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1 **Title:** Genomic characterization of urethritis-associated *Neisseria meningitidis* shows
2 that a wide range of *N. meningitidis* strains can cause urethritis

3 **Running title:** Genomics of urethritis-associated *N. meningitidis*

4 **Authors:** Kevin C. Ma,[a] Magnus Unemo,[b] Samo Jeverica,[c] Robert D. Kirkcaldy,[d]
5 Hideyuki Takahashi,[e] Makoto Ohnishi,[e] Yonatan H. Grad[a,f]#

6 **Affiliations:**

7 a. Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of
8 Public Health, Boston, USA

9 b. WHO Collaborating Centre for Gonorrhoea and other STIs, National Reference
10 Laboratory for Sexually transmitted Infections, Faculty of Medicine and Health, Örebro
11 University, Örebro, Sweden

12 c. Institute for Microbiology and Immunology, Medical Faculty, University of Ljubljana,
13 Ljubljana, Slovenia

14 d. Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD and
15 TB Prevention, CDC, Atlanta, Georgia, USA

16 e. Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan

17 f. Division of Infectious Diseases, Brigham and Women's Hospital and Harvard Medical
18 School, Boston, USA

19

20 #Address correspondence to Yonatan H. Grad (ygrad@hsph.harvard.edu).

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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 gonococcus, raising the question of the extent to which these strains contain features that promote adaptation to the genitourinary niche, making them gonococcal-like and distinguishing them from other *N. meningitidis*. 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* is possible across a range of meningococcal genotypes. Previously identified allelic patterns in urethritis-associated *N. meningitidis* may reflect genetic diversity in the underlying meningococcal population rather than novel adaptation to the urogenital tract.

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INTRODUCTION

The genus *Neisseria* comprises many commensal as well as two primarily pathogenic species. Pathogenic *Neisseria* include *N. 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* classically defined as commensals can cause disease, and *N. meningitidis* defined as pathogenic routinely persist asymptomatically in carriers (1). Accordingly, the meningococcus inhabits the nasopharynx commensally in about 10 percent 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 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* 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* isolated from genitourinary infection (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.

METHODS

Sample collection

Non-gonococcal *Neisseria* isolates associated with men with urethritis (n=39 *N. meningitidis* and n=1 *N. lactamica*) were obtained from the US Centers for Disease Control and Prevention's Gonococcal Isolate Surveillance Project (CDC 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 identified by Kraken (9) as non-gonococcal. WHO CC and Japanese isolates were identified through routine culture-based diagnosis of samples from men with urethritis. Isolates were sequenced as described below. Sequences have been deposited at Genbank under the

accession numbers in Supplemental Table S1. 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) (see Table 1). PubMLST isolates were located by querying the database for isolates where "species=Neisseria meningitidis" and "source=urethral". Isolates were named (or re-named for previously published isolates; see Table 1) according to country of origin and year of isolation.

DNA Sequencing and Analysis

DNA was prepared from isolates and Nextera libraries constructed using standard protocols, with sequencing performed on the Illumina platform. FASTQ reads were quality trimmed using Sickle (https://github.com/najoshi/sickle), underwent quality control in FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and *de novo* assembled using SPAdes (version 3.6.2) (10). A genus-level phylogeny was constructed using PhyloPhlAn (11), incorporating reference strains selected from a *Neisseria*-wide taxonomy (12). A species-level maximum likelihood phylogeny was constructed with RAxML (version 8.2.8) (13) using a core genome alignment generated by Roary (version 3.6.0) with a minimum BLASTP percent identity of 90 (14). Meningococcal finetyping was conducted using meningotype (v0.7-beta) and computational serogrouping results corrected when additional information was available (see *Candidate gene analysis*) (15). Phylogenetic trees were visualized and annotated in FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

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116 *Candidate gene analysis*

117 An *aniA* reference nucleotide sequence from MC58 (AE002098.2) was used in a
118 BLASTn query with default parameters against a BLAST database built using the
119 contigs from the *N. meningitidis* urethritis isolate genomes. Top hits were extracted from
120 the contigs. Samples with no BLAST hits were denoted as missing. Each nucleotide
121 sequence was queried against the PubMLST database to identify its allelic number.
122 Sequences which did not match a PubMLST allele were translated to assess whether
123 the peptide was truncated. Nucleotide sequences were aligned using MAFFT (version
124 7.017) (16) and phylogenetic trees constructed in Geneious (version 9.1.7) (17) using
125 the neighbor-joining method.

