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Comparison of *Neisseria gonorrhoeae* Multiantigen Sequence Typing and *porB* Sequence Analysis for Identification of Clusters of *N. gonorrhoeae* Isolates[▽]†

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porB **DNA sequence analysis and *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) methods were compared for their abilities to discriminate strains and to identify epidemiologically congruent pairs of *N. gonorrhoeae*. Both methods provided high-level discrimination of strains. NG-MAST further differentiated large *porB*-based clusters. However, considerations of cost suggest that *porB* DNA sequence analysis is a useful tool for preliminary molecular analysis of the epidemiology of *N. gonorrhoeae*.**

Molecular typing methods that differentiate *Neisseria gonorrhoeae* isolates, coupled with traditional epidemiological methods, have been used to identify circulating clusters of strains and transmission networks (3, 24). DNA sequence analysis of various genes is currently the method of choice for distinguishing *N. gonorrhoeae* strains, as it provides unambiguous and reproducible information and high discriminatory power, and data can be stored or shared electronically, permitting reliable comparisons to be made between laboratories (10, 11, 23). *porB* DNA sequence analysis is now commonly used for studying the molecular epidemiology of *N. gonorrhoeae* (1, 9, 13, 15, 16, 19, 20, 23) and involves the sequencing of either the entire gene or various regions of *porB* (8, 17–19). The *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) methodology, which has been applied since 2004 (2, 9, 13, 20, 21), is based on limited DNA sequence analyses of two highly polymorphic loci, *porB* and *tbpB* (11). The NG-MAST database, available online (www.ng-mast.net), allows public access for sequence submission and the assignment of sequence types either for *porB* or *tbpB* individually or for the assignment of strain types (STs) using a combination of the two loci (11). The objective of the present study was to compare NG-MAST with *porB* DNA sequence analysis (~82% of the full-length *porB* gene) to identify circulating clusters of *N. gonorrhoeae* isolates in Shanghai, China, and to evaluate the correlation between self-reported sexual contacts and genotypes of *N. gonorrhoeae* isolates.

The *N. gonorrhoeae* isolates (n = 199) were collected from males with gonorrhea (n = 157) and their positive female partners (n = 42), as previously described (8, 25). These isolates included 39 pairs and one triplet (one male with two female partners) from patients with self-reported sexual contacts. The identification and growth of *N. gonorrhoeae* isolates were described previously (8, 25). DNA was extracted from the isolates and used for PCR amplification of *porB* and *tbpB*. Amplicons were analyzed as previously described (8, 11, 25). *porB* DNA sequence analysis covered ~82% of the nucleotides encoding surface-exposed loops I to VII and interspace regions II to VII as described and analyzed previously (8, 15, 22), and *porB* DNA sequences were deposited in the GenBank database (8). Each isolate was arbitrarily assigned a *porB* DNA sequence type (PST), and isolates were sorted into groups based on PSTs; groups also defined common clusters of isolates. For NG-MAST analysis, DNA sequences of *porB* and *tbpB* fragments as described previously were submitted to the NG-MAST database (www.ng-mast.net; last accessed 2 May 2008) (11). NG-MAST STs were assigned for each *N. gonorrhoeae* isolate, and STs new to the NG-MAST database were identified. Simpson's index of diversity (ID) was used to determine the discriminatory abilities of each method (4, 7). To minimize clonal effects, only *porB* DNA sequences and STs from isolates from male patients (n = 157) were used in ID analyses.

