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## Identification of *Neisseria meningitidis* by MALDI-TOF MS may not be reliable.

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1 The identification of *Neisseria meningitidis* by MALDI-TOF may not be reliable.

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7 Running title: Identification of meningococci by MALDI-TOF

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25 **Abstract**

26 *Objectives:* The Matrix Laser Desorption/Ionization Time of Flight Mass Spectrometry  
27 (MALDI-TOF/MS) technique is increasingly used in hospital laboratories for routine  
28 identification of microorganisms. However, its performance is variable particularly for highly  
29 variable species such as *Neisseria meningitidis*. Reliable identification of *N. meningitidis* is  
30 crucial for the management of invasive meningococcal disease by rapid implementation of  
31 treatment and preventive measures among close contacts. We aim here in this study to assess  
32 and improve *N. meningitidis* identification by MALDI-TOF by enriching the databases with  
33 reference strains identified using whole genome sequencing (WGS) as a gold standard.

34 *Methods:* We first built-up a collection of 24 strains from several species of *Neisseria* genus  
35 that we characterized by WGS. This collection was added to the available database to test by  
36 MALDI-TOF/MS another collection of 32 clinical isolates received between 2015 and 2017  
37 at the French National Reference Laboratory for Meningococci.

38 *Results:* Using the commercially available library of mass spectrometry profiles (MSP), only  
39 67% (47-82%, 95% CI) of concordance was observed at the species level between MALDI-  
40 TOF and WGS characterization. However, when the new enriched reference collection was  
41 used on the second subset of isolates, the identification of *N. meningitidis* was significantly  
42 improved (p=0.0016) showing 92% (75-98%, 95% CI) of specificity while it was of 52% (34-  
43 70%, 95% CI) with the manufacturer's database alone.

44 *Conclusions:* Our data highlight the need to update the available MALDI-TOF database by  
45 high quality references to enhance the identification of *N. meningitidis* and avoid unwarranted  
46 preventive measures or missing them.

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48

49 **Keywords:**

50 **MALDI-TOF, whole genome sequencing, *Neisseria meningitidis*, identification**

51

52 **Introduction**

53 The Matrix Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-  
54 TOF/MS) technique is increasingly used in hospital clinical laboratories as routine tool for  
55 microbial identification. It is based on generating a unique protein mass spectrum of a given  
56 microorganism and comparing it to a library of spectra from known reference microorganisms  
57 (1-3). MALDI-TOF/MS is a simple, rapid and cost-effective identification technique(4). It is  
58 also time saving by identifying organism in positive blood culture broths or urine samples (5-  
59 7).

60 Bacterial identification is quantified by the score obtained by comparison to a library of main  
61 spectra profiles (MSP) present in the database. This score reflects the level of reliability of the  
62 identification at the genus or species levels. However, the reliability of the identification  
63 depends on the representativeness of the reference strains included in the MSP library. This  
64 issue is critical for genus and species showing high diversity and frequent horizontal DNA  
65 exchanges such as the genus *Neisseria* (8). A global gene pool has been suggested to be  
66 shared by the species of this genus including the pathogenic species *Neisseria meningitidis*  
67 and *Neisseria gonorrhoeae*(9). Indeed, MALDI-TOF/MS as well as the conventional  
68 phenotypic and biochemical testing for the characterization of *Neisseria* species can lead to  
69 misidentifications that may be also due to poor representativeness of available MSPs from  
70 *Neisseria* species other than pathogenic *Neisseria* species (10-12). The high degree of genetic  
71 relatedness between *N. meningitis*, *Neisseria polysaccharea*, *N. gonorrhoeae*, *Neisseria*  
72 *lactamica* and *Neisseria cinerea* species promoted the suggestion that these species form a

73 single genospecies on the basis of the stability of DNA heteroduplex to the S1 nuclease  
74 activity (13). However, next generation sequencing era and the analysis of whole genome  
75 sequences (WGS) data showed that species of the genus *Neisseria* could be robustly identified  
76 by a set of 53 genes encoding ribosomal protein subunits (ribosomal multilocus sequence  
77 typing, rMLST). The rMLST scheme represents an effective and rapid method for taxonomic  
78 classification (14). We aimed in this work to assess the use of MALDI-TOF/MS in the  
79 identification of *Neisseria* species and to improve the specificity of *N. meningitidis*  
80 identification by MALDI-TOF/MS. The analysis was conducted using the WGS data as  
81 reference to ascertain the bacterial identification.