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

131 Capsule region A genes were first analyzed using the PubMLST genome query
132 tool (18). Isolates with all genes for a particular capsule locus, as defined in (19), were
133 denoted as complete. Isolates that lacked capsule genes but matched a particular
134 capsule null locus (cnl) allele as defined by PubMLST were indicated accordingly. Many
135 isolates possessed a subset of the capsule genes or lacked capsule genes but did not
136 match a cnl allele in the PubMLST database. These isolates were further characterized

using a combination of BLASTn to detect capsule genes with novel alleles not in the PubMLST database and ISmapper using reference insertion sequences from ISfinder (20, 21). Non-cnl isolates which lacked capsule genes were analyzed by mapping sequencing reads to the capsule region A reference sequences in Harrison et al. (2013) in Geneious (version 9.1.7). Genes in capsule regions B and C were analyzed via BLASTn and Geneious (version 9.1.7).

Recombination detection

The pan and core genomes of urethritis isolates and gonococcal isolates selected from the WHO reference panel (22) was constructed using Roary (14) with 95% identity and MAFFT (version 7.017) (16) core genome alignment parameters. Genes in the core genome were selected for further analysis using fastGEAR (23). fastGEAR under default settings was run on each core gene alignment individually, and each gene was scored based on the number of meningococcal isolates which harbored at least one recent recombination of 200 bp or more in size and log(Bayes Factor) greater than 0.5 from the gonococcal lineage. Genes with the highest number of putative directional recombinations were further analyzed via MAFFT (version 7.017) (16) alignments and nucleotide neighbor-joining trees in Geneious (version 9.1.7) (17). 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.

RESULTS AND DISCUSSION

Isolate metadata and population structure

Our sample set included 39 isolates of urethritis-associated *N. meningitidis* and one isolate of urethritis-associated *N. lactamica,* all obtained from male patients, and collected over a decade and across eight countries (Table 1). The average age of patients from isolates where 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 1237 genes, of which 1177 were included in the core genome alignment, whereas the core genome of the 39 meningococcal isolates comprised 1384 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 (Figure 1), with computational sequence typing revealing seven clonal complexes and 19 sequence types in this dataset (Table 1).

Of the meningococcal strains, 13 isolates (33.3%) belonged to the hyperinvasive lineages ST-11 (ET-37) or ST-41/44 complex (Lineage 3), and 17 (43.6%) belonged to the emerging invasive lineages 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 from Toh et al. (2017) and the men who have sex with men (MSM)-associated strain NmFR12-1 from Taha et al. (2016). 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

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was represented by a cluster of 12 isolates from Japan and one isolate from the US (NmUS02-1). One isolate (NmUS03-2) harbored an unknown sequence type. In this isolate, every gene queried as part of the MLST was associated with an allele in the PubMLST database (*abcZ*: 7, *adk*: 16, *aroE*: 55, *fumC*: 10, *gdh*: 3, *pdhC*: 56, *pgm*: 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 closest to gonococcus formed a distinct clade and were all grouped under ST-198 (Supplemental Figure S1). 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 (24). The presence of three such unusual isolates in our dataset raise the possibility that urethritis is another manifestation of this clade's pathogenicity.