The IDs of *porB*-based analyses and NG-MAST for *N. gonorrhoeae* isolates from male patients were 0.942 and 0.982, respectively. Among the 199 clinical isolates, including female partners, 81 PSTs were identified (see Table S1 in the supplemental material). Isolates were separated into nine groups based on their PSTs (Fig. 1A). Group I included 46 (23%) isolates each having a distinct PST. Group II comprised 24 *porB* DNA sequences with two isolates per PST, i.e., 48 isolates (24%) having 24 PSTs. Group III contained 12 isolates (6%) with four PSTs, with each PST comprising three isolates. Group V included 10 isolates (5%) divided into two *porB* groups (i.e., each PST with five isolates). Isolates in groups IV (n = 4; 2%), VI (n = 6; 3%), VII (n = 14; 7%), VIII (n = 17; 9%), and IX (n = 42; 21%) had an identical PST in each

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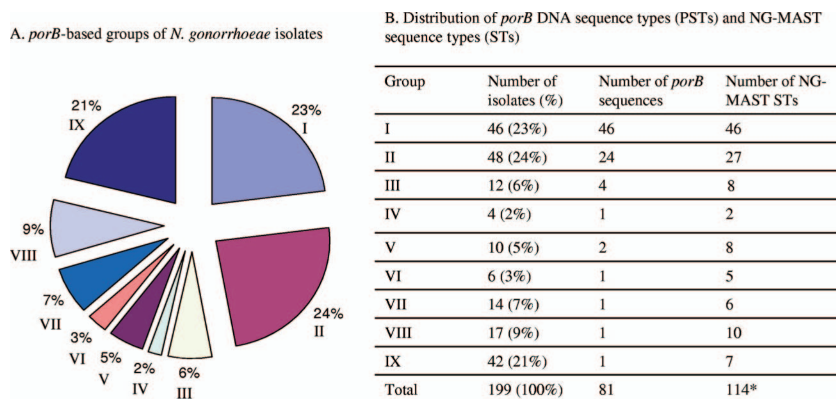

B. Distribution of *porB* DNA sequence types (PSTs) and NG-MAST sequence types (STs)

Group	Number of isolates (%)	Number of *porB* sequences	Number of NG-MAST STs
I	46 (23%)	46	46
II	48 (24%)	24	27
III	12 (6%)	4	8
IV	4 (2%)	1	2
V	10 (5%)	2	8
VI	6 (3%)	1	5
VII	14 (7%)	1	6
VIII	17 (9%)	1	10
IX	42 (21%)	1	7
Total	199 (100%)	81	114*

FIG. 1. *porB*-based groups and distributions of PSTs and NG-MAST STs of 199 *N. gonorrhoeae* isolates. (A) *porB*-based groups. (B) Distribution of *porB* PSTs and NG-MAST STs. Isolates were grouped based on the number of isolates with identical *porB* PSTs. For group I, each isolate has a distinct PST (n = 46). For group II, each two isolates have an identical PST (n = 48). For group III, each three isolates have an identical PST (n = 12). For group IV, four isolates have an identical PST. For group V, each five isolates have an identical PST (n = 10). For group VI, six isolates have an identical PST. For group VII, 14 isolates have an identical PST. For group VIII, 17 isolates have an identical PST. For group IX, 42 isolates have an identical PST. Percentages represent the proportions of total isolates. *, some NG-MAST STs were further differentiated by *porB* typing.

group, and PSTs were distinct between the groups (Fig. 1A and B).

Among the 199 *N. gonorrhoeae* isolates, 114 NG-MAST STs were identified, and 71 (62%) of these STs had not been previously reported (Fig. 1B; see also Table S1 in the supplemental material). Groups of *N. gonorrhoeae* isolates identified by *porB*-based analysis and NG-MAST were compared; a discordance of clusters between PSTs and NG-MAST STs was noted (see vertical double arrows in Table S1 in the supplemental material). Each isolate in group I (n = 46) exhibited a distinct ST. There were 24 PSTs in group II and 27 NG-MAST STs with 2 PSTs distinguished by NG-MAST. Isolates in group III (n = 12) with four PSTs were further differentiated into eight STs, with three of the PSTs being further differentiated by NG-MAST. Each of the two group V PSTs (n = 10) were differentiated into four different NG-MAST STs, for a total of eight STs from group V. Isolates with a single PST in groups IV (n = 4), VI (n = 6), VII (n = 14), VIII (n = 17), and IX (n = 42) were further differentiated into 2, 5, 6, 10, and 7 NG-MAST STs, respectively (Fig. 1B; see also Table S1 in the supplemental material). On the other hand, a few isolates with identical NG-MAST STs were further differentiated by *porB* DNA sequence analysis. ST567 (two isolates), ST641 (four isolates), ST1691 (six isolates), and ST2066 (three isolates) were differentiated into two PSTs, respectively.

