Genomic Characterization of Urethritis-Associated *Neisseria meningitidis* Shows that a Wide Range of *N. meningitidis* Strains Can Cause Urethritis

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ABSTRACT *Neisseria meningitidis*, typically a resident of the oro- or nasopharynx and the causative agent of meningococcal meningitis and meningococcemia, is capable of invading and colonizing the urogenital tract. This can result in urethritis, akin to the syndrome caused by its sister species, *N. gonorrhoeae*, the etiologic agent of gonorrhea. Recently, meningococcal strains associated with outbreaks of urethritis were reported to share genetic characteristics with the gonococcus, raising the question of the extent to which these strains contain features that promote adaptation to the genitourinary niche, making them gonococcus-like and distinguishing them from other *N. meningitidis* strains. Here, we analyzed the genomes of 39 diverse *N. meningitidis* isolates associated with urethritis, collected independently over a decade and across three continents. In particular, we characterized the diversity of the nitrite reductase gene (*aniA*), the factor H-binding protein gene (*fHbp*), and the capsule biosynthetic locus, all of which are loci previously suggested to be associated with urogenital colonization. We observed notable diversity, including frameshift variants, in *aniA* and *fHbp* and the presence of intact, disrupted, and absent capsule biosynthetic genes, indicating that urogenital colonization and urethritis caused by *N. meningitidis* are possible across a range of meningococcal genotypes. Previously identified allelic patterns in urethritis-associated *N. meningitidis* strains may reflect genetic diversity in the underlying meningococcal population rather than novel adaptation to the urogenital tract.

KEYWORDS *Neisseria gonorrhoeae*, *Neisseria meningitidis*, bacterial genomics, urethritis

The genus *Neisseria* comprises many commensal organisms as well as two primarily pathogenic species. Pathogenic *Neisseria* species include *Neisseria gonorrhoeae* (also known as the gonococcus), the etiologic agent of gonorrhea, and *N. meningitidis* (also known as the meningococcus), the etiologic agent of meningococcal meningitis and meningococcemia. However, the distinction between commensal and pathogen is imprecise: *Neisseria* strains classically defined as commensals can cause disease, and *N. meningitidis* strains defined as pathogenic routinely persist asymptomatically in carriers (1). Accordingly, the meningococcus inhabits the nasopharynx commensally in about 10% of the population; this carriage state is likely the source for symptomatic cases of

meningococcal disease (1). Furthermore, although *N. meningitidis* and *N. gonorrhoeae* were conventionally thought to occupy distinct human ecological niches, case reports in the literature across several decades have indicated that *N. meningitidis* is capable of invading and colonizing the urethra and in doing so results in urethritis akin to that caused by gonococcal infection (2–4). Oral sex has been strongly associated with many such cases, suggesting that pathogenesis depends on orogenital contact (2, 5, 6).

N. meningitidis strains isolated from cases of urethritis serve as natural experiments well-suited for advancing our understanding of how *Neisseria* strains diverge and specialize for ecological niches within their human hosts. Investigating the dynamics and mechanisms by which these atypical isolates have potentially adapted could also improve epidemiological characterization of the transmission networks of pathogenic *Neisseria*. Whole-genome sequencing offers one approach for both understanding the epidemiology of *N. meningitidis*-associated urethritis and interrogating the genetic basis of possible adaptation of these meningococcal lineages. Recent studies employing genomics have suggested that particular alleles of nitrite reductase (AniA), the factor H-binding protein (fHbp), and the capsule are associated with *N. meningitidis* strains isolated from genitourinary infections (2, 3, 7, 8). However, because these studies focused primarily on genetically related isolates, their power to distinguish genuine adaptive features from shared features due to population structure was limited. We thus assembled and sequenced a diverse collection of urethritis-associated meningococcal strains to assess whether any previously identified or novel genetic signals could explain the unusual pathogenesis of these lineages.

RESULTS AND DISCUSSION

Isolate metadata and population structure. Our sample set included 39 isolates of urethritis-associated *N. meningitidis* and 1 isolate of urethritis-associated *N. lactamica*, all collected from male patients over a decade across eight countries (Table 1). The average age of the patients for whom metadata were available ($n = 24$) was 30.8 years, with a range of 21 to 52 years. All except one of the patients had symptoms of urethritis (Table 1). The core genome of all 40 isolates comprised 1,237 genes, of which 1,177 were included in the core genome alignment, whereas the core genome of the 39 meningococcal isolates comprised 1,384 genes. The maximum likelihood phylogeny constructed using a concatenated core genome alignment and rooted using the *N. lactamica* isolate (NlUS07-1) indicated a diverse sampling of urethritis-associated neisserial strains (Fig. 1), with computational sequence typing revealing seven clonal complexes and 19 sequence types (STs) in this data set (Table 1).