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97 **Methods.**

98 **Strain collections**

99 We used two collections of strains: The first one (reference collection) corresponded to 24  
100 strains from the collection of the French National Reference Laboratory for Meningococci  
101 from 1979 to 2004 with at least one strain of each *Neisseria* species present in our collection.  
102 These strains were characterized initially by conventional phenotypic and biochemical testing  
103 (PubMLST *Neisseria* database: id38974, id38975, id38976, id38977, id38978, id38979,  
104 id38980, id38981, id38982, id38983, id38984, id38985, id38986, id39715, id39716, id39717,  
105 id39718, id39719, id39720, id39721, id39722, id39723, id39724, id39725).

106 The second collection (test collection) corresponded to 25 non-*N. meningitidis* strains,  
107 isolated between 2015 and 2017 as well as 7 *N. meningitidis* strains representing each of the  
108 serogroups A, B, C, W, X, Y in addition to a non-groupable strain (PubMLST *Neisseria*  
109 database: id40248, id40261, id40270, id41220, id42055, id42057, id42058, id42059, id42060,  
110 id42061, id42062, id42198, id42421, id43987, id45355, id45356, id46114, id49258, id49259,  
111 id51008, id51545, id51557, id51558, id51584, id52432, id53422, id57256, id57266, id57277,  
112 id58872, id58873, id58874).

113

114 **Whole genome sequencing and data analysis**

115 Genomic DNA was extracted with the MagNA Pure 96 system (Roche Molecular System,  
116 Pleasanton, USA). Libraries preparation were performed with the Nextera<sup>®</sup> XT DNA library  
117 Preparation Kit (Illumina, San Diego, USA) and whole genome sequencing was processed  
118 with Illumina technology (NextSeq 500, Illumina) with paired-end strands of 150 bp and with  
119 a sequencing depth of 50X.

120 All de novo assemblies were performed with SPAdes (CAB, St. Petersburg State University,  
121 Russia) and genome comparison was performed using the BIGSdb Genome Comparator tool  
122 available in PubMLST platform (15). Core genomes were compared with 50 isolates from  
123 several species of *Neisseria*, available in PubMLST *Neisseria* database (id5354, id14740,  
124 id19080, id19081, id19083, id19087, id19088, id19089, id19090, id19095, id19096, id19097,  
125 id19100, id19940, id20515, id20516, id21038, id21039, id21040, id21041, id21042, id21044,  
126 id21045, id21046, id21048, id21049, id21061, id21063, id21064, id26870, id26871, id26876,  
127 id27632, id28271, id29271, id36140, id36153, id36167, id36173, id36174, id36179, id36317,  
128 id37587, id40839, id41201, id41237, id41396, id41409, id41588, id49340). A matrix of  
129 allelic distance was computed based on rMLST scheme and viewed in SplitsTree4 (version  
130 4.14.6) using the Neighbor-Net algorithm (16).

131

### 132 **MALDI-TOF/MS analysis**

133 Ribosomal proteins extractions were performed according to the manufacturer's instructions  
134 with a formic acid and acetonitrile treatment. Proteins were spotted in duplicate on the  
135 MALDI biotarget and co-crystallized with HCCA matrix. Spectra were acquired with a  
136 MALDI Biotyper Microflex LT (Bruker Daltonik GmbH, Bremen, Germany).

137 In order to build a local database, the strains selected (reference collection) were spotted 8  
138 times in the same target and then read 4 times, generating 32 spectra for each strain. A  
139 minimum of 20 spectra for each strain were selected after quality control procedures, in order  
140 to create a single Main Spectra Profile (MSP) for each strain of our database (FlexAnalysis  
141 software, version 3.4).

142 Strain identification was evaluated by the score generated from the Bruker Biotyper software  
143 (MBT Compass and Explorer, version 4.1.80) against all biotypes available in the Bruker's  
144 database (7311 MSP available).

145 A score comprised between 2.0 to 3.0 leads to a reliable identification at the genus and  
146 species level. If the score is comprised between 1.7 and 2.0, the identification of the genus  
147 remains probable and finally if the score is under 1.7, the reliability is not sufficient to let a  
148 genus identification.

149 Specificity and sensitivity were calculated using a two-way table as previously described (17)  
150 with the genomic identification as a gold standard. We used Chi<sup>2</sup>-Mc Nemar test to calculate  
151 the significance of the differences among the specificities of the tests used to identify bacterial  
152 isolates (WGS and MALDI-TOF/MS).

