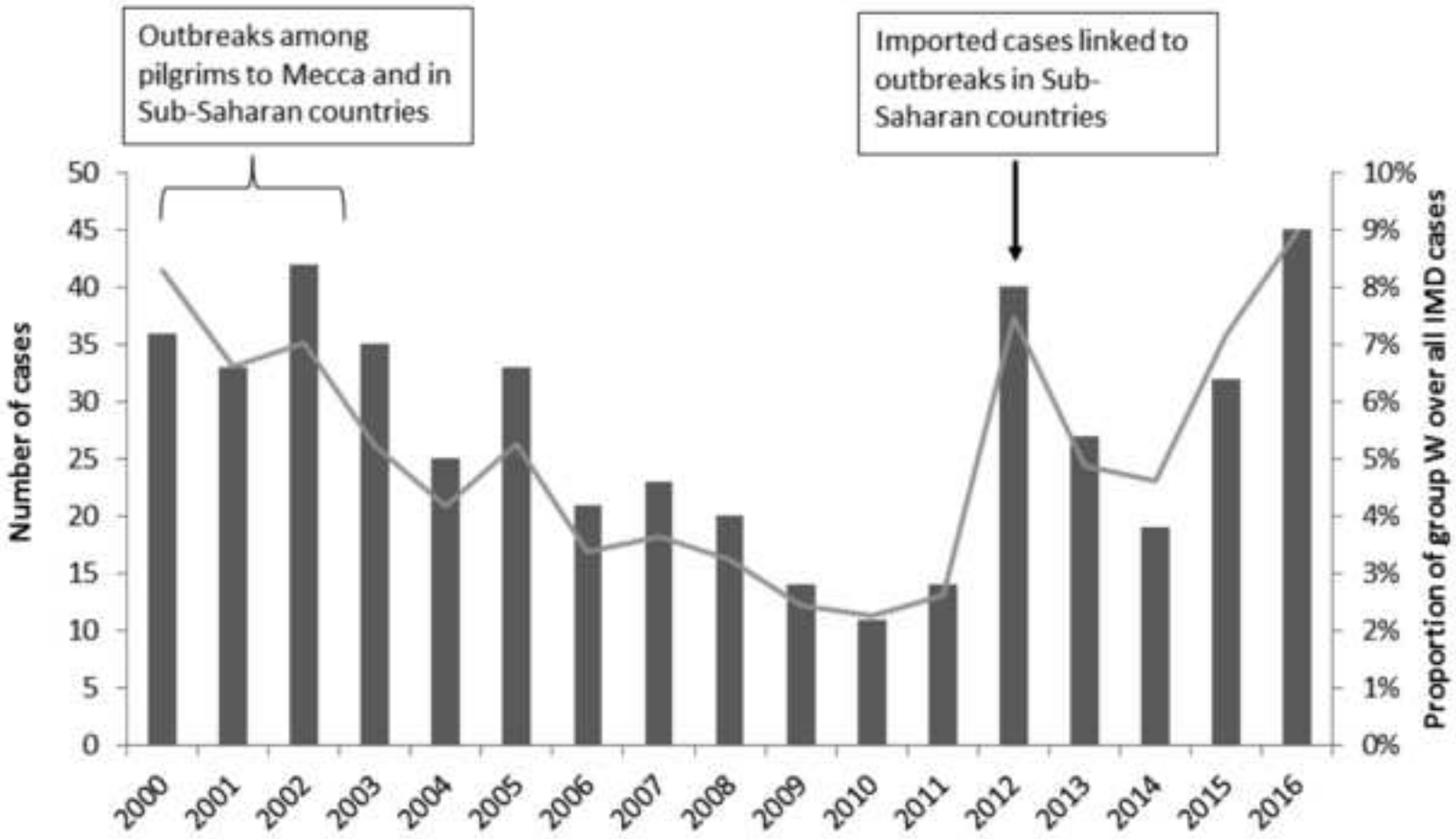

Outbreaks among pilgrims to Mecca and in Sub-Saharan countries
Imported cases linked to outbreaks in Sub-Saharan countries
Number of cases
Proportion of group W over all IMD cases
0
5
10
15
20
25
30
35
40
45
50
0%
1%
2%
3%
4%
5%
6%
7%
8%
9%
10%
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