Of the meningococcal strains, 13 isolates (33.3%) belonged to the hyperinvasive lineage ST-11 (electrophoretic type 37 [ET-37]) or ST-41/44 complex (lineage 3), and 17 (43.6%) belonged to the emerging invasive lineage ST-23 (cluster A3), ST-213, or ST-269. The hyperinvasive ST-11 (ET-37) lineage included previously reported urethritis-associated *N. meningitidis* strains, such as NmUS16-1 and NmUS16-2 collected by Toh et al. in 2017 (2) and the men who have sex with men (MSM)-associated strain NmFR12-1 collected by Taha et al. in 2016 (7). Three isolates from Italy obtained through PubMLST (NmIT14-1, NmIT14-2, NmIT14-3) were also part of this clonal complex. The emerging invasive lineage ST-23 was represented by a cluster of 12 isolates from Japan and 1 isolate from the United States (NmUS02-1). One isolate (NmUS03-2) harbored an unknown sequence type. In this isolate, every gene queried as part of multilocus sequence typing (MLST) was associated with an allele in the PubMLST database (*abcZ*, $n = 7$; *adk*, $n = 16$; *aroE*, $n = 55$; *fumC*, $n = 10$; *gdh*, $n = 3$; *pdhC*, $n = 56$; *pgm*, $n = 16$); however, the combination of alleles together was novel. The remaining isolates belonged to rare clonal complexes. The three *N. meningitidis* urethritis isolates (NmJP12-3, NmSL15-1, NmJPb05-1) phylogenetically the closest to the gonococcus formed a distinct clade and were all grouped under ST-198 (see Fig. S1 in the supplemental material). Prior literature has indicated that, out of *cnl* meningococcal strains, ST-198 isolates in particular have been able to cause invasive meningococcal disease, despite being unencapsulated (9). The presence of three such unusual isolates
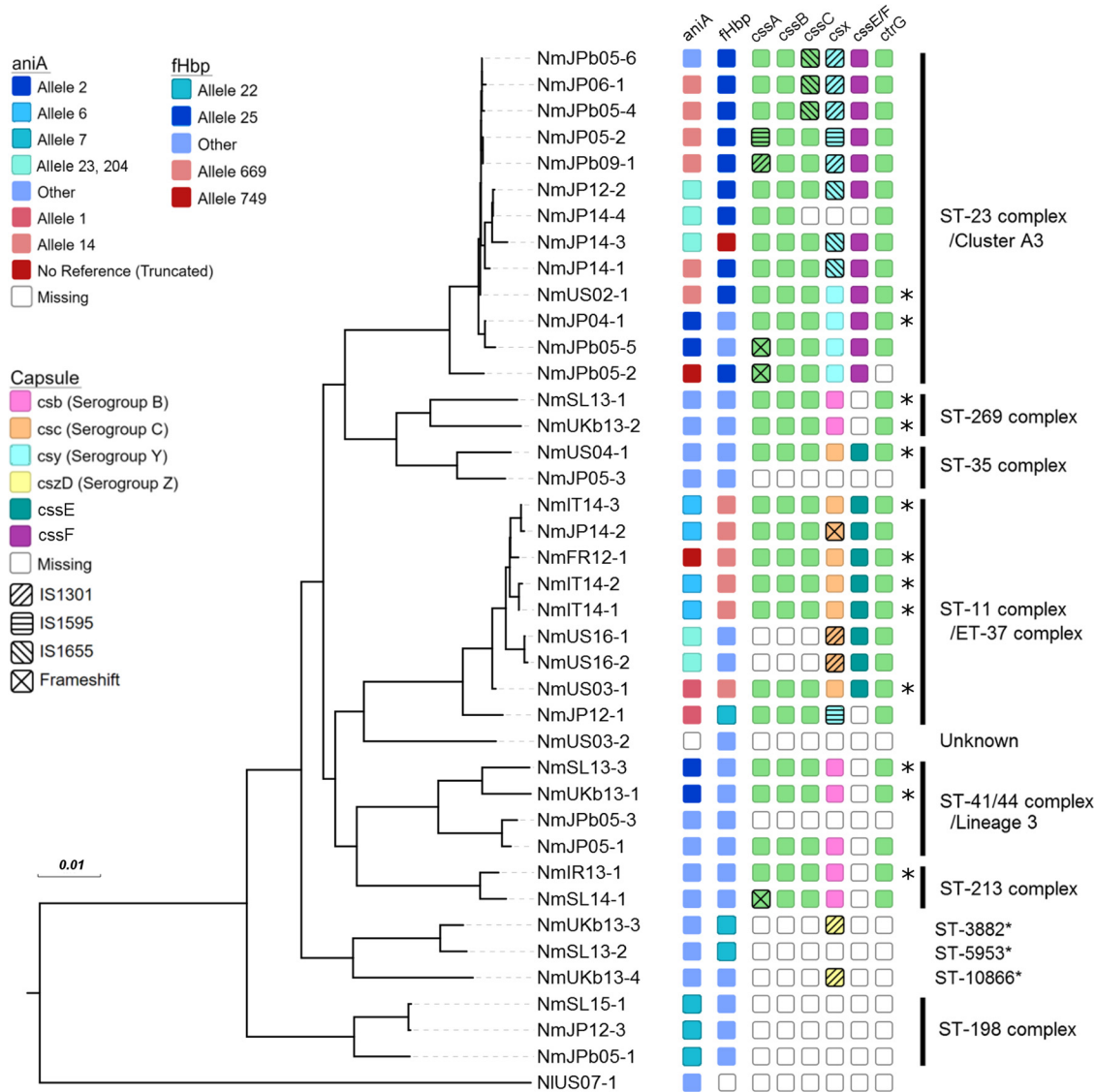
FIG 1 Core genome phylogeny and genomic characterization of *aniA*, *fHbp*, and capsular region A of urethritis-associated *N. meningitidis* and *N. lactamica* isolates. For *aniA* and *fHbp*, blue shading indicates a full gene product and red shading indicates a truncated gene product. Alleles associated with truncated genes and alleles associated with full genes found in three or more isolates are specifically denoted in the key on the left; all other alleles were grouped into the "other" category. *aniA* alleles 23 and 204 are putatively gonococcal in origin. For capsule region A, *csx* variants which determine the serogroup (where *csx* can be *csb*, *csc*, *csy*, or *cszD*), the presence of either *cssE* or *cssF*, and capsule gene disruption via insertion sequence or frameshift are specified by the colors and symbols in the key. *, strains predicted to encode capsules; unshaded boxes, missing genes. Clonal complexes are indicated along the right, with asterisks indicating no associated clonal complex.