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## 166 **Results**

### 167 **WGS characterization of the collection**

168 We have selected several strains (N=24) from several species of the genus *Neisseria* that  
169 mainly were not or poorly represented in the available database of MSP in the Bruker  
170 MALDI-TOF/MS system (Table 1). The identification of these strains was initially  
171 performed using the conventional biochemical identification of *Neisseria* (18) as indicated in  
172 Materials and Methods. We next used WGS on all these strains and extract the rMLST that  
173 analyses the genetic relationships on the basis of the polymorphism of 53 *rps* genes encoding  
174 ribosomal proteins. This analysis allowed identification of species by comparison to rMLST  
175 profiles already available on the BIGSdb platform as previously described (19). This analysis  
176 compared the strains of our collection to the 50 strains belonging to different species of the  
177 genus *Neisseria* that were available in the BIGSdb database. The neighbor-joining tree using  
178 rMLST data showed species-specific clusters as previously described (Figure A) (20). This  
179 genetic identification was consistent with the initial biochemical identification for only 17/24  
180 of our collection of strains (71%). However, for 7 isolates, no consistent identification was  
181 confirmed comparing genetic to phenotypic identification (isolates highlighted with blue  
182 circles, Figure A). One strain (id38974) initially identified as *N. perflava* appeared to belong  
183 to the *N. mucosa* cluster by rMLST and thus further underlines the difficulty in the  
184 identification of *Neisseria* species by the conventional biochemical tests. Three other strains  
185 (id38979; id39722 and id39723) that were biochemically identified respectively as *N. flava*,  
186 *N. sicca* and *N. perflava*, clustered together in the *N. subflava* group by rMLST (*N. subflava*  
187 *biovars*)(14). The sequence analysis of *rplF* gene (encoding the 50S ribosomal protein L6)  
188 also assigned all of these three isolates as *N. subflava* (20). One isolate that was identified as  
189 *N. polysaccharea* (id39718) was characterized by *rplF* sequence as *N. bergeri*, a closely

190 species related to *N. polysaccharea* (14). And finally, the two isolates (id39719 and id39720)  
191 identified as *Neisseria denitrificans* and *Neisseria canis* clustered together on the rMLST-  
192 based tree but were separated from the 50 isolates in the BIGSdb database (Figure A).  
193 In order to refine the rMLST identification of our set of 24 strains, particularly for the three  
194 last strains mentioned above, a core genome MLST (cgMLST) analysis was performed  
195 (Figure B), that analyses the genetic relationships on the basis of polymorphism of the core  
196 genome (1605 genes cgMLST) (15). This analysis confirmed the data obtained with the  
197 rMLST analysis. In addition, the strain (id39718) that was not identified by rMLST (but was  
198 identified as *N. bergeri* by *rplF* sequencing) clustered by cgMLST with the *N. polysaccharea*  
199 group (Figure B). Finally, the two strains that were identified initially by biochemical tests as  
200 *N. denitrificans* and *N. canis* (but were not identified by rMLST) clustered with the *Neisseria*  
201 species and are more closely related to commensal species isolated from animal hosts. These  
202 two isolates were assigned as *Neisseria sp.*, in absence of reference strains available in the  
203 database (Table 1).

204

#### 205 **MALDI-TOF/MS characterization**

206 We next performed the MALDI-TOF analysis to identify the 24 isolates using the Microflex  
207 Biotyper Bruker system that allowed the identification at genus level for 96% of our set of  
208 strains (23/24 strains with a score above 1.7). Among these 23 isolates, 19 isolates reached a  
209 score higher than 2, allowing a species level identification for 79 % (19/24) of our reference  
210 collection. The remaining strain showed a score under 1.7 that do not permit species  
211 identification (Table 1).

212 The MALDI-TOF identification of the 24 strains was consistent with the genomic analysis for  
213 16/24 strains (67% of total strains) at species level (Table 1). Indeed, most of isolates (5/8

214 isolates) that unmatched between MALDI-TOF and genomic data, showed MALDI-TOF  
215 scores higher than 2 (Table 1). Most striking is that 5 isolates were identified by MALDI-TOF  
216 as *N. meningitidis* with scores ranging between 1.65 and 2.35 but were identified by rMLST  
217 and cgMLST as *N. polyscharrea*, *N. bergeri* or *N. cinerea* (Table 1). The *N. meningitidis*  
218 strain that was included within the 24 strains (id38983) was correctly identified with a score  
219 of 2.58. These data indicate low specificity (78%) for *N. meningitidis* identification by  
220 MALDI-TOF, although it showed high sensitivity (100%).