Figure2

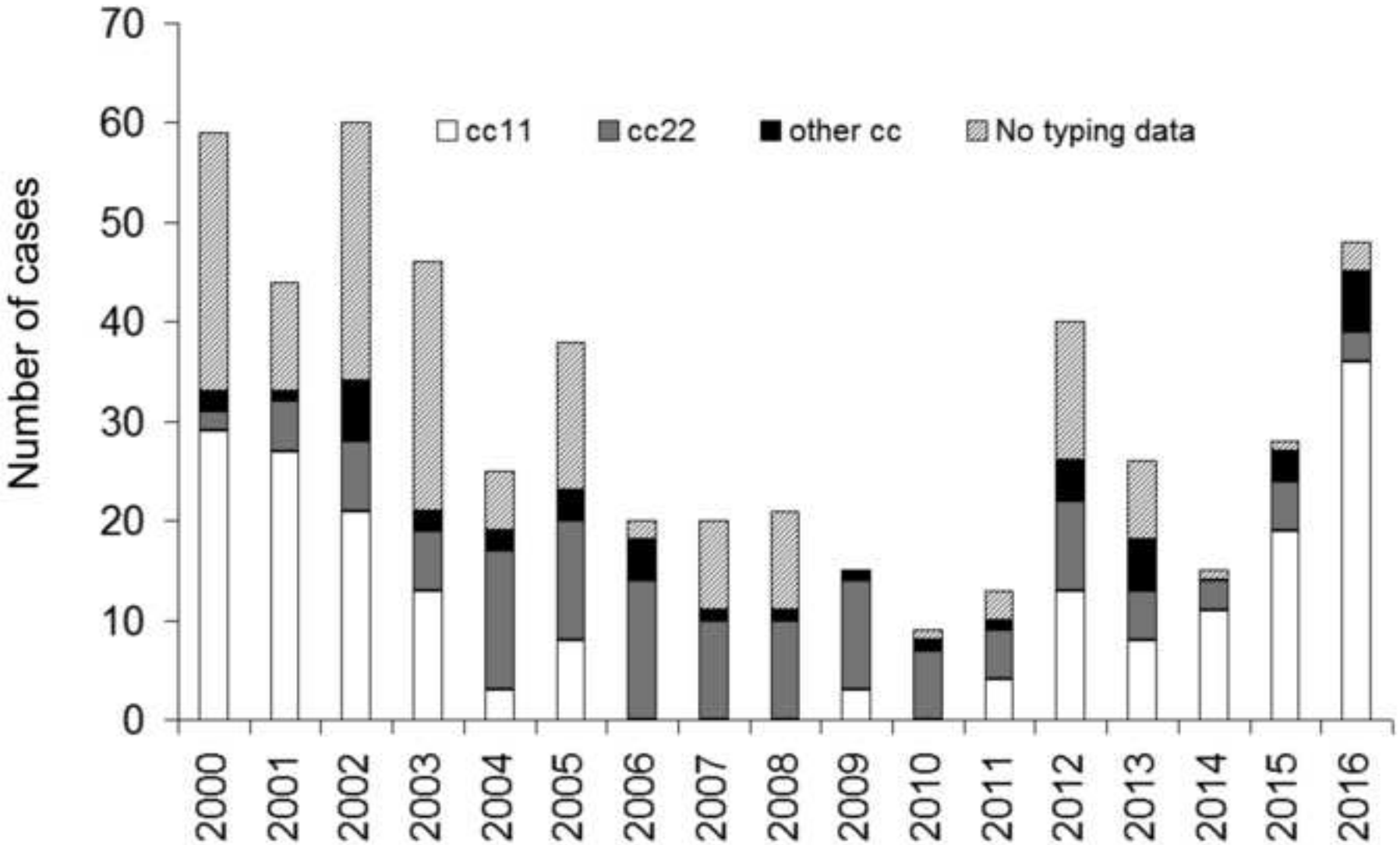

Number of cases
70
60
50
40
30
20
10
0
cc11
cc22
other cc
No typing data
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

