Methods for Identifying Neisseria meningitidis Carriers: A Multi-Center Study in the African Meningitis Belt

Nicole E. Basta1,2,3*, James M. Stuart4, Maria C. Nascimento4, Olivier Manigart4, Caroline Trotter5, Musa Hassan-King4, Daniel Chandramohan4, Samba O. Sow4, Abdoulaye Berthe6, Ahmed Bedru7, Yenenes K. Tekletision7,8, Jean-Marc Collard9, Jean-François Jusot6, Aldiouma Diallo10, Hubert Basséne10, Doumagoum M. Daugla11, Khadidia Gamougam11, Abraham Hodgson12, Abdulai A. Forgor13, Babatunji A. Omotara14, Galadima B. Gadzama15, Eleanor R. Watkins8, Lisa S. Rebbetts8, Kanny Diallo6,8, Noel S. Weiss3,16, M. Elizabeth Halloran3,17, Martin C. J. Maiden8, Brian Greenwood4

Abstract

Objective: Detection of meningococcal carriers is key to understanding the epidemiology of Neisseria meningitidis, yet no gold standard has been established. Here, we directly compare two methods for collecting pharyngeal swabs to identify meningococcal carriers.

Methods: We conducted cross-sectional surveys of schoolchildren at multiple sites in Africa to compare swabbing the posterior pharynx behind the uvula (U) to swabbing the posterior pharynx behind the uvula plus one tonsil (T). Swabs were cultured immediately and analyzed using molecular methods.

Results: One thousand and six paired swab samples collected from schoolchildren in four countries were analyzed. Prevalence of meningococcal carriage was 6.9% (95% CI: 5.4-8.6%) based on the results from both swabs, but the observed prevalence was lower based on one swab type alone. Prevalence based on the T swab or the U swab alone was similar (5.2% (95% CI: 3.8-6.7%) versus 4.9% (95% CI: 3.6-6.4%) respectively (p=0.6)). The concordance between the two methods was 96.3% and the kappa was 0.61 (95% CI: 0.50-0.73), indicating good agreement.

Conclusions: These two commonly used methods for collecting pharyngeal swabs provide consistent estimates of the prevalence of carriage, but both methods misclassified carriers to some degree, leading to underestimates of the prevalence.

Citation: Basta NE, Stuart JM, Nascimento MC, Manigart O, Trotter C, et al. (2013) Methods for Identifying Neisseria meningitidis Carriers: A Multi-Center Study in the African Meningitis Belt. PLoS ONE 8(10): e78336. doi:10.1371/journal.pone.0078336

Editor: Jose Antonio Stoute, Pennsylvania State University College of Medicine, United States of America

Received August 6, 2013; Accepted September 19, 2013; Published October 23, 2013

Copyright: © 2013 Basta et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This research was funded by the Bill & Melinda Gates Foundation Grant 51251 (www.gatesfoundation.org), the Wellcome Trust Grant 086546 (www.wellcome.ac.uk), NIH Grant R37AI032042 (support for MEH, NEB), NIH Early Independence Award 1DP5OD009162 (Office of the Director and the National Institute of Dental and Craniofacial Research; support for NEB) (www.nih.gov), and the RAPIDD program of the Science & Technology Directorate (Department of Homeland Security and the Fogarty International Centre; support for NEB)(http://www.fic.nih.gov). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

* E-mail: nbasta@princeton.edu
Introduction

Devastating, large-scale meningitis outbreaks have occurred in the African meningitis belt every 5-12 years for the past century, resulting in an annual incidence as high as 1,000 cases per 100,000 population during epidemics [1-3]. Typical annual incidence in non-epidemic periods ranges from 1 to 20 cases per 100,000 population [2]. Asymptomatic carriers of Neisseria meningitidis serve as a reservoir for persistence and spread of the bacterium in the population. Understanding the epidemiology and natural history of carriers is central to understanding the epidemiology of meningococcal disease. Interest in this area has increased in recent years, particularly in the context of understanding the effects of conjugate vaccines on carriage [4-8]. In the African meningitis belt, where efforts to prevent major meningococcal epidemics caused by serogroup A meningococci have intensified with the introduction of a new serogroup A polysaccharide-protein conjugate vaccine [18], Prior to the implementation of large, cross-sectional carriage surveys, a multi-center pilot study was conducted in schoolchildren to compare the two pharyngeal swabbing methods for detecting carriage of N. meningitidis described above.

Materials and Methods

Ethics Statement

The study was approved by the Ethics Committee at the London School of Hygiene & Tropical Medicine and the relevant ethical committees at each of the seven participating African centers (Comité National d'Ethique pour la Recherche en Santé (CNERS) in Senegal; Research & Ethical Committee of the University of Maiduguri Teaching Hospital in Nigeria; Comité Consultatif National d'Ethique in Niger; Comité d'Ethique de la Faculté de Médecine et Pharmacie D'Odonto-Stomatologie de l'Université de Bamako in Mali; Navrongo Health Research Center Institutional Review Board in Ghana; Armauer Hansen Research Institute/All Africa Leprosy Rehabilitation and Training Center (AHRI/ALERT) Ethical Review Committee in Ethiopia, and in Chad approval was granted by a committee established to oversee MenAfriCar studies by the Ministry of Health since no formal ethical committee was in place in the country at the time). During enrollment, staff explained the purpose and nature of the study, and a parent or guardian provided written, informed consent for conducting surveillance of carriage, and a clearer understanding of the relationship between carriage, outbreaks of invasive disease, and immunity is needed [14].