221

### 222 **Improvement of the MALDI-TOF diagnosis of *N. meningitidis***

223 The 32 isolates from the test collection (see Methods) were sequenced and were further  
224 identified by rMLST and cgMLST (Figure A, Figure B and Table 2). All these strains (the 25  
225 non-*N. meningitidis* strains and the 7 *N. meningitidis* strains) were subsequently tested by  
226 MALDI-TOF and identified using both databases (the original database and the extended one  
227 as described in Methods section). All the 7 *N. meningitidis* strains were correctly identified by  
228 both databases. For the 25 “non-*N. meningitidis*” isolates and using the original Bruker  
229 database, the identification reached the genus level for all tested strains and 20/25 (80%) at  
230 species level (Table 2). However, 12/25 (48%) strains were misidentified as *N. meningitidis*  
231 (indicated with red stars in Figure A) leading to a specificity of *N. meningitidis* identification  
232 of 52% (34-70%, 95% CI). When the extended database was used for the identification of  
233 spectra, only two of the 12 false positive *N. meningitidis* were still identified as *N.*  
234 *meningitidis* (id40248 and id45356) with scores >2. Both strains were identified as *N.*  
235 *polysaccharea* by cgMLST (Table 2). However, two strains (id42059 and id43987) were  
236 identified as *N. bergeri* with the extended database (scores of 1.89 and 2.17 respectively)  
237 whereas they were characterized as *N. cinerea* and *N. polysaccharea* on the basis of genomic

238 database respectively. Using these results, the extended database allowed an identification of  
239 *N. meningitidis* by MALDI-TOF within these 32 strains with a specificity of 92% (75-98%,  
240 95% CI) and a sensitivity of 100% (65-100%, 95% CI).

241

## 242 **Discussion**

243 In this work we described a new MALDI-TOF database that is based on WGS as a gold  
244 standard to identify bacterial species. Indeed, our data suggest that identification may be  
245 problematic for bacterial species such as those of the genus *Neisseria* where free and frequent  
246 horizontal DNA exchanges may occur. Indeed, the data clearly showed that the currently  
247 available database showed low specificity for the identification of *N. meningitidis* resulting in  
248 a high level of false positive diagnosis of this species. Concordantly, some strains tested in  
249 our study that were misidentified as *N. meningitidis* by MALDI-TOF/MS (*N. cinerea* and *N.*  
250 *polysaccharea*) were from invasive infections and were isolated from blood (Table 1 and 2).  
251 The performance of MALDI-TOF can be improved by enriching the Bruker database with  
252 spectra from poorly represented species and from species that are close to *N. meningitidis*.  
253 Indeed, most of isolates identified as *N. meningitidis* by mass spectrometry, actually belonged  
254 all to *N. cinerea* species and *N. polysaccharea* polyphyletic group. With the expansion of  
255 Bruker MSP by our database (MSP based on genomic data analysis), we were able to improve  
256 the diagnosis of *N. meningitidis* with higher specificity (92% versus 52%). Enriching the  
257 database of the MALDI-TOF Bruker system was also reported to improve the identification  
258 of *N. gonorrhoeae* (21). Our work presents the originality of using WGS data as a gold  
259 standard to validate the identification of bacterial species. Genomic-based identification may  
260 be suggested as a gold standard to build up new collection of reference strains that can be  
261 used to construct spectra databases. However our work included a limited number of isolates

262 belonging to non-*Neisseria meningitidis* species and this may explain that two isolates were  
263 still identified as *N. meningitidis* by using the expanded spectral database, whereas these  
264 isolates clustered within the *N. polysaccharea* group. This highlights the need to increment  
265 and frequently update the MSP database based on genome comparison analysis, in order to  
266 enhance the performance of *Neisseria* species identification by mass spectrometry and  
267 conducting similar work on other commercially available databases is needed. Our work is  
268 highly relevant as bacterial identification by MALDI-TOF/MS is becoming widely used in  
269 clinical microbiology laboratories due to its simplicity, rapidity and low cost. Indeed, it  
270 provides within few minutes a bacterial identification on microbial colonies or blood cultures.  
271 This diagnosis is of concern as *Neisseria meningitidis* can be encountered in invasive  
272 bacterial disease leading to unwarranted uses of prophylactic antibiotics. Our new database is  
273 expected to improve the quality of this rapid diagnosis and should hence be beneficial for  
274 rapid management of meningococcal disease.

