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Letter to the Editor

Genomic analysis of *Neisseria meningitidis* ST23 serogroup Y isolated from the semen

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Dear Editor,

Neisseria meningitidis (*Nm*) is an obligate human pathogen that can cause widespread epidemic outbreaks and is a leading cause of life-threatening infections such as meningococcal meningitis and septicemia [1]. This pathogen can occasionally cause urethritis [2]. Here, we isolated *Nm* (R22) from the semen of a 28-year-old infertile man, a patient at Nini Hospital (Tripoli, Lebanon), and analyzed the isolate using whole-genome sequencing. We also compared the genome of the isolate with the reference genomes of *Neisseria* spp. available in the GenBank database to screen for potential genetic determinants that might contribute to urogenital pathogenesis and infertility.

The isolate (R22) was recovered from semen on chocolate PolyViteX medium (BioMérieux®) and was identified as *Nm* by MALDI-ToF (Bruker®). Maximum likelihood phylogenetic analysis of the 16S rRNA gene sequences [3] clustered R22 within the *Nm* group, suggesting a high similarity with the *Nm* DE10444 strain isolated from a 16-year-old woman with invasive meningococcal disease (Fig. 1A) [4]. R22 was susceptible to amoxicillin, cefotaxime, ciprofloxacin, and rifampicin. No other pathogenic organisms were found in the semen sample using standard protocols.

The genomic DNA of *Nm* was sequenced using MiSeq Technology (Illumina). The draft genome was assembled by the A5 pipeline, organized by mauve alignment, and annotated by Prokka and RAST as described previously [5]. Virulence factors, plasmids, and antimicrobial resistance genes (ARGs) were evaluated using ABRicate version 1.0.1 (https://github.com/tseemann/abricate). We used Genome-to-Genome Distance Calculator (GGDC) (http://ggdc.dsmz.de) to estimate the similarity between the genome of the R22 strain and reference genomes (*Neisseria mucosa* 19696, *N. gonorrhoeae* FA1090, *Neisseria flavescens* NRL30031H2, *Nm* alpha 710, *Nm* alpha 14, *Nm* MC58, *Nm* 8013, *Nm* Z2491, and *Nm* DE10444). The mean levels of relatedness between the genome sequences were measured using OrthoANI (Orthologous Average Nucleotide Identity) (https://www.ezbiocloud.net/tools/orthoani). The clustered regularly-interspaced short palindromic repeats (CRISPR) associated (Cas) system was detected using CRISPR-Cas Finder (https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/Index). Multilocus sequence typing (MLST) was also performed (https://cge.cbs.dtu.dk//services/MLST/). All raw results are publicly available (https://doi.org/10.5281/zenodo.7213782).

Nm R22 belongs to ST23 serogroup Y. No plasmids or ARGs were found. The genome size is 2,150,675 bp long with 51.6% GC content. Of the 2046 predicted genes, 1954 were protein-coding genes, 53 tRNA, 2 rRNA, and 37 other RNA. Several loci in this genome were predicted to encode hypothetical surface proteins and virulence factors such as those containing the genes for capsule biosynthesis, ABC transporter, surface adhesion proteins (including type IV Pili and Opc adhesion molecules), and iron sequestration. Furthermore, ten loci in the genome of R22 were predicted to encode transposases and insertional elements. R22 shared the minimum orthologous genes with *N. flavescens* NRL30031/H210 (1318) and the maximum with *Nm* DE10444 (1714). R22 isolate was closely related to *Nm* DE10444 with an Average Genomic Identity of Orthologous Gene Sequences (AGIOS) value of 50.53%, sharing 1714 orthologues genes. Additionally, the *in-silico* DNA-DNA hybridization (*is*DDH) values varied from 75.7% with *Nm* MC58 to 98.9% with *Nm* DE10444 and the OrthoANI values ranged from 83.96% with *Neisseria macacae* ATCC 33926 to 99.88% with *Nm* DE10444 (Fig. 1B).

Pan-genome analysis of *Nm* R22 with the 106 other genomes (91 *Nm* and 15 *N. gonorrhoeae*) was performed as described previously [6]. R22 clustered with *Nm* DE10444 and revealed the difference between *N. gonorrhoeae* and *Nm,* where several genes were present in gonococcus but absent in meningococcus strains, including R22 (Fig. S1). Indeed, 375 genes were identified as specific genes of gonococcus that encode for enzymes, hypothetical proteins, transposases, CRISPR system, and transporters. A total number of 1310 SNPs were identified by comparing *Nm* R22 with *Nm* DE10444 (Fig. S2). These SNPs were annotated by functional class as follows: 714 missense SNPs (75.1%), 28 nonsense

Abbreviations: Nm, *Neisseria meningitidis*; ARGs, Antimicrobial Resistance Genes; GGDC, Genome-to-Genome Distance Calculator; MLST, Multilocus sequence typing; ORF, Open Reading Frame; *N. gonorrhoeae*, *Neisseria gonorrhoeae*; OrthoANI, Orthologous Average Nucleotide Identity; CRISPR, Clustered Regularly-Interspaced Short Palindromic Repeats; AGIOS, Average Genomic Identity of Orthologous Gene Sequences; *is*DDH, *in-silico*DNA-DNA hybridization.