Nitrite reductase (aniA) and Factor-H binding protein (fHbp)

The nitrite reductase gene *aniA* of *N. meningitidis* is often frameshifted in clinical isolates but 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 2006-2012 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 population structure. We thus investigated the allelic

diversity of *aniA* and characterized the prevalence of frameshifted variants to understand 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 (AE002098.2) reference genome showed a drop in coverage to zero for *aniA* and the loci immediately adjacent. Excluding NmFR12-1 (original name: LNP26948) from Taha et al. (2016), we found that 24.3% (9/37) isolates harbor truncated *aniA*, indicating that intact *aniA* is not necessary for urogenital colonization (Figure 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 (Supplemental Figure S2). Intriguingly, 12.8% (5/39) isolates harbored genes with suspected gonococcal origins (as determined by BLASTing the nucleotide sequences against 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. (2017) contained allele 204 (Figure 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 find similarly 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 dataset, all isolates contained the *fHbp* gene, and considerable diversity was present, with all three Novartis

variants represented. Only a minority of samples (6/38, excluding NmFR12-1) harbored truncated *fHbp* (alleles 669 and 749 in Figure 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 (ST-11 complex) as NmFR12-1, suggesting that the association of urethritis with *fHbp* truncation is likely due to relatedness between strains (Figure 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, both of which are genes previously characterized as divergent between *N. meningitidis* and *N. gonorrhoeae* (25, 26). 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 poly-G residue portions of the promoter (26), and Aspholm et al. found that in the meningococcus, a SNP resulting in CcoP truncation was present (25). In both cases, *N. meningitidis* associated with urethritis in our dataset harbored genes with wild-type meningococcal characteristics (i.e., intact *porA* gene and promoter and truncated *ccoP*), suggesting that gonococcal-like *porA* pseudogene and *ccoP* are not strictly necessary for neisserial colonization of the urogenital tract.

248 *Capsule*

249 The meningococcal capsule is important in the pathogenesis of meningococcal
250 disease in that it facilitates bacterial survival by promoting evasion of the immune
251 system; however, capsule expression also appears to detrimentally impact adhesion
252 and entry into human cells (27). Because capsule disruptions have been previously
253 found in urethritis-associated *N. meningitidis* (2), we characterized the biosynthetic
254 locus in our isolates with a particular focus on genes in region A, which comprises
255 capsule biosynthesis enzymes. For serogroups B, C, W, and Y, region A genes include
256 *cssA*, *cssB*, and *cssC*, which function in cytidine-5′-monophosphate-N-acetylneuraminic
257 acid synthesis, as well as a *csx* (where *x* can be *b, c, w,* or *y*) gene which encodes a
258 serogroup-specific polymerase (19). We also characterized genes in region B,
259 comprising capsule translocation genes c*trE* and *ctrF*, and genes in region C,
260 comprising capsule export genes *ctrA-D.* We considered present, intact genes in
261 regions A through C to be necessary for production of the capsule.

262 NmSL15-1, NmJPb05-1, NmJP12-3, and NmJPb05-3 contained the cnl allele 2,
263 where the cnl is defined to be an approximately 113 bp intergenic region that has
264 replaced regions A and C of the capsule (Figure 1) (19). Three of these isolates
265 (excluding NmJPb05-3) belonged to the ST-198 clade; among the rare cases of
266 unencapsulated *N. meningitidis* associated with invasive disease, isolates from the ST-
267 198 clade are frequently found (24). The ST-198 clade isolates also lacked region B
268 (*ctrE*, *ctrF*) genes, whereas NmJPb05-3 possessed them. Three other isolates
269 (NmSL13-2 in ST-5953, NmUS03-2 with unknown sequence type, and NmJP05-3 in the
270 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-nt 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, IS1301-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 harbor one or two insertion sequence disruptions in the middle of the *cssA*, *cssC*, or *csy* genes. Within this clade, the distribution of insertion sequence families and the pattern of genes disrupted appeared to mirror the core-genome maximum-likelihood phylogeny (Figure 1).

The remaining 14 isolates harbored an intact capsule Region A, of which six (NmSL13-1, NmSL13-3, NmJP05-1, NmIR13-1, NmUKb13-1, and NmUKb13-2) belonged to serogroup B, six (NmUS04-1, NmUS03-1, NmFR12-1, NmIT14-1, NmIT14-2, and NmIT14-3) belonged to serogroup C, and two (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* genes (NmUS01-2), 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 capsular phenotype (28). 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* are predicted to be encapsulated. Isolate NmFR12-1 from Taha et al. (originally denoted as 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 from solely genomic assessment, in keeping with our observation, we find other reports of encapsulated urethritis-associated *N. meningitidis* throughout the literature (29-31).