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#### 276 **Transparency Declaration**

277 All authors report no conflicts of interest relevant to this article.

278

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283 Sequence Typing website (<https://pubmlst.org/neisseria/>) developed by Keith Jolley and sited  
284 at the University of Oxford (15). The development of this site has been funded by the  
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286 Foundation Meningococcus Genome Library (<http://www.meningitis.org/research/genome>)  
287 developed by Public Health England, the Wellcome Trust Sanger Institute and the University  
288 of Oxford.

### 289 **Author contributions**

290 E.H. and M.K.T. conceived and designed the experiments; E.H and Y.B. performed the  
291 experiments; E.H. and M.K.T. analysed the data; E.H wrote the manuscript with input from  
292 M.K.T. All authors reviewed and edited the manuscript.

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363 **Legends of figures and tables**

364 **Figure.** Characterization of isolates by genome analysis. Neighbor-joining analysis of rMLST  
365 (A.) and cgMLST (B.) of our two collections (reference and test) (56 isolates) with 50  
366 available isolates in the BIGSdb database. Discordance between genomic diagnosis and  
367 phenotypic and biochemical tests are indicated with blue circles or branches (N=7).  
368 Concordant identification between genomic data analysis and MALDI-TOF identification  
369 with Bruker's database are indicated with green branches. Red branches stand for a  
370 discordance between genomic diagnosis and MALDI-TOF identification with Bruker's  
371 database. Misidentification of *N. meningitidis* by MALDI-TOF diagnosis with Bruker's  
372 database are indicated with red stars.

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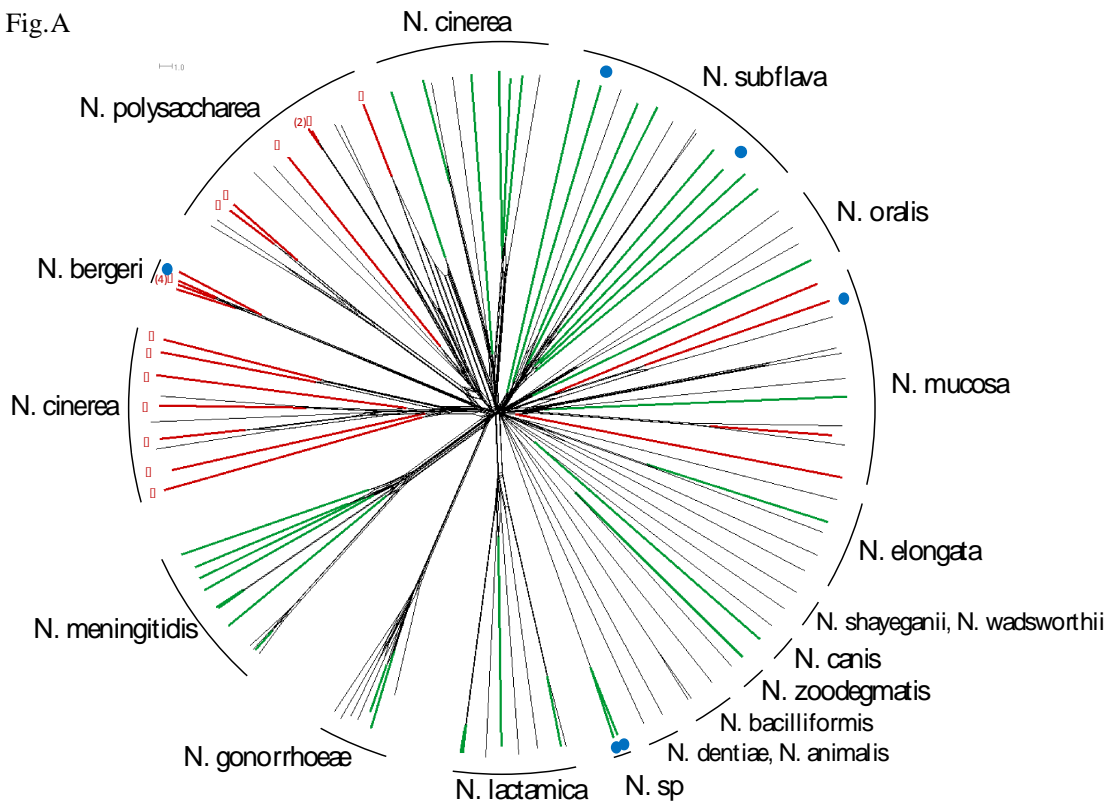
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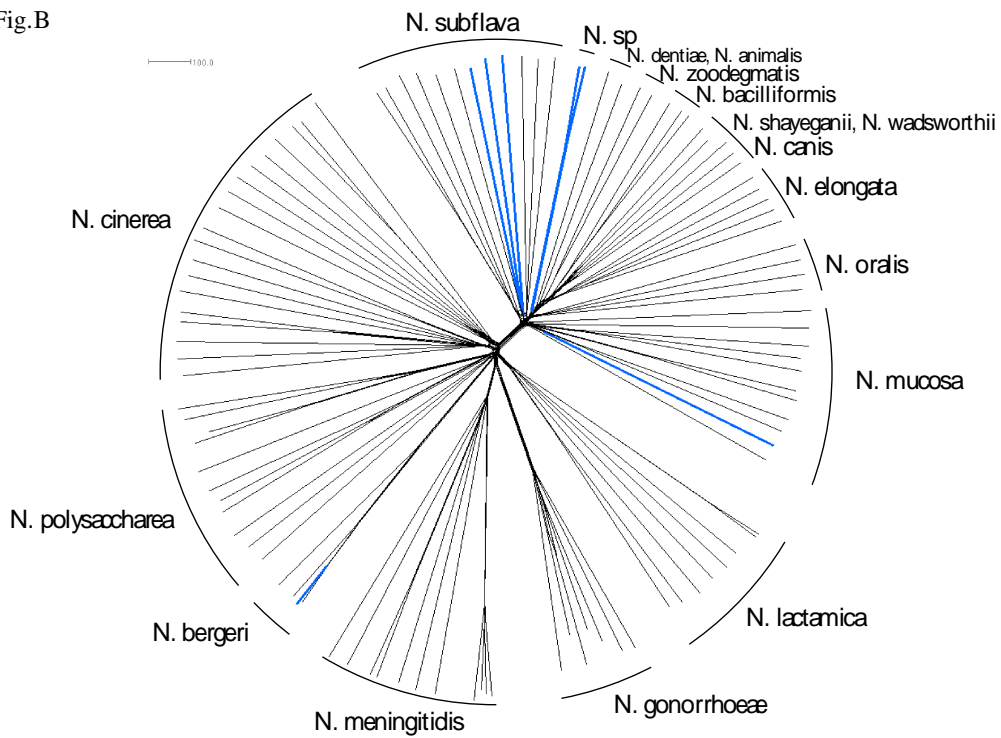