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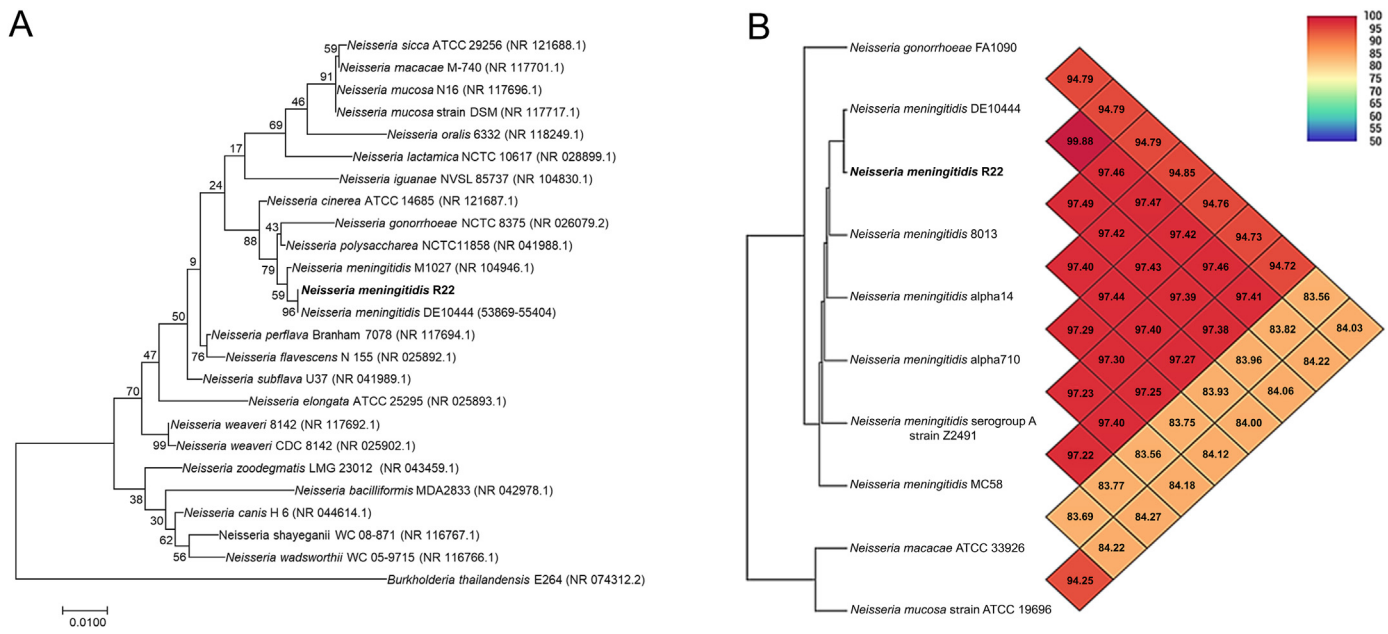

Fig. 1. Sequence analysis of the 16S rRNA gene of *Neisseria meningitidis* R22. 1A. Maximum likelihood phylogenetic tree based on the 16s rRNA gene sequence highlighting the position of *N. meningitidis* R22 relative to other species of *Neisseria* using *Burkholderia thailandensis* as an outgroup. Sequences were aligned using ClustalW and MEGA 7 with default parameters. All positions containing gaps and missing data were eliminated. Phylogenetic inferences were obtained using the maximum likelihood method with 1000 times. Bootstrap values >50% are given at nodes. Bar, 0.020 substitutions per nucleotide position. 1B. Heatmap generated with OrthoANI values that were calculated using the OAT software. A Pairwise OrthoANI comparison with genome sequences of the *N. meningitidis* R22 with other established species of *Neisseria* showed that R22 shared 99.88% similarity with *Neisseria meningitidis* DE10444, 97.49% with *Neisseria meningitidis* 8013 and 97.42% with *Neisseria meningitidis* alpha 14.

(3%), and 208 silent (21.9%), where a high number of these SNPs was detected in the coding region (1268). Regarding the missense SNPs, we found that the adhesin *mafA* in R22 was affected by a stop-gained mutation, which shifted the open reading frame (ORF) and affected protein function.

In conclusion, genes corresponding to infertility and thriving in urogenital infections were not identified in R22, indicating an unlikely association with infertility. Host factors might be the main contributors to different tissue tropisms of meningococcus and the observed infertility; however, further studies are required to confirm these explanations.

Ethics declaration

This investigation was approved by the ethical committee of the Doctoral School of Science and Technology/Lebanese University authorized by the Lebanese Ministry of Public Health (CE-EDST-4–2016) agreement with Lebanese legislation. Oral and written informed consent was obtained from the patient. All data were analyzed anonymously.

Author contributions

Conceptualization, **MO, JMR, and MH.**; methodology, **MK, MO, and RR**; software, **MK, RR; and SMD**; validation, **MO, IIK, RR, AS, SMD, JMR, and MH**; formal analysis, **MK, MO, and MH**; investigation, **MK, MO, IIK, and RR**; resources, **MO, JMR, and MH**; data curation, **MK and MH**; writing—original draft preparation, **MK, MO, and RR**; writing—review and editing, **IIK, AS, SMD, JMR, and MH**; visualization, **MK and RR**; supervision, **MO, AS, SMD, and JMR**; project administration, **MO**; funding acquisition, **MO, JMR, and MH**. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2023.101129.

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