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 (32); the most common of these mosaic variants is known as the *penA* XXXIV allele. Deghmane et al. (2017) 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. 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 in Deghmane et al (33). 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 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 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 WHO gonococcal reference strains (22). Genes that contained signals of gonococcal-to-meningococcal recombination as detected by fastGEAR in multiple urethritis-associated meningococcal isolates were further investigated. Overall, we found that of the 1237 identified meningococcal core genes, the majority (1198, or 96.8%) contain 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, detected recombination loci for *dtpT* were only weakly supported (Bayes Factors 1.2 to 2.0). Recombinations in *infB* were more strongly supported (Bayes Factor 10.6 to 61.9), but were generally found in meningococcal lineages phylogenetically closest to gonococcal

lineages, suggesting identity by descent could also be a possible explanation. Because signals of recombination spanning over the entire gene (e.g., as found above in *aniA* and *norB*) may be overlooked by fastGEAR, we also examined genes for which at least five meningococcal-derived genes were clustered *a priori* into gonococcal lineages. Out of these results, we identified only *aniA* and *norB* as 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* 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*, 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 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

361 truncated proteins in nearly a quarter of our isolates implies *N. meningitidis* can survive
362 in the microaerophilic environment of the urethra without nitrite reduction. We observed
363 two instances of putative gonococcal-to-meningococcal recombination of the *norB-aniA*
364 gene cassette, which may promote anaerobic growth in the urogenital tract (8). The
365 previously reported association of *fHbp* disruption with urethritis-associated *N.*
366 *meningitidis* appears to be a result of population structure, as the majority of our non-
367 ST-11 complex strains harbored intact *fHbp*. Finally, we observed substantial diversity
368 in the capsule, including intact open reading frames, insertion sequence disruptions,
369 frameshift mutations, and cnl, showing urogenital colonization is possible across a
370 range of capsular phenotypes.

371 Based on our findings, a phylogenetically diverse array of *N. meningitidis* can
372 cause urethritis, affirming that the textbook niche specifications of *Neisseria* species are
373 too narrow. We note several questions unanswered in this study that may be promising
374 avenues for future investigation. First, these results do not address whether certain
375 lineages of *N. meningitidis* may be better adapted to growth and transmission once
376 within the urogenital niche. Second, because this is a convenience sample, population-
377 level prevalence and mutational diversity cannot be inferred. Epidemiological studies
378 that evaluate the incidence of meningococcal urethritis, meningococcal nasopharyngeal
379 carriage, and sexual behaviors will help distinguish whether increased rates of urethritis
380 may be due to “spillover” from higher carriage rates, from higher frequency of orogenital
381 contact, or from lineage-specific adaptation to the urogenital niche. Subsequent
382 functional analyses will then be required to characterize the role of candidate genes and
383 alleles (8). Third, the association of *N. meningitidis* with cases of urethritis does not

384 necessarily imply a causal link; for instance, co-infection with other sexually transmitted
385 diseases that cause non-gonococcal urethritis (e.g., *Chlamydia trachomatis,*
386 *Mycoplasma genitalium,* etc.) can occur. In some but not all urethritis cases described
387 here, testing was done to rule out coinfection with *C. trachomatis* (Table 1). Future
388 studies confirming causation of urethritis by *N. meningitidis* should aim likewise to rule
389 out other causes of NGU. With the increased incidence of *N. meningitidis*-associated
390 cases of urethritis, as reported by Bazan et al. (2016, 2017) and Toh et al. (2017),
391 leading to the concern about the emergence of meningococcal lineages adapted to
392 urogenital infection and transmission, such studies will be critical for informing the
393 appropriate clinical and public health responses.

394

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Table 1: Patient metadata and strain finetyping

Isolate names in parenthesis are original names of isolates from source database or publications. MSM=men who have sex with men. MSW=men who have sex with women. Patient sexual orientation and results of testing for *Chlamydia trachomatis* are indicated when available. '-' in finetype indicates computational serogrouping yielded no results. '*' indicates novel *porA* sequence.