The congruence of genetic types and sexual connections identified by contact tracing was analyzed. Thirty *porB* PSTs and 36 NG-MAST STs were identified among 81 isolates comprising 40 sexual contacts (39 pairs and one trio) (see Table S2 in the supplemental material). *porB* PSTs in the majority of isolates having sexual connections (37 pairs and the trio) were identical, while two pairs (pairs 141/141F and 41/41F) had different PSTs (see isolates in boldface type in Table S2 in the supplemental material). NG-MAST typing revealed that the majority of pairs (37/39 pairs) and the trio had identical STs (see Table S2 in the supplemental material). As expected, the two pairs with different PSTs also had different NG-MAST STs. Noticeably, each of three PSTs (i.e., PST15 [nine pairs], PST67 [three pairs], and PST71 [two pairs and the trio]) were differentiated into three NG-MAST STs (see open boxes in Table S2 in the supplemental material), although seven of nine pairs of PST15 also had an identical NG-MAST ST (i.e., ST421).

This study shows that both *porB* DNA sequence analysis and NG-MAST analysis have high discriminatory powers sufficient to distinguish *N. gonorrhoeae* isolates and to identify circulating clusters of strains. Among isolates with identified epidemiological links, both methods were congruent with epidemiological findings. These results validate the use of *porB* DNA sequence analysis for epidemiological studies, in agreement with data from previous studies (1, 5, 8, 11, 13, 15, 18–21, 23). NG-MAST further differentiated *porB* types due to the sequence variations present in *tbpB* alleles (6), which contributed to a different NG-MAST ST. However, it is uncertain whether the differences detected in *tbpB* using NG-MAST are indicative of epidemiologically distinct groups of isolates, and further study is required. The majority of isolates in the largest *porB*-based cluster (PST15; n = 42) exhibited a single NG-MAST ST (ST421; n = 27), demonstrating that the two methods are congruent in defining predominant clusters of transmission. Other isolates of this large *porB* cluster (n = 15) displayed six distinct NG-MAST STs, which warrants further epidemiological investigation to determine whether these isolates are truly related or not.

porB DNA sequence analysis is less costly and quicker to perform, as this method can be determined by one PCR and two DNA sequencing reactions (one reaction for each strand). NG-MAST typing involves two PCR and four DNA sequencing reactions. *porB* DNA sequences are associated with two gonococcal *porB* isoforms (i.e., *porB1a* and *porB1b*) (8) and would additionally provide isolate information with clinical relevance, as *porB* serovar analysis was formerly used (18). However, the association between *porB* sequence types and serovar types remains to be elucidated (17). It should be noted that the analysis of various lengths of *porB* DNA sequence can produce different discriminatory abilities. Olsen et al. previ-

ously reported that an analysis of the entire *porB* DNA sequence had an ID of 97.8% (13); however, four sequencing reactions (two for each DNA strand) were required to ascertain the entire *porB* gene sequence. By analyzing DNA sequences of defined regions covering ~82% of the *N. gonorrhoeae porB* gene, we obtained an ID of 94.2%, which we consider to provide sufficiently high discrimination for identifying transmission clusters of *N. gonorrhoeae* (4, 7). This method also confirmed epidemiologically identified sexual contacts (8).