in our data set raises the possibility that urethritis is another manifestation of this clade's pathogenicity.

Nitrite reductase gene (*aniA*) and factor H-binding protein gene (*fHbp*). The nitrite reductase gene (*aniA*) of *N. meningitidis* is often frameshifted in clinical isolates but is intact in *N. gonorrhoeae*, suggesting that AniA may play an important role for homeostasis in the microaerophilic environment of the urogenital tract (7). In keeping with this hypothesis, four of the five isolates in a collection of urethritis-associated *N. meningitidis* isolates collected in France and Germany from 2006 to 2012 harbored an intact *aniA* gene and exhibited active nitrite reductase activity *in vitro* (7). However, because these strains were closely related, the association of intact *aniA* with urogenital infection could instead be due to the population structure. We thus investigated the allelic diversity of *aniA* and characterized the prevalence of frameshifted variants to under-

stand whether intact *aniA* is associated with urogenital colonization. We found that the *aniA* gene was present in all isolates except NmUS03-2; mapping the sequencing reads of this isolate to the MC58 reference genome (GenBank accession number AE002098.2) showed a drop in coverage to zero for *aniA* and the loci immediately adjacent. Excluding NmFR12-1 (original name, LNP26948) from Taha et al. (7), we found that 24.3% (9/37) of the isolates harbored a truncated *aniA*, indicating that intact *aniA* is not necessary for urogenital colonization (Fig. 1). Full-length *aniA* exhibited a range of alleles, whereas truncated *aniA* largely belonged to allele 14 (6/9 isolates); this was also evident in the nucleotide phylogeny, where most of the truncated *aniA* sequences clustered together (Fig. S2). Intriguingly, 12.8% (5/39) of the isolates harbored genes with suspected gonococcal origins (as determined by BLAST analysis of the nucleotide sequences against the sequences in the nr/nt database). These five isolates were split into two clades, each harboring a distinct allele: NmJP14-3, NmJP14-4, and NmJP12-2 contained allele 23, and NmUS16-1 and NmUS16-2 from Toh et al. (2) contained allele 204 (Fig. 1). Tzeng et al. recently reported that NmUS16-1, NmUS16-2, and additional urethritis-associated isolates in the same lineage contained gonococcal nitric oxide reductase genes (*norB*), in addition to gonococcal *aniA* (8). In NmJP12-2, NmJP14-3, and NmJP14-4, we similarly found that *norB* is gonococcal in origin, implying that the entire *norB*-*aniA* cassette was acquired via horizontal recombination.