Isolate	Source	Country	Year	Age	Symptoms	Additional Patient Information	Finetype
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* (+)	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		W: P1.5,2: F1-1: ST-10651
NmJP12-2	NIID	Japan	2012	28	Urethritis	*C. trachomatis* (-)	Y: P1.5-2,10-40: F4-1: ST-23
NmJP12-3	NIID	Japan	2012	33	Balanoposthitis	*C. trachomatis* (-)	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		C: P1.5-1,10-8: F3-6: ST-11
NmJP14-3	NIID	Japan	2014	29	Urethritis	*C. trachomatis* (-)	Y: P1.5-2,10-1: F4-1: ST-11120
NmJP14-4	NIID	Japan	2014		Urethritis	*C. trachomatis* (-)	-: P1.5-2,10-1: F4-1: ST-23
NmJPb05-1	NIID	Japan	<2005		Urethritis		-: P1.5-1,2-5: F5-5: ST-823
NmJPb05-2	NIID	Japan	<2005	21	Urethritis		Y: P1.5-1,2-2: F5-8: ST-23
NmJPb05-3	NIID	Japan	<2005	25	Urethritis		-: P1.5-1,1: F1-7: ST-2045
NmJPb05-4	NIID	Japan	<2005	52	Urethritis		Y: P1.5-2,10-1: F4-1: ST-23
NmJPb05-5	NIID	Japan	<2005	32	Urethritis		Y: P1.5-2,10-1: F4-1: ST-23
NmJPb05-6	NIID	Japan	<2005	25	Urethritis		Y: P1.5-2,10-1: F4-1: ST-23
NmJPb09-1	NIID	Japan	<2009		Urethritis		Y: P1.5-2,10-1: F4-1: ST-23
NmSL13-1	WHO	Slovenia	2013	46	Urethritis	MSM	B: P1.19,15: F3-6: ST-3091
NmSL13-2	WHO	Slovenia	2013	20	Urethritis	MSM, *C. trachomatis* (-)	-: P1.18-1,*: F5-7: ST-5953
NmSL13-3	WHO	Slovenia	2013	30	Urethritis		B: P1.7-2,4: F1-5: ST-41
NmSL14-1	WHO	Slovenia	2014	28	Carriage	MSM / MSW, *C. trachomatis* (-)	B: P1.22,14: F5-5: ST-213
NmSL15-1	WHO	Slovenia	2015	32	Urethritis	MSW, *C. trachomatis* (-)	-: P1.18,25-1: F5-5: ST-198
NmUS02-1	CDC	USA	2002	23	Urethritis	MSW	Y: P1.5-2,10-1: F4-1: ST-23
NmUS03-1	CDC	USA	2003	40	Urethritis	MSW	C: P1.5,2: F3-6: ST-11
NmUS03-2	CDC	USA	2003	30	Urethritis	MSW	-: P1.19,15: F1-18: ST--
NmUS04-1	CDC	USA	2004	24	Urethritis	MSW	C: P1.7-2,13-2: F1-7: ST-278
NIUS07-1	CDC	USA	2007	33	Urethritis	MSW	
NmFR12-1 (LNP26948)	Taha et al. 2016	France	2012	25	Urethritis		C: P1.5-1,10-1: F3-6: ST-10482
NmUKb13-1 (NM9853)	Harrison et al. 2017	UK	2011-2013		Urethritis		B: P1.7-2,4: F1-5: ST-41
NmUKb13-2 (NM8525)	Harrison et al. 2017	UK	2011-2013		Urethritis		B: P1.19-1,15-11: F5-1: ST-269
NmUKb13-3 (NM10492)	Harrison et al. 2017	UK	2011-2013		Urethritis		Z: P1.18,25-15: F5-7: ST-3882

NmUKb13-4 (NM10763)	Harrison et al. 2017	UK	2011-2013	Urethritis	Z: P1.22-4,14-13: F5-7: ST-10866
NmUS16-1 (NM-1)	Toh et al. 2017	USA	2016	Urethritis	C: P1.5-1,10-8: F3-6: ST-11
NmUS16-2 (NM-2)	Toh et al. 2017	USA	2016	Urethritis	C: P1.5-1,10-8: F3-6: ST-11

