The supplemental material:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Reference Protein** | **PMGA** | **Gene** | **Mutation** | **Associated Antibiotic Resistance(s)** | **References** |
| DNA gyrase subunit A | NEIS1320 | gyrA | T91I | Ciprofloxacin | 1-4 |
| DNA gyrase subunit A | NEIS1320 | gyrA | T173A | Ciprofloxacin; Levofloxacin | 4 |
| DNA gyrase subunit A | NEIS1320 | gyrA | T91F | Ciprofloxacin | 3 |
| DNA gyrase subunit A | NEIS1320 | gyrA | D95A | Ciprofloxacin | 3 |
| DNA gyrase subunit A | NEIS1320 | gyrA | D95N | Ciprofloxacin | 2-3 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | F504L | Penicillin; Ampicillin | 5-6 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | A510V | Penicillin; Ampicillin | 5-6 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | I515V | Penicillin; Ampicillin | 5-6 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | H541N | Penicillin; Ampicillin | 5-6 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | I566V | Penicillin; Ampicillin | 6 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | I312M | Cephalosporins; Penicillins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | V316T | Cephalosporins; Penicillins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | N512Y | Cephalosporins; Penicillins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | G545S | Cephalosporins; Penicillins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | A501V | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | A501P | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | A311V | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | V316P | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | T483S | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | G482S | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | G542S | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | P551S | Cephalosporins | 7 |
| Penicillin Binding Protein 2 | NEIS1753 | penA | P551L | Cephalosporins | 7 |
| Penicillin Binding Protein 1 | NEIS0414 | ponA | L421P | Penicillins | 7 |
| DNA topoisomerase IV subunit A | NEIS1525 | parC | D86N | Ciprofloxacin  | 8 |
| DNA topoisomerase IV subunit A | NEIS1525 | parC | S87I | Ciprofloxacin  | 8 |
| DNA topoisomerase IV subunit A | NEIS1525 | parC | S87R | Ciprofloxacin  | 8 |
| DNA topoisomerase IV subunit A | NEIS1525 | parC | E91G | Ciprofloxacin  | 8 |
| RNA Polymerase Beta-subunit | NEIS0123 | rpoB | S548F | Rifampin | 9 |
| RNA Polymerase Beta-subunit | NEIS0123 | rpoB | H552R | Rifampin | 9 |
| RNA Polymerase Beta-subunit | NEIS0123 | rpoB | H552N | Rifampin | 9 |
| RNA Polymerase Beta-subunit | NEIS0123 | rpoB | S557D | Rifampin | 9 |
| RNA Polymerase Beta-subunit | NEIS0123 | rpoB | D542V | Rifampin | 9 |
| RNA Polymerase Beta-subunit | NEIS0123 | rpoB | H552Y | Rifampin | 10 |
| RNA Polymerase Beta-subunit | NEIS0123 | rpoB | G560S | Rifampin | 10 |

Table S1. List of alleles associated with antibiotic resistance among *N. meningitidis* strains

**References:**

1. Wu et al. 2009 Emergence of ciprofloxacin-resistant Neisseria meningitidis in North America. N Engl J Med 360:886-892
2. Hong et al. 2013 Target gene sequencing to define the susceptibility of *Neisseria meningitidis* to ciprofloxacin. Antimicrob Agents Chemother 57:1961-1964
3. Chen et al. 2015 Shifts in the antibiotic susceptibility, serogroups, and clonal complexes of *Neisseria meningitidis* in Shanghai, China: A Time Trend Analysis of the Pre-Quinolone and Quinolone Eras. PLoS Medicine 12(6):e1001838
4. Castanheira et al. 2012 Evaluation of quinolone resistance–determining region mutations and efflux pump expression in *Neisseria meningitidis* resistant to fluoroquinolones. Diagn Microbiol Infect Dis. 72:263-266.
5. Taha et al. 2007 Target gene sequencing to characterize the penicillin G susceptibility of *Neisseria meningitidis*., Antimicrob Agents Chemother 51:2784–2792
6. Deghmane et al. 2017 Emergence of meningococci with reduced susceptibility to third-generation cephalosporins., J Antimicrob Chemother 72:95-98.
7. Zapun et al. 2016 Resistance to β-Lactams in *Neisseria* ssp due to chromosomally encoded penicillin-binding proteins. Antibiotics (Basel) 5:35. doi: 10.3390/antibiotics5040035.
8. Chen et al. 2020 Meningococcal quinolone resistance originated from several commensal *Neisseria* species. Antimicrob Agents Chemother 64:1494-519.
9. Taha et al. 2010 Multicenter study for defining the breakpoint for rifampin resistance in *Neisseria meningitidis* by rpoB sequencing. Antimicrob Agents Chemother 54:3651-3658
10. Taha et al. 2006 Rifampin-resistant *Neisseria meningitidis*. Emerg Infect Dis 12: 859-860.

Table S2. Clinicals vs. Isolates WGS cost comparison per specimen

|  |  |  |
| --- | --- | --- |
| **Items** | **Clinical, $** | **Isolate, $** |
|  | **Enrichment** |
| EquiPhi (Thermo Fisher) | 4.75 | - |
| Pyrophosphatase (Thermo Fisher) | 1.33 | - |
| dNTP (Roche) | 0.82 | - |
| SWGA primers (IDT) | 0.62 | - |
| Magnetic beads (Beckman) | 1.97 | - |
|  | **Fragmentation** |
| Tubes (Covaris) | 4.65 | 4.65 |
| Caps (Covaris) | 0.47 | 0.47 |
|  | **Library Prep** |
| NEBNext Ultra IILibrary Preparation Kit (NEB) | 23.39 | 23.39 |
| Magnetic beads (Beckman) | 2.4 | 2.4 |
|  | **Whole Genome Sequencing** |
| NovaSeq 6000 SP Reagent Kit v1.5 (500 cycles) | 21a  | 5.46b |
| NEBNext Indices (NEB) | 5.2 | 5.2 |
| **Total** | 66.6 | 41.6 |

aThe cost is based on multiplexity value of 200 specimens loaded per single cartridge for enriched clinical specimens

bThe cost is based on multiplexity value of 768 specimens loaded per single cartridge for isolates



Figure S1. Scheme of the workflow used in the study. Stars designate three types of qPCR: performed on crude specimen (direct); on extracted DNA (traditional); on enriched specimens (SWGA). Orange boxes - bench work; Blue boxes – data analysis.



Fig. S2

The number of cgMLST loci present in genome assemblies as a function of Ct *sodC* value measured after SWGA procedure (bottom axis) or corresponding genome titer (upper axis). Genome assemblies with more than 1,400 cgMLST loci generally had over 95% accuracy at those loci that is required for accurate molecular typing of *N. meningitidis* (Itsko *et al*., 2020. Full Molecular Typing of Neisseria meningitidis Directly from Clinical Specimens for Outbreak Investigation. J Clin Microbiol 58, DOI:10.1128/JCM.01780-20). The arrows demonstrate the rational for cut off of Ct *sodC* 16.



Fig. S3

Success rate of full molecular typing for *N. meningitidis* present in CSF specimen as a function of Ct *sodC* value measured before SWGA (bottom axis) or corresponding genome titer (upper axis). Each bin represents 2 Ct units or fourfold difference in titer.