Frameshifted *fHbp* has been associated with urethritis isolates and results in significant effects on virulence in a mouse model (7). In our data set, all isolates contained the *fHbp* gene, and considerable diversity was present, with all three Novartis variants being represented. Only a minority of samples (6/38, excluding NmFR12-1) harbored truncated *fHbp* (alleles 669 and 749 in Fig. 1), indicating that this particular genotype is not strongly associated with urogenital colonization. Furthermore, 5/6 isolates with truncated *fHbp* (NmIT14-1, NmIT14-2, NmIT14-3, NmUS03-1, and NmJP14-2) contained the same allele (allele 669) and belonged to the same clonal complex (the ST-11 complex) as NmFR12-1, suggesting that the association of urethritis with *fHbp* truncation is likely due to relatedness between strains (Fig. 1).

Additional genetic features present in *N. gonorrhoeae* but not generally found in *N. meningitidis* may also be responsible for the potential urogenital adaptation undergone by our urethritis isolates. We therefore examined the allelic distribution in our sample set of the class 1 outer membrane porin PorA and the membrane-bound *c*-type cytochrome CcoP, the genes for both of which were previously characterized as divergent between *N. meningitidis* and *N. gonorrhoeae* (10, 11). Feavers and Maiden found that in the gonococcus, the expression of the PorA protein was inactivated via frameshift mutations and deletions of parts of the TATAAT box and the poly(G) residue portions of the promoter (11), and Aspholm et al. found that in the meningococcus, a single nucleotide polymorphism resulting in CcoP truncation was present (10). In both cases, *N. meningitidis* strains associated with urethritis in our data set harbored genes with wild-type meningococcal characteristics (i.e., an intact *porA* gene and promoter and truncated *ccoP*), suggesting that a gonococcus-like *porA* pseudogene and *ccoP* are not strictly necessary for neisserial colonization of the urogenital tract.

Capsule. The meningococcal capsule is important in the pathogenesis of meningococcal disease, in that it facilitates bacterial survival by promoting evasion of the immune system; however, capsule expression also appears to detrimentally impact adhesion and entry into human cells (12). Because capsule disruptions have previously been found in urethritis-associated *N. meningitidis* strains (2), we characterized the biosynthetic locus in our isolates, with a particular focus on genes in region A, which comprises capsule biosynthesis enzymes. For serogroups B, C, W, and Y, region A genes include *cssA*, *cssB*, and *cssC*, which function in cytidine-5′-monophosphate-*N*-acetylneuraminic acid synthesis, as well as a *csx* (where *csx* can be *csb*, *csc*, *csw*, or *csy*) gene which encodes a serogroup-specific polymerase (13). We also characterized genes in region B, comprising capsule translocation genes *ctrE* and *ctrF*, and genes in region

C, comprising capsule export genes *ctrABCD*. We considered present, intact genes in regions A through C to be necessary for production of the capsule.

Isolates NmSL15-1, NmJPb05-1, NmJP12-3, and NmJPb05-3 contained *cnl* allele 2, where *cnl* is defined to be an approximately 113-bp intergenic region that has replaced regions A and C of the capsule (Fig. 1) (13). Three of these isolates (excluding NmJPb05-3) belonged to the ST-198 clade; among the rare cases of unencapsulated *N. meningitidis* isolates associated with invasive disease, isolates from the ST-198 clade are frequently found (9). Isolates in the ST-198 clade also lacked region B (*ctrE*, *ctrF*) genes, whereas NmJPb05-3 possessed them. Three other isolates (NmSL13-2 in ST-5953, NmUS03-2 with an unknown sequence type, and NmJP05-3 in the ST-35 complex) also lacked capsule region A genes but did not possess any of the characteristic *cnl* alleles present in the PubMLST database. While NmSL13-2 and NmUS03-2 belonged to unusual sequence types and possessed intact region B and C genes, NmJP05-3 lacked region C genes and was closely related to an encapsulated meningococcus in the ST-35 complex (NmUS04-1), suggesting that loss of the capsule occurred due to recombination.

Out of the isolates in which capsule genes were present, 17 harbored some type of disruption. Four of these 17 strains contained a frameshift mutation in a capsule region A gene: NmJPb05-2, NmJPb05-5, and NmSL14-1 contained various insertions in non-homopolymeric regions of the *cssA* gene, resulting in a truncated peptide product, and NmJP14-2 contained a 1-nucleotide deletion resulting in truncation of the *csc* gene. The remaining 13 isolates contained insertion sequence-mediated disruptions. In NmUS16-1, NmUS16-2, NmUKb13-3, and NmUKb13-4, IS*1301*-mediated disruption of the *csx* (where *csx* is *csb*, *csc*, *csy*, or *cszD*) gene was also associated with complete disruption of the upstream *cssABC* operon. In the ST-23 complex, a cluster of eight related isolates from Japan harbored one or two insertion sequence disruptions in the middle of the *cssA*, *cssC*, or *csy* gene. Within this clade, the distribution of insertion sequence families and the pattern of genes disrupted appeared to mirror the core genome maximum likelihood phylogeny (Fig. 1).