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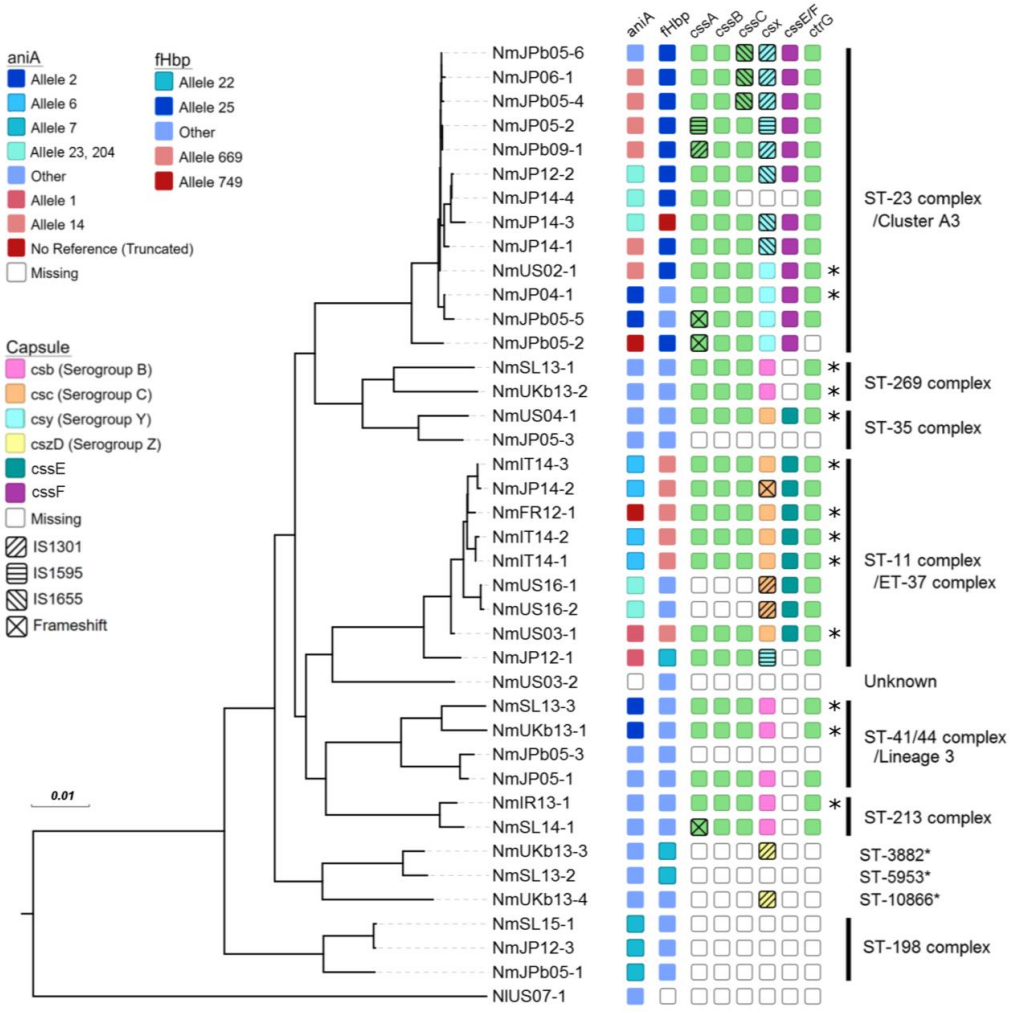

Figure 1: Core-genome phylogeny and genomic characterization of *aniA*, *fHbp*, and capsular region A of urethritis-associated *N. meningitidis* and *N. lactamica*. For *aniA* and *fHbp*, blue shading indicates a full gene product and red indicates a truncated gene product. Alleles associated with truncated genes and alleles associated with full genes found in three or more isolates were specifically denoted in the legend; all other alleles were grouped into the "other" category. *aniA* alleles 23 and 204 are putatively gonococcal in origin. For the capsule region A, *csx* variants which determine 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 legend. Starred isolates indicate strains predicted to encode capsules. Unshaded boxes indicate missing genes. Clonal complexes are indicated along the right, with starred sequence types indicating no associated clonal complex.