Fig.A



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Fig.B



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382 **Table 1.** Characterization of our collection of reference strains by biochemical tests, rMLST  
383 analysis, cgMLST analysis and MALDI-TOF. *N. meningitidis* misidentifications by MALDI-  
384 TOF are highlighted in grey. PubMLST id (i) indicates the strain was isolated from an  
385 invasive site.

| PubMLST id | Biochemical identification            | rMLST identification    | cgMLST identification   | BRUKER MALDI-TOF identification  | BRUKER MALDI-TOF mean score |
|------------|---------------------------------------|-------------------------|-------------------------|----------------------------------|-----------------------------|
| 38974 (i)  | <i>N. perflava</i>                    | <i>N. mucosa</i>        | <i>N. mucosa</i>        | <i>N. macacae</i>                | 2,13                        |
| 38975 (i)  | <i>N. lactamica</i>                   | <i>N. lactamica</i>     | <i>N. lactamica</i>     | <i>N. lactamica</i>              | 2,51                        |
| 38976      | <i>N. subflava</i>                    | <i>N. subflava</i>      | <i>N. subflava</i>      | <i>N. subflava</i>               | 2,41                        |
| 38977      | <i>N. mucosa</i>                      | <i>N. mucosa</i>        | <i>N. mucosa</i>        | <i>N. macacae</i>                | 2,15                        |
| 38978      | <i>N. cinerea</i>                     | <i>N. cinerea</i>       | <i>N. cinerea</i>       | <i>N. cinerea</i>                | 2,48                        |
| 38979      | <i>N. flava</i>                       | <i>N. subflava</i>      | <i>N. subflava</i>      | <i>N. subflava</i>               | 2,57                        |
| 38980 (i)  | <i>N. polysaccharea</i>               | <i>N. polysaccharea</i> | <i>N. polysaccharea</i> | <i>N. meningitidis</i>           | 2,31                        |
| 38981      | <i>N. polysaccharea</i>               | <i>N. polysaccharea</i> | <i>N. polysaccharea</i> | <i>N. meningitidis</i>           | 2,34                        |
| 38982      | <i>N. gonorrhoeae</i>                 | <i>N. gonorrhoeae</i>   | <i>N. gonorrhoeae</i>   | <i>N. gonorrhoeae</i>            | 2,53                        |
| 38983 (i)  | <i>N. meningitidis</i>                | <i>N. meningitidis</i>  | <i>N. meningitidis</i>  | <i>N. meningitidis</i>           | 2,58                        |
| 38984      | <i>N. cinerea</i>                     | <i>N. cinerea</i>       | <i>N. cinerea</i>       | <i>N. cinerea</i>                | 2,53                        |
| 38985 (i)  | <i>N. sp. close to N. lactamica</i>   | <i>N. lactamica</i>     | <i>N. lactamica</i>     | <i>N. lactamica</i>              | 2,57                        |
| 38986 (i)  | <i>N. cinerea</i>                     | <i>N. cinerea</i>       | <i>N. cinerea</i>       | <i>N. meningitidis</i>           | 1,65                        |
| 39715      | <i>N. mucosa</i>                      | <i>N. mucosa</i>        | <i>N. mucosa</i>        | <i>N. subflava</i>               | 1,89                        |
| 39716      | <i>N. canis</i>                       | <i>N. canis</i>         | <i>N. canis</i>         | <i>N. canis</i>                  | 2,23                        |
| 39717 (i)  | <i>N. cinerea</i>                     | <i>N. cinerea</i>       | <i>N. cinerea</i>       | <i>N. cinerea</i>                | 2,22                        |
| 39718      | <i>N. polysaccharea</i>               | <i>N. bergeri</i>       | <i>N. bergeri</i>       | <i>N. meningitidis</i>           | 2,35                        |
| 39719      | <i>N. denitrificans</i>               | <i>N. sp.</i>           | <i>N. sp.</i>           | <i>Bergeriella denitrificans</i> | 1,76                        |
| 39720      | <i>N. canis</i>                       | <i>N. sp.</i>           | <i>N. sp.</i>           | <i>Bergeriella denitrificans</i> | 1,85                        |
| 39721      | <i>N. cinerea</i>                     |                         | <i>N. cinerea</i>       | <i>N. cinerea</i>                | 2,35                        |
| 39722 (i)  | <i>N. sicca</i>                       | <i>N. subflava</i>      | <i>N. subflava</i>      | <i>N. subflava</i>               | 2,43                        |
| 39723 (i)  | <i>N. perflava</i>                    | <i>N. subflava</i>      | <i>N. subflava</i>      | <i>N. subflava</i>               | 2,52                        |
| 39724      | <i>N. cinerea</i>                     | <i>N. cinerea</i>       | <i>N. cinerea</i>       | <i>N. meningitidis</i>           | 1,84                        |
| 39725      | <i>N. sp. close to N. zoodegmatis</i> | <i>N. zoodegmatis</i>   | <i>N. zoodegmatis</i>   | <i>N. zoodegmatis</i>            | 2,59                        |