The remaining 14 isolates harbored an intact capsule region A; 6 of these isolates (NmSL13-1, NmSL13-3, NmJP05-1, NmIR13-1, NmUKb13-1, and NmUKb13-2) belonged to serogroup B, 6 (NmUS04-1, NmUS03-1, NmFR12-1, NmIT14-1, NmIT14-2, and NmIT14-3) belonged to serogroup C, and 2 (NmJP04-1 and NmUS03-1) belonged to serogroup Y. While the assemblies of three of these isolates contained frameshift mutations in either the *csb* (NmSL13-1 and NmJP05-1) or *csy* (NmUS01-2) gene, these mutations were found in homopolymeric tracts of C or A residues that play a role in reversible phase-variable expression, hindering exact prediction of the capsular phenotype (14). With the exception of NmJP05-1, which contained a frameshift mutation in *ctrA*, all 13 of these isolates also harbored intact region B and C genes; thus, in our sample set, up to one-third of the urethritis-associated *N. meningitidis* strains are predicted to be encapsulated. Isolate NmFR12-1 from Taha et al. (originally denoted LNP26948) was previously confirmed to produce capsule serogroup C (7), and isolate NmSL13-3 from this study was confirmed to produce capsule serogroup B via slide agglutination. Although we cannot indicate for certain capsule production for the other strains solely from a genomic assessment, in keeping with our observation, we found other reports of encapsulated urethritis-associated *N. meningitidis* strains throughout the literature (15–17).

Horizontal gene transfer. Horizontal gene transfer offers a critical source of genetic diversity for neisserial adaptation to environmental pressures, especially with respect to antibiotics. Reduced susceptibility and resistance to the third-generation cephalosporins arises primarily through interspecies horizontal recombination of the *penA* locus (18); the most common of these mosaic variants is known as the *penA* XXXIV allele. Deghmane et al. identified this allele in invasive and urethritis-associated meningococci with decreased susceptibility to cefotaxime and ceftriaxone, two of the drugs used to treat meningococcal infection (19). In our sample set, we identified two isolates

(NmIT14-3 and NmJP14-2) that contain the *penA* XXXIV allele. These isolates were within the ST-11 complex and clustered closely together on the phylogenetic tree; furthermore, they shared the same fine type (C:P1.5-1,10-8:F3-6:ST-11) as the invasive meningococcal isolates identified by Deghmane et al. (19). The absence of the *penA* XXXIV allele in other urethritis-associated isolates suggests that the acquisition of this allele is not related exclusively to urogenital infection and can instead be explained by the clonal spread and diversification of a C:P1.5-1,10-8:F3-6:ST-11 ancestral strain that acquired the *penA* XXXIV allele from *N. gonorrhoeae*.

Recombination of other genomic fragments from the gonococcus, which has adapted for sexual transmission, may confer advantages for meningococcal colonization of the genitourinary niche. To undertake a systematic approach for investigating horizontal gene transfer events, we analyzed the core genome shared between our 39 isolates and 13 of the World Health Organization (WHO) gonococcal reference strains (20). Genes that contained signals of gonococcal-to-meningococcal recombination, as detected by the fastGEAR method, in multiple urethritis-associated meningococcal isolates were further investigated. Overall, we found that of the 1,237 identified meningococcal core genes, the majority (1,198, or 96.8%) contained no signal of gonococcal-to-meningococcal recombination. Of the remaining 39 genes, 33 contained only one or two recombination events. *dtpT* and *infB* each contained 10 instances of detected recombination; however, the detected recombination loci for *dtpT* were only weakly supported (Bayes factors, 1.2 to 2.0). Recombinations in *infB* were more strongly supported (Bayes factors, 10.6 to 61.9) but were generally found in meningococcal lineages phylogenetically the closest to gonococcal lineages, suggesting that identity by descent could also be a possible explanation. Because signals of recombination spanning over the entire gene (e.g., as found in *aniA* and *norB* as described above) may be overlooked by fastGEAR, we also examined genes for which at least five meningococcus-derived genes were clustered *a priori* into gonococcal lineages. Out of these results, we identified only *aniA* and *norB* to be hits after filtering out genes with low levels of diversity (as defined by genes with 90% of sites or greater identical). Thus, recombination in the core genome does not appear to be a necessary component of adaptation to the urogenital environment.