Figure3

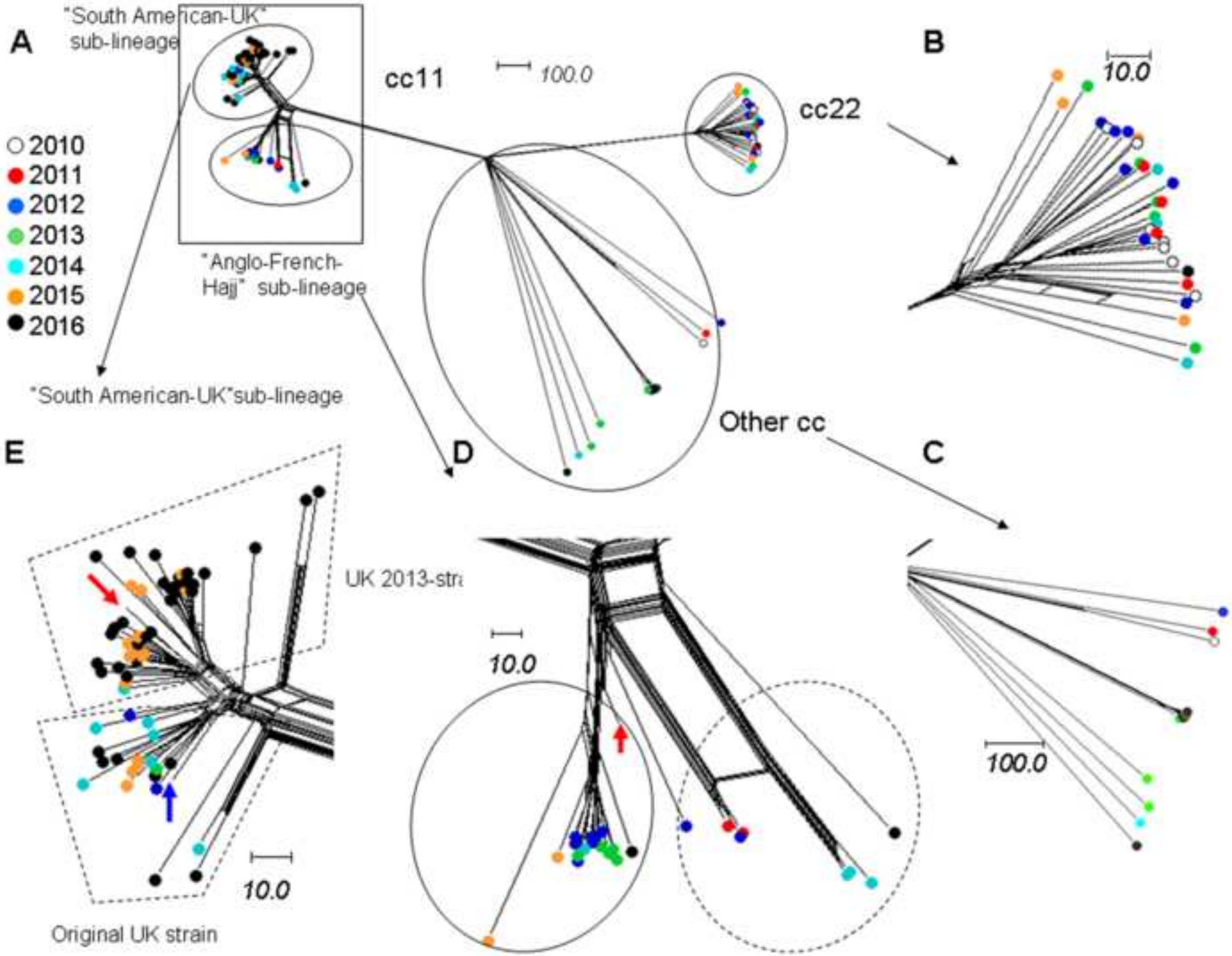

A
"South American-UK" sub-lineage
cc11
100.0
cc22
B
10.0
2010
2011
2012
2013
2014
2015
2016
"Anglo-French-Hajj" sub-lineage
"South American-UK"sub-lineage
Other cc
E
D
C
UK 2013-str
10.0
10.0
100.0
Original UK strain