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393 **Table 2.** Improvement of MALDI-TOF identification against a test collection of strains by  
394 implementing profiles (reference collection) to the manufacturer's database. *N. meningitidis*  
395 misidentifications by MALDITOF are highlighted in grey. PubMLST id (i) indicates the  
396 strain was isolated from an invasive site.  
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| PubMLST id | Biochemical identification | rMLST identification | cgMLST identification | BRUKER MALDI-TOF identification | BRUKER MALDI-TOF mean score | BRUKER+MSP MALDI-TOF identification | BRUKER+MSP MALDI-TOF mean score |
|------------|----------------------------|----------------------|-----------------------|---------------------------------|-----------------------------|-------------------------------------|---------------------------------|
| 40248      | N. sp.                     | N. polysaccharea     | N. polysaccharea      | N. meningitidis                 | 2,29                        | N. meningitidis                     | 2,29                            |
| 40261 (i)  | N. sicca                   | N. subflava          | N. subflava           | N. subflava                     | 2,16                        | N. subflava                         | 2,32                            |
| 40270 (i)  | N. meningitidis            | N. subflava          | N. subflava           | N. subflava                     | 2,28                        | N. subflava                         | 2,43                            |
| 41220      | N. polysaccharea           | N. bergeri           | N. bergeri            | N. meningitidis                 | 2,21                        | N. bergeri                          | 2,61                            |
| 42055 (i)  | N. mucosa                  | N. oralis            | N. oralis             | N. oralis                       | 2,34                        | N. oralis                           | 2,34                            |
| 42057      | N. cinerea                 | N. cinerea           | N. cinerea            | N. meningitidis                 | 1,97                        | N. cinerea                          | 2,19                            |
| 42058      | N. cinerea                 | N. cinerea           | N. cinerea            | N. meningitidis                 | 1,97                        | N. cinerea                          | 2,22                            |
| 42059      | N. cinerea                 | N. cinerea           | N. cinerea            | N. meningitidis                 | 1,91                        | N. bergeri                          | 1,89                            |
| 42060      | N. sicca                   | N. subflava          | N. subflava           | N. subflava                     | 2,26                        | N. subflava                         | 2,29                            |
| 42061      | N. sp.                     | N. cinerea           | N. cinerea            | N. cinerea                      | 2,24                        | N. cinerea                          | 2,28                            |
| 42062      | N. sp.                     | N. cinerea           | N. cinerea            | N. meningitidis                 | 1,92                        | N. cinerea                          | 2,13                            |
| 42198      | N. lactamica               | N. lactamica         | N. lactamica          | N. lactamica                    | 2,39                        | N. lactamica                        | 2,43                            |
| 42421 (i)  | N. cinerea                 | N. cinerea           | N. cinerea            | N. meningitidis                 | 1,86                        | N. cinerea                          | 2,24                            |
| 43987      | N. polysaccharea           | N. polysaccharea     | N. polysaccharea      | N. meningitidis                 | 2,22                        | N. bergeri                          | 2,17                            |
| 45355      | N. mucosa                  | N. mucosa            | N. mucosa             | N. macacae                      | 2,06                        | N. mucosa                           | 2,55                            |
| 45356      | N. polysaccharea           | N. polysaccharea     | N. polysaccharea      | N. meningitidis                 | 2,22                        | N. meningitidis                     | 2,22                            |
| 46114 (i)  | N. mucosa                  | N. mucosa            | N. mucosa             | N. mucosa                       | 2,25                        | N. mucosa                           | 2,45                            |
| 49258      | N. cinerea                 | N. cinerea           | N. cinerea            | N. cinerea                      | 2,39                        | N. cinerea                          | 2,40                            |
| 49259      | N. polysaccharea           | N. bergeri           | N. bergeri            | N. meningitidis                 | 2,22                        | N. bergeri                          | 2,55                            |
| 51008 (i)  | N. meningitidis            | N. meningitidis      | N. meningitidis       | N. meningitidis                 | 2,47                        | N. meningitidis                     | 2,47                            |
| 51545      | N. gonorrhoeae             | N. gonorrhoeae       | N. gonorrhoeae        | N. gonorrhoeae                  | 2,35                        | N. gonorrhoeae                      | 2,35                            |
| 51557 (i)  | N. meningitidis            | N. meningitidis      | N. meningitidis       | N. meningitidis                 | 2,48                        | N. meningitidis                     | 2,48                            |
| 51558 (i)  | N. meningitidis            | N. meningitidis      | N. meningitidis       | N. meningitidis                 | 2,57                        | N. meningitidis                     | 2,57                            |
| 51584      | N. meningitidis            | N. meningitidis      | N. meningitidis       | N. meningitidis                 | 2,47                        | N. meningitidis                     | 2,47                            |
| 52432      | N. sicca                   | N. subflava          | N. subflava           | N. subflava                     | 2,32                        | N. subflava                         | 2,08                            |
| 53422 (i)  | N. meningitidis            | N. meningitidis      | N. meningitidis       | N. meningitidis                 | 2,52                        | N. meningitidis                     | 2,52                            |
| 57256 (i)  | N. elongata                | N. elongata          | N. elongata           | N. elongata                     | 2,51                        | N. elongata                         | 2,51                            |
| 57266 (i)  | N. meningitidis            | N. meningitidis      | N. meningitidis       | N. meningitidis                 | 2,56                        | N. meningitidis                     | 2,56                            |
| 57277 (i)  | N. meningitidis            | N. meningitidis      | N. meningitidis       | N. meningitidis                 | 2,53                        | N. meningitidis                     | 2,53                            |
| 58872      | N. lactamica               | N. lactamica         | N. lactamica          | N. lactamica                    | 2,37                        | N. lactamica                        | 2,37                            |
| 58873      | N. polysaccharea           | N. polysaccharea     | N. polysaccharea      | N. meningitidis                 | 2,36                        | N. polysaccharea                    | 2,33                            |
| 58874      | N. polysaccharea           | N. bergeri           | N. bergeri            | N. meningitidis                 | 2,13                        | N. bergeri                          | 2,24                            |

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