Conclusion. An increase in *N. meningitidis*-associated cases of urethritis raises the question of an emerging urethrotropic meningococcal clade (4, 6). Recent genomic analyses of *N. meningitidis* strains isolated from cases of urethritis have suggested that particular alleles of *aniA* and *fHbp* and disruptions in the capsule biosynthetic enzymes may be associated with the atypical pathogenesis of these strains (2, 7). To further investigate these associations, we assembled and sequenced a broad convenience sample collection of urethritis-associated *N. meningitidis* strains collected independently over a decade and across three continents. We found that most isolates belonged to hyperinvasive or emerging invasive clonal complexes, with the remainder being associated with either no clonal complex or unusual ones, such as the *cnl* invasive ST-198 complex. We found that the nitrite reductase gene *aniA* is generally intact, but the presence of frameshifts resulting in truncated proteins in nearly a quarter of our isolates implies that *N. meningitidis* can survive in the microaerophilic environment of the urethra without nitrite reduction. We observed two instances of putative gonococcal-to-meningococcal recombination of the *norB-aniA* gene cassette, which may promote anaerobic growth in the urogenital tract (8). The previously reported association of an *fHbp* disruption with urethritis-associated *N. meningitidis* appears to be a result of the population structure, as the majority of our non-ST-11 complex strains harbored intact *fHbp*. Finally, we observed substantial diversity in the capsule, including intact open reading frames, insertion sequence disruptions, frameshift mutations, and *cnl*, showing that urogenital colonization is possible across a range of capsular phenotypes.

On the basis of our findings, a phylogenetically diverse array of *N. meningitidis* strains can cause urethritis, affirming that the textbook niche specifications of *Neisseria*

species are too narrow. We note several questions, unanswered in this study, that may be promising avenues for future investigation. First, these results do not address whether certain lineages of *N. meningitidis* may be better adapted to growth and transmission once they are within the urogenital niche. Second, because the sample used in this study was a convenience sample, population-level prevalence and mutational diversity could not be inferred. Epidemiological studies that evaluate the incidence of meningococcal urethritis, meningococcal nasopharyngeal carriage, and sexual behaviors will help distinguish whether increased rates of urethritis may be due to spillover from individuals with higher carriage rates or a higher frequency of orogenital contact or lineage-specific adaptation to the urogenital niche. Subsequent functional analyses will then be required to characterize the role of candidate genes and alleles (8). Third, the association of *N. meningitidis* with cases of urethritis does not necessarily imply a causal link; for instance, coinfection with other sexually transmitted organisms that cause nongonococcal urethritis (e.g., *Chlamydia trachomatis*, *Mycoplasma genitalium*) can occur. In some but not all urethritis cases described here, testing was done to rule out coinfection with *C. trachomatis* (Table 1). Future studies confirming the causation of urethritis by *N. meningitidis* should likewise aim to rule out other causes of nongonococcal urethritis. With the increased incidence of *N. meningitidis*-associated cases of urethritis, as reported by Bazan et al. (4, 6) and Toh et al. (3), leading to concern about the emergence of meningococcal lineages adapted to urogenital infection and transmission, such studies will be critical for informing the appropriate clinical and public health responses.

MATERIALS AND METHODS

Sample collection. Nongonococcal *Neisseria* isolates associated with men with urethritis (39 *N. meningitidis* isolates and 1 *N. lactamica* isolate) were obtained from the U.S. Centers for Disease Control and Prevention's (CDC's) Gonococcal Isolate Surveillance Project (GISP) (*n* = 5), the World Health Organization (WHO) Collaborating Centre (CC) for Gonorrhoea and Other Sexually Transmitted Infections (*n* = 5), the Japanese National Institute of Infectious Diseases (NIID) (*n* = 19), and from prior studies (*n* = 11). GISP isolates were strains presumed on collection to be gonococcus but after sequencing were identified by Kraken (21) to be nongonococcal. WHO CC and Japanese isolates were identified through routine culture-based diagnosis by the use of samples from men with urethritis. Isolates were sequenced as described below. Previously published sequences included urethritis-associated *N. meningitidis* genome sequences described in prior publications (*n* = 7) and sequences found in the online PubMLST database (*n* = 4) (Table 1). PubMLST isolates were located by querying the database for isolates by the use of *Neisseria meningitidis* for the species and urethral for the source. Isolates were named (or renamed for previously published isolates; Table 1) according to the country of origin and year of isolation.

DNA sequencing and analysis. DNA was prepared from the isolates, and Nextera libraries were constructed using standard protocols, with sequencing being performed on an Illumina platform. FASTQ format reads were quality trimmed using the Sickle tool (https://github.com/najoshi/sickle), underwent quality control in FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and de novo assembled using the SPAdes (version 3.6.2) genome assembler (22). A genus-level phylogeny was constructed using the PhyloPhlAn method (23), incorporating reference strains selected from a *Neisseria*-wide taxonomy (24). A species-level maximum likelihood phylogeny was constructed with the RAxML (version 8.2.8) tool (25) using a core genome alignment generated by Roary analysis (version 3.6.0) with a minimum BLASTP identity of 90% (26). Meningococcal fine typing was conducted using the meningotype (v0.7-beta) program, and computational serogrouping results were corrected when additional information was available (see "Candidate gene analysis" below) (27). Phylogenetic trees were visualized and annotated in FigTree software (http://tree.bio.ed.ac.uk/software/figtree/).