Standardized guidelines should be established if *porB* DNA sequence analysis is to be used for widely studying *N. gonorrhoeae* molecular epidemiology, to facilitate interlaboratory comparisons, and to differentiate *N. gonorrhoeae* clusters from different geographical regions. An independent *N. gonorrhoeae porB* DNA sequence database has not yet been established; a publicly accessible *N. gonorrhoeae porB* database is needed for depositing of sequence data and for sequence type assignment, as well as for spatial and temporal characterizations of *N. gonorrhoeae* isolate distribution. Furthermore, some common *porB* type assignments should be established between NG-MAST-assigned types and *porB* types based on larger *porB* segments. Overall, *porB* DNA sequence analysis is a useful tool for determining *N. gonorrhoeae* molecular epidemiology, particularly in resource-limited settings. NG-MAST analysis can be used if higher discrimination is required for the further differentiation of large *porB*-based clusters of *N. gonorrhoeae*. Opa typing is another highly discriminatory method that has been used for the molecular typing of *N. gonorrhoeae* (12, 14). However, DNA sequencing methods are replacing the Opa typing approach, which differentiates *N. gonorrhoeae* isolates based on banding patterns of amplified/restriction endonuclease-digested *opa* genes (14).

Nucleotide sequence accession numbers. The *porB* DNA sequences reported here were deposited in the GenBank database under accession numbers EF540591 to EF540669 and EU719202 to EU719208. *tbpB* and *porB* sequences for NG-MAST analysis have been submitted to the NG-MAST database.