Figure4

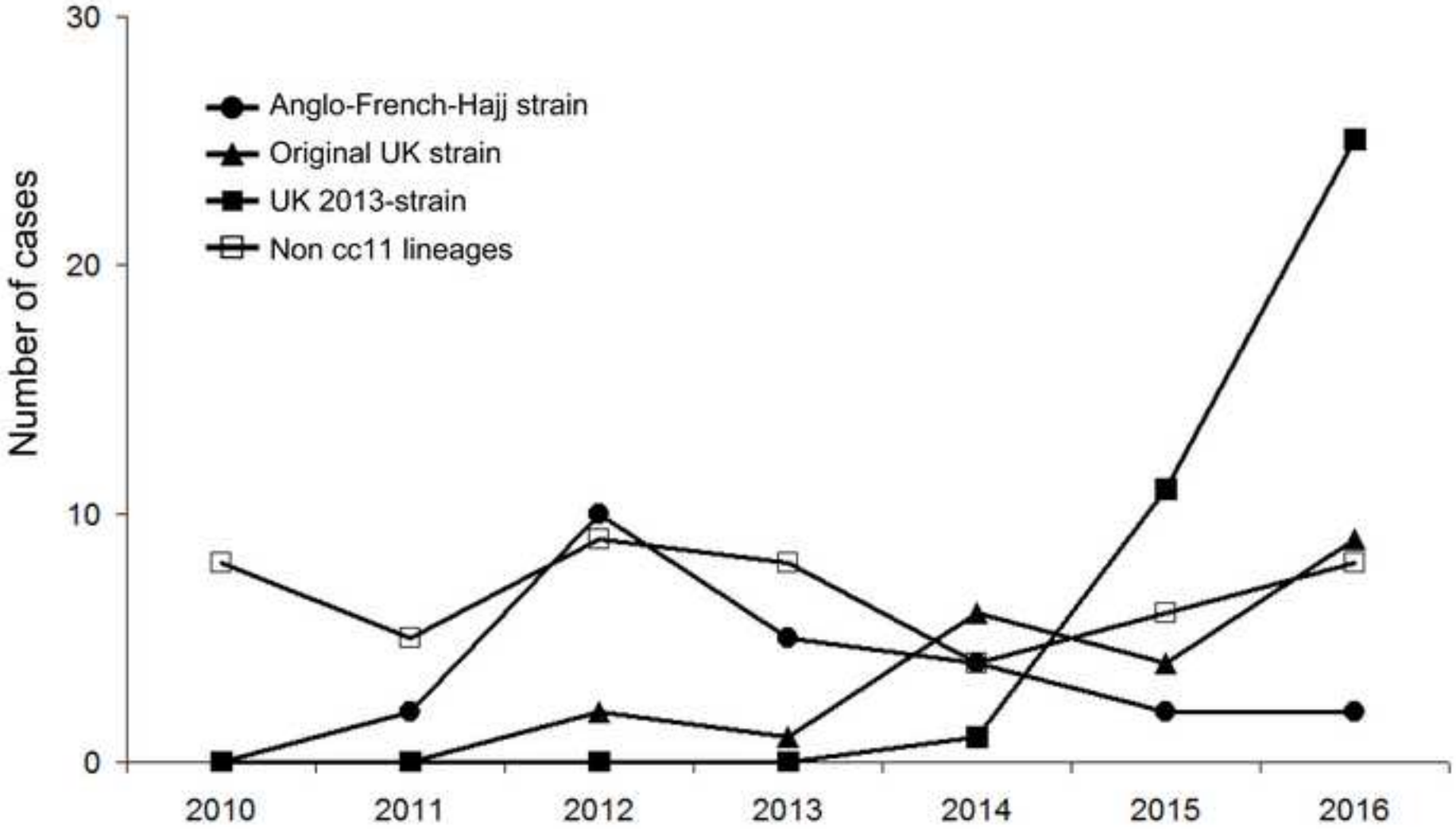

30
20
10
0
Number of cases
Anglo-French-Hajj strain
Original UK strain
UK 2013-strain
Non cc11 lineages
2010
2011
2012
2013
2014
2015
2016