Candidate gene analysis. An *aniA* reference nucleotide sequence from MC58 (GenBank accession number AE002098.2) was used in a BLASTn query with default parameters against a BLAST database built using the contigs from the *N. meningitidis* urethritis isolate genomes. Top hits were extracted from the contigs. Samples with no BLAST hits were denoted as missing. Each nucleotide sequence was queried against the PubMLST database to identify its allelic number. Sequences which did not match a PubMLST allele were translated to assess whether the peptide was truncated. Nucleotide sequences were aligned using the MAFFT (version 7.017) multiple-sequence alignment program (28) and phylogenetic trees constructed in Geneious (version 9.1.7) software (29) using the neighbor-joining method.

fHbp allele 1 (Pfizer subfamily B, Novartis variant family 1), as defined in the PubMLST database (locus *fHbp*), was used in a BLASTn query with default parameters against the *N. meningitidis* urethritis isolate contig database. Top BLASTn results were extracted from the contigs and queried against the PubMLST database to identify its allelic number.

Capsule region A genes were first analyzed using the PubMLST genome query tool (30). Isolates with all genes for a particular capsule locus, as defined elsewhere (13), were denoted as complete. Isolates that lacked capsule genes but matched a particular capsule null locus (*cnl*) allele, as defined by PubMLST,

TABLE 1 Patient metadata and strain fine typing

Isolate[a]	Source, yr (reference)	Country of origin	Yr of isolation	Patient age (yr)	Symptoms	Additional patient information[b]	Fine type[c]
NmIR13-1 (12028_13)	PubMLST	Ireland	2013	50	Urethritis		B:P1.22,14:F5-5:ST-10981
NmIT14-1 (PE5)	PubMLST	Italy	2014		Urethritis		C:P1.5-1,10-8:F3-6:ST-11
NmIT14-2 (PE6)	PubMLST	Italy	2014		Urethritis		C:P1.5-1,10-8:F3-6:ST-11
NmIT14-3 (PE7)	PubMLST	Italy	2014		Urethritis		C:P1.5-1,10-8:F3-6:ST-11
NmJP04-1	NIID	Japan	2004	21	Urethritis	C. trachomatis positive	Y:P1.5-2,10-1:F4-1:ST-23
NmJP05-1	NIID	Japan	2005		Urethritis		B:P1.21-2,2-33:F1-7:ST-687
NmJP05-2	NIID	Japan	2005		Urethritis		Y:P1.5-2,10-1:F4-1:ST-23
NmJP05-3	NIID	Japan	2005	30	Urethritis		--:P1.18-1,3:F4-1:ST-35
NmJP06-1	NIID	Japan	2006	29	Urethritis		Y:P1.5-2,10-1:F4-1:ST-23
NmJP12-1	NIID	Japan	2012		Urethritis		Y:P1.5-2,10-1:F4-1:ST-23
NmJP12-2	NIID	Japan	2012	28	Urethritis	C. trachomatis negative	Y:P1.5-2,10-40:F4-1:ST-23
NmJP12-3	NIID	Japan	2012	33	Balanoposthitis	C. trachomatis negative	Y:P1.18,25-34:F5-5:ST-198
NmJP14-1	NIID	Japan	2014	34	Urethritis		Y:P1.5-2,10-15:F4-1:ST-23
NmJP14-2	NIID	Japan	2014		Urethritis		Y:P1.5-2,10-1:F4-1:ST-23
NmJP14-3	NIID	Japan	2014		Urethritis		C:P1.5-1,10-8:F3-6:ST-11
NmJP14-4	NIID	Japan	2014	29	Urethritis	C. trachomatis negative	Y:P1.5-2,10-1:F4-1:ST-11120
NmJPb05-1	NIID	Japan	Before 2005	21	Urethritis	C. trachomatis negative	--:P1.5-2,10-1:F4-1:ST-23
NmJPb05-2	NIID	Japan	Before 2005	25	Urethritis	C. trachomatis negative	--:P1.5-1,2-5:F5-5:ST-823
NmJPb05-3	NIID	Japan	Before 2005	52	Urethritis		Y:P1.5-1,2-2:F5-8:ST-23
NmJPb05-4	NIID	Japan	Before 2005	25	Urethritis		--:P1.5-1,1:F1-7:ST-2045
NmJPb05-5	NIID	Japan	Before 2005	32	Urethritis		Y:P1.5-2,10-1:F4-1:ST-23
NmJPb05-6	NIID	Japan	Before 2005	25	Urethritis		Y:P1.5-2,10-1:F4-1:ST-23
NmJPb09-1	NIID	Japan	Before 2009		Urethritis		--:P1.18,25-1:F5-5:ST-198
NIUS07-1	CDC	USA	2007	33	Urethritis	MSW	Y:P1.5-2,10-1:F4-1:ST-23
NmUS04-1	CDC	USA	2004	24	Urethritis	MSW	C:P1.5,2:F3-6:ST-11
NmUS03-2	CDC	USA	2003	30	Urethritis	MSW	Y:P1.5-2,10-1:F4-1:ST-23
NmUS03-1	CDC	USA	2003	40	Urethritis	MSW	--:P1.19,15:F1-18:ST-
NmUS02-1	CDC	USA	2002	23	Urethritis	MSW	C:P1.7-2,13-2:F1-7:ST-278
NmSL15-1	WHO	Slovenia	2015	32	Carriage	MSM/MSW, C. trachomatis negative	B:P1.19,15:F3-6:ST-3091
NmSL14-1	WHO	Slovenia	2014	28	Urethritis	MSM, C. trachomatis negative	--:P1.18-1,*:F5-7:ST-5953
NmSL13-3	WHO	Slovenia	2013	30	Urethritis		B:P1.7-2,4:F1-5:ST-41
NmSL13-2	WHO	Slovenia	2013	20	Urethritis		B:P1.22,14:F5-5:ST-213
NmSL13-1	WHO	Slovenia	2013	46	Urethritis	MSM	--:P1.18,25-1:F5-5:ST-198
NmFR12-1 (LNP26948)	Taha et al., 2016 (7)	France	2012	25	Urethritis		Y:P1.5-2,10-1:F4-1:ST-23
NmUKb13-1 (NM9853)	Harrison et al., 2017 (3)	UK	2011–2013	25	Urethritis		C:P1.5-1,10-8:F3-6:ST-10482
NmUKb13-2 (NM8525)	Harrison et al., 2017 (3)	UK	2011–2013		Urethritis		B:P1.7-2,4:F1-5:ST-41
NmUKb13-3 (NM10492)	Harrison et al., 2017 (3)	UK	2011–2013		Urethritis		B:P1.19-1,15-11:F5-1:ST-269
NmUKb13-4 (NM10763)	Harrison et al., 2017 (3)	UK	2011–2013		Urethritis		Z:P1.18,25-15:F5-7:ST-3882
NmUS16-1 (NM-1)	Toh et al., 2017 (2)	USA	2016		Urethritis		Z:P1.22-4,14-13:F5-7:ST-10866
NmUS16-2 (NM-2)	Toh et al., 2017 (3)	USA	2016		Urethritis		C:P1.5-1,10-8:F3-6:ST-11