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REFERENCES

1. **Bash, M., P. Zhu, S. Gulati, D. McKnew, P. Rice, and F. Lynn.** 2005. *por* variable-region typing by DNA probe hybridization is broadly applicable to epidemiologic studies of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **43:**1522–1530.
2. **Bilek, N., I. Martin, G. Bell, G. Kinghorn, C. Ison, and B. Spratt.** 2007. Concordance between *Neisseria gonorrhoeae* genotypes recovered from known sexual contacts. J. Clin. Microbiol. **45:**3564–3567.
3. **Choudhury, B., C. Risley, A. Ghani, C. Bishop, H. Ward, K. Fenton, C. Ison, and B. Spratt.** 2006. Identification of individuals with gonorrhoea within sexual networks: a population-based study. Lancet **368:**139–146.
4. **Dillon, J., M. Rahman, and K. Yeung.** 1993. Discriminatory power of typing schemes based on Simpson's index of diversity for *Neisseria gonorrhoeae*. J. Clin. Microbiol. **31:**2831–2833.
5. **Fredlund, H., L. Falk, M. Jurstrand, and M. Unemo.** 2004. Molecular genetic methods for diagnosis and characterisation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: impact on epidemiological surveillance and interventions. APMIS **112:**771–784.
6. **Harrison, O., M. Maiden, and B. Rokbi.** 2008. Distribution of transferrin binding protein B gene (*tbpB*) variants among *Neisseria* species. BMC Microbiol. **8:**66.
7. **Hunter, P., and M. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
8. **Liao, M. M., K. Bell, W. M. Gu, Y. Yang, N. F. Eng, W. K. Fu, L. Wu, C. G. Zhang, Y. Chen, A. M. Jolly, and J. A. R. Dillon.** 2008. Clusters of circulating *Neisseria gonorrhoeae* strains and association with antimicrobial resistance in Shanghai. J. Antimicrob. Chemother. **61:**478–487.
9. **Lundbäck, D., H. Fredlund, T. Berglund, B. Wretlind, and M. Unemo.** 2006. Molecular epidemiology of *Neisseria gonorrhoeae*—identification of the first presumed Swedish transmission chain of an azithromycin-resistant strain. APMIS **114:**67–71.
10. **Maiden, M.** 2006. Multilocus sequence typing of bacteria. Annu. Rev. Microbiol. **60:**561–588.
11. **Martin, I., C. Ison, D. Aanensen, K. Fenton, and B. Spratt.** 2004. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J. Infect. Dis. **189:**1497–1505.
12. **Morris, A., H. Palmer, and H. Young.** 2008. Opa-typing can identify epidemiologically distinct subgroups within *Neisseria gonorrhoeae* multi-antigen sequence type (NG-MAST) clusters. Epidemiol. Infect. **136:**417–420.
13. **Olsen, B., R. Hadad, H. Fredlund, and M. Unemo.** 2008. The *Neisseria gonorrhoeae* population in Sweden during 2005—phenotypes, genotypes and antibiotic resistance. APMIS **116:**181–189.
14. **O'Rourke, M., C. Ison, A. Renton, and B. Spratt.** 1995. Opa-typing: a high-resolution tool for studying the epidemiology of gonorrhoea. Mol. Microbiol. **17:**865–875.
15. **Pérez-Losada, M., R. Viscidi, J. Demma, J. Zenilman, and K. Crandall.** 2005. Population genetics of *Neisseria gonorrhoeae* in a high-prevalence community using a hypervariable outer membrane *porB* and 13 slowly evolving housekeeping genes. Mol. Biol. Evol. **22:**1887–1902.
16. **Posada, D., K. Crandall, M. Nguyen, J. Demma, and R. Viscidi.** 2000. Population genetics of the *porB* gene of *Neisseria gonorrhoeae*: different dynamics in different homology groups. Mol. Biol. Evol. **17:**423–436.
17. **Unemo, M., P. Olcén, J. Albert, and H. Fredlund.** 2003. Comparison of serologic and genetic *porB*-based typing of *Neisseria gonorrhoeae*: consequences for future characterization. J. Clin. Microbiol. **41:**4141–4147.
18. **Unemo, M., P. Olcén, T. Berglund, J. Albert, and H. Fredlund.** 2002. Molecular epidemiology of *Neisseria gonorrhoeae*: sequence analysis of the *porB* gene confirms presence of two circulating strains. J. Clin. Microbiol. **40:**3741–3749.
19. **Unemo, M., P. Olcén, J. Jonasson, and H. Fredlund.** 2004. Molecular typing of *Neisseria gonorrhoeae* isolates by pyrosequencing of highly polymorphic segments of the *porB* gene. J. Clin. Microbiol. **42:**2926–2934.
20. **Unemo, M., A. Sjöstrand, M. Akhras, B. Gharizadeh, E. Lindbäck, N. Pourmand, B. Wretlind, and H. Fredlund.** 2007. Molecular characterization of *Neisseria gonorrhoeae* identifies transmission and resistance of one ciprofloxacin-resistant strain. APMIS **115:**231–241.
21. **Unemo, M., V. Vorobieva, N. Firsova, T. Ababkova, I. Leniv, B. Haldorsen, H. Fredlund, and V. Skogen.** 2007. *Neisseria gonorrhoeae* population in Arkhangelsk, Russia: phenotypic and genotypic heterogeneity. Clin. Microbiol. Infect. **13:**873–878.
22. **van der Ley, P., J. Heckels, M. Virji, P. Hoogerhout, and J. Poolman.** 1991. Topology of outer membrane porins in pathogenic *Neisseria* spp. Infect. Immun. **59:**2963–2971.
23. **Viscidi, R., J. Demma, J. Gu, and J. Zenilman.** 2000. Comparison of sequencing of the *por* gene and typing of the *opa* gene for discrimination of *Neisseria gonorrhoeae* strains from sexual contacts. J. Clin. Microbiol. **38:**4430–4438.
24. **Ward, H., C. Ison, S. Day, I. Martin, A. Ghani, G. Garnett, G. Bell, G. Kinghorn, and J. Weber.** 2000. A prospective social and molecular investigation of gonococcal transmission. Lancet **356:**1812–1817.
25. **Yang, Y., M. Liao, W. Gu, K. Bell, L. Wu, N. Eng, C. Zhang, Y. Chen, A. Jolly, and J. Dillon.** 2006. Antimicrobial susceptibility and molecular determinants of quinolone resistance in *Neisseria gonorrhoeae* isolates from Shanghai. J. Antimicrob. Chemother. **58:**868–872.