Figure5

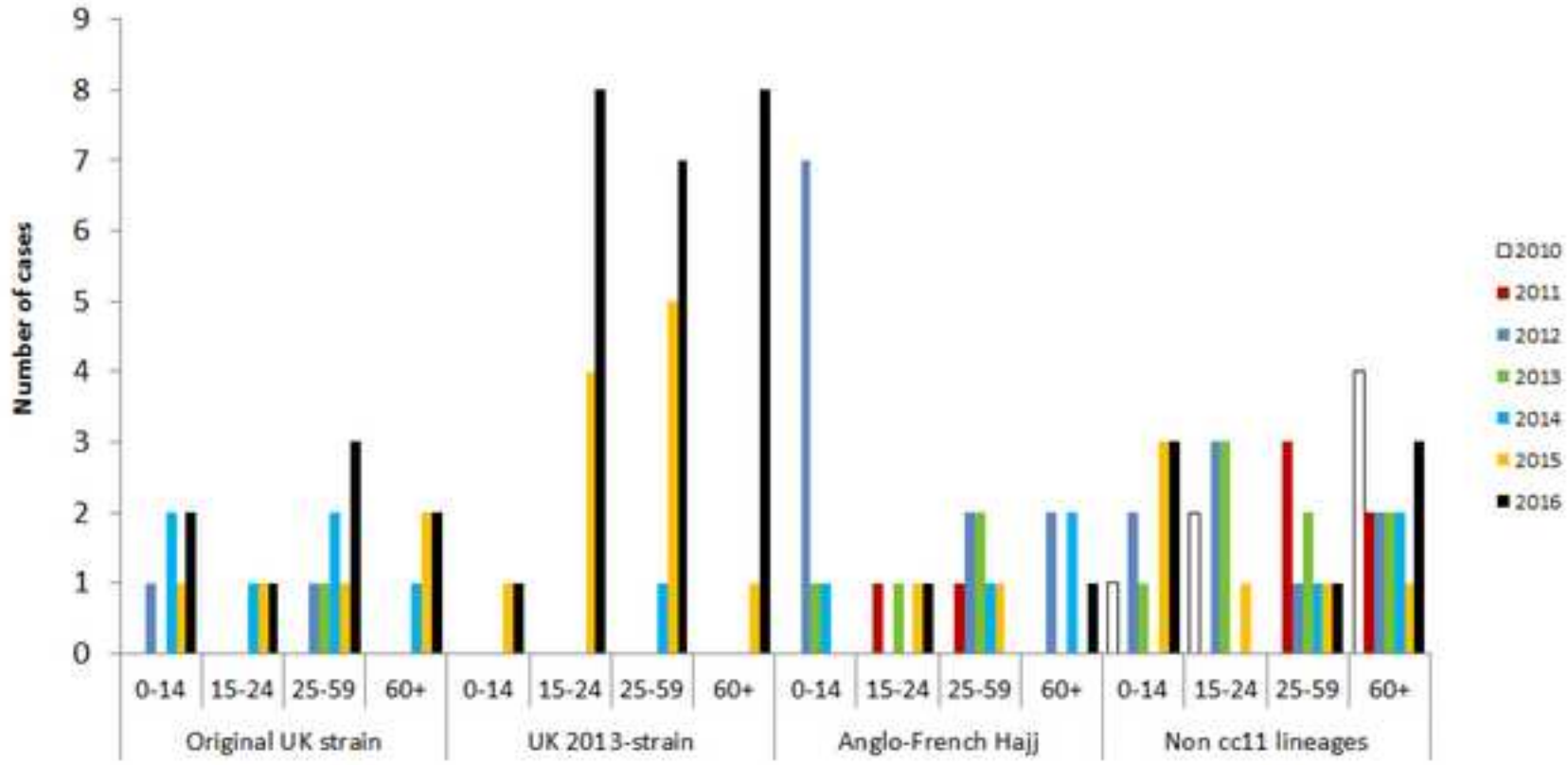
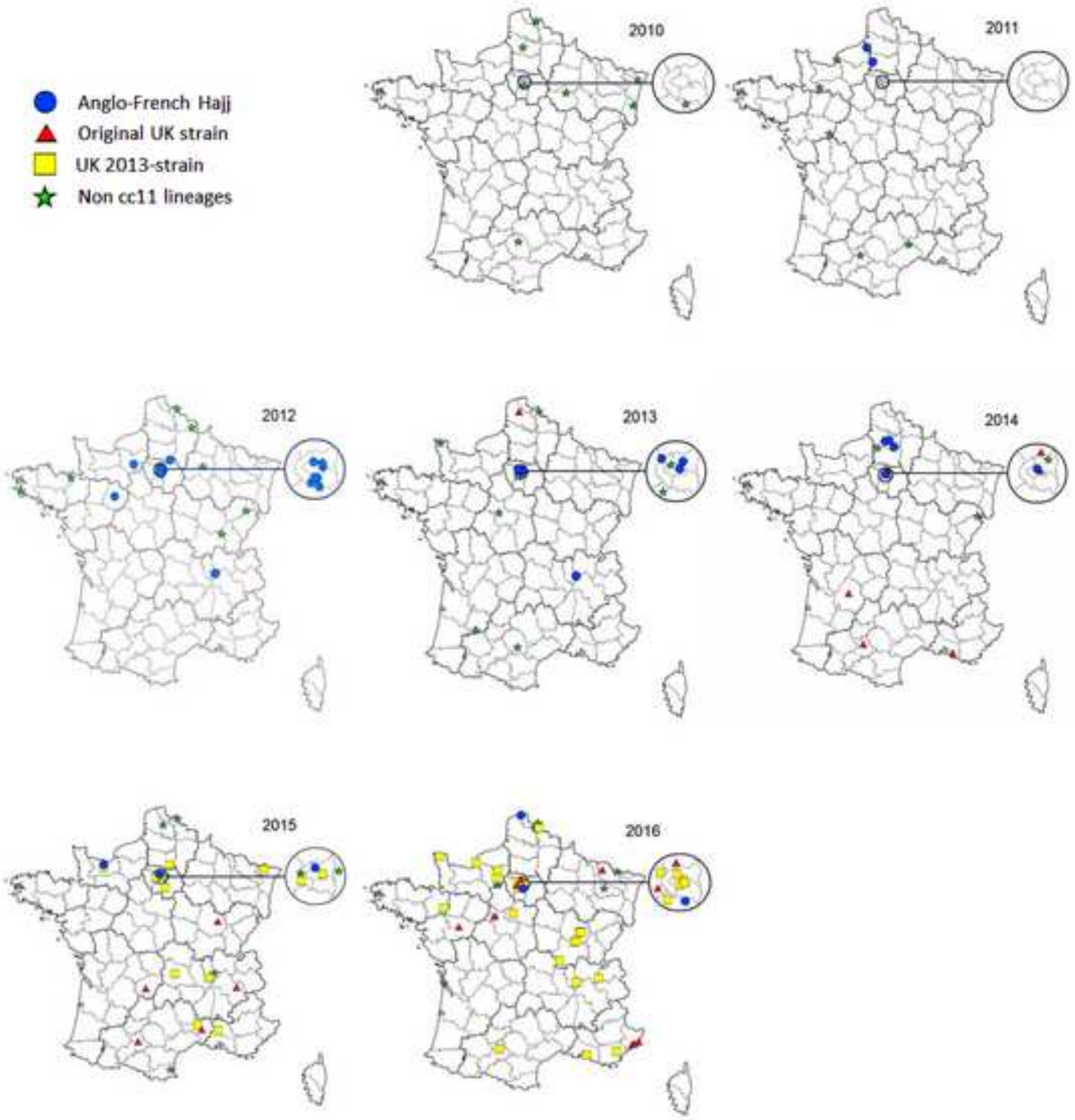

Number of cases
9
8
7
6
5
4
3
2
1
0
0-14
15-24
25-59
60+
Original UK strain
UK 2013-strain
Anglo-French Hajj
Non cc11 lineages
2010
2011
2012
2013
2014
2015
2016

Figure6


Anglo-French Hajj
Original UK strain
UK 2013-strain
Non cc11 lineages
2010
2011
2012
2013
2014
2015
2016

Supplementary Table

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