[a] Isolate names in parentheses are the original names of the isolates from the source database or publication.

[b] Patient sexual orientation and results of testing for *Chlamydia trachomatis* are indicated when available. MSM, men who have sex with men; MSW, men who have sex with women.

[c] —, computational serogrouping yielded no results; *, novel *porA* sequence.

were indicated accordingly. Many isolates possessed a subset of the capsule genes or lacked capsule genes but did not match a *cnl* allele in the PubMLST database. These isolates were further characterized using a combination of the BLASTn program to detect capsule genes with novel alleles not in the PubMLST database and the ISmapper program using reference insertion sequences from the ISfinder database (31, 32). Non-*cnl* isolates which lacked capsule genes were analyzed by mapping sequencing reads to the capsule region A reference sequences in the work of Harrison et al. (13) in the Geneious (version 9.1.7) program. Genes in capsule regions B and C were analyzed by use of BLASTn and the Geneious (version 9.1.7) program.

Recombination detection. The pangenome and core genome of the urethritis isolates and gonococcal isolates selected from the WHO reference panel (20) were constructed using Roary analysis (26) with 95% identity and the MAFFT (version 7.017) program (28) core genome alignment parameters. Genes in the core genome were selected for further analysis using fastGEAR (33). fastGEAR under default settings was run on each core gene alignment individually, and each gene was scored on the basis of the number of meningococcal isolates which harbored at least one recent recombination of 200 bp or more in size and a log(Bayes factor) greater than 0.5 from the gonococcal lineage. Genes with the highest number of putative directional recombinations were further analyzed via the MAFFT (version 7.017) (28) alignments and nucleotide neighbor-joining trees in Geneious (version 9.1.7) (29). Genes present in meningococcal strains that were initially clustered into gonococcal lineages via fastGEAR were also examined, as these could also represent instances of horizontal recombination.

Accession number(s). Newly determined sequences have been deposited in GenBank, and the accession numbers can be found via BioProject record number PRJNA384053 (see also Table S1 in the supplemental material).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM .01018-17.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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