Methods for Identifying N. meningitidis Carriers

While sample collection methods for identifying cases of invasive meningococcal disease from cerebrospinal fluid are well established, questions remain about the most effective method for collecting pharyngeal swabs to identify asymptomatic carriers. Methods used in previous studies have not been consistent, with variation in both the region of the pharynx sampled and the treatment of the swab immediately following collection [5,6,15,16]. A recent review compared common collection methods and found that collecting the swab through the mouth rather than through the nose, touching the swab to the posterior pharynx wall alone instead of the tonsils alone, and plating the sample immediately rather than using transport medium improved the identification of carriers [17]. However, the authors of the review argued that further evidence is needed to directly assess whether there is a difference between collecting the sample by swabbing the posterior pharynx alone or in combination with swabbing the tonsils. No previous study has directly addressed this question. In addition, the ecology of pharyngeal carriage of meningococcal and other bacteria is not well understood. Whether swabbing the tonsils and the posterior pharynx would improve the detection of carriers by sampling two surfaces or whether other bacteria living on the tonsils might inhibit growth of the meningococcus is not known.

Evaluating methods for collecting swabs to identify N. meningitidis carriage is key to identification of carriers, to reducing misclassification, and to implementing large-scale studies of carriage, including appropriately powered longitudinal studies. The African Meningococcal Carriage Consortium (MenAfriCar, www.menafricar.org) is an international collaboration that was established in 2009 to define the epidemiology of meningococcal carriage across the meningitis belt before and after the introduction of PsA-TT vaccine [18]. Prior to the implementation of large, cross-sectional carriage surveys, a multi-center pilot study was conducted in schoolchildren to compare the two pharyngeal swabbing methods for detecting carriage of N. meningitidis described above.

Methods for Identifying N. meningitidis Carriers
Modified Thayer-Martin (TM) selective agar plates were prepare locally using Gonococci agar base (Oxoid CM0367B), hemoglobin powder (Oxoid LP053B) containing 3mg/liter vancomycin, 7.5mg/liter colistin, 12.5 U/liter nystatin, 5mg/liter trimetoprim lactate (Oxoid SR00991E) and Vitox enrichment supplement (Oxoid SR0090A) (Thermo Scientific, UK) [19]. Swabs were plated immediately in the field onto TM plates, returned to the laboratory within six hours, and incubated in 5% CO$_2$ at 35-37°C for 24-48 hours to determine growth. A single colony with morphology typical of *N. meningitidis* (large or medium size, blue-grey color, and mucoid in appearance) was selected, sub-cultured on a blood agar plate (BAP), streaked, and incubated in 5% CO$_2$ at 35-37°C for an additional 18-24 hours. BAPs were prepared locally with blood agar base number 2 (Oxoid, CM0271, Thermo Scientific, UK) supplemented with 5% defibrinated sheep’s blood. The colonies remaining on the TM selective agar plate were collected with a sterile plastic loop, suspended in a cryotube containing 1mL of Brain heart infusion (BHI) broth supplemented with 15% glycerol and stored at -80°C. The remaining colonies from the BAP were emulsified in 0.5 mL phosphate buffered saline (PBS) in microcentrifuge tubes, boiled for 20 minutes to release DNA, cooled, divided into four aliquots in 250μL tubes and stored at -20°C for future molecular testing.

During the pilot survey in Mali, the selected colonies sub-cultured onto BAP underwent oxidase testing and Gram staining to ensure that they were Gram-negative diplococci. However, DNA samples prepared from the selected colonies sent from Mali to the University of Oxford for molecular testing showed that these samples contained DNA from many organisms that were not Gram-negative diplococci. This prompted the inclusion of three additional biochemical tests in a new protocol circulated to all the sites: γ-glutamyl transferase activity (GGT) (Rosco Diagnostica, Denmark) for identification of presumptive *N. meningitidis*, β-galactosidase activity with ortho-nitrophenyl-β-D-galactopyranoside (ONPG) (Rosco Diagnostica, Denmark) for identification of *Neisseria lactamica*, and butyrate esterase activity (Tributyrin) (Rosco Diagnostica, Denmark) to further distinguish *Moraxella* species from *Neisseria* species which was the main cause of the initial misidentification [18]. The new protocol piloted in Mali in June 2010 indicated that the introduction of the biochemical tests improved species identification. The new protocol incorporating the biochemical tests was introduced in the rest of the sites two months later using samples from the original pilot study that had been stored in BHI broth supplemented with glycerol at -80°C. These samples were thawed at room temperature, vortex mixed briefly, and plated on TM plates followed by BAPs as described above. Growth from all oxidase positive, Gram negative diplococci samples were harvested into microcentrifuge tubes containing 1 mL PBS, placed in a boiling water bath for 20 minutes to release DNA and inactivate nucleases, cooled, divided into lots and stored at -20°C for molecular testing. Heat killed cell suspensions, prepared from all oxidase-positive, Gram-negative diplococci from each site (49 from Ethiopia, 188 from Mali, 23 from Niger, 95 from Senegal) were sent to the University of Oxford for molecular characterization [18].

A swab was positive for *N. meningitidis* if the *rplF* sequence-based assay, described previously in [18], identified *N. meningitidis*. A participant was classified as a positive carrier if at least one of the samples provided met this definition. Data for each center were collected and managed locally using Microsoft Excel; data were cleaned and merged centrally using STATA for Mac version 12.1 (StataCorp LP 2012). Data were analyzed to determine the overall prevalence of *N. meningitidis* among the swabs collected and among the participants overall and at each center using standard statistical measures and calculating the exact binomial 95% confidence intervals (CIs) (also known as the Clopper-Pearson CIs) [20] for the point prevalence estimates. The two methods for collecting pharyngeal swabs were compared by calculating the concordance, kappa, and applying McNemar’s test for paired samples to test the null hypothesis that there was no difference between the proportion of positive samples observed using one method compared to the other. Analyses were performed combined (pooling all samples) and by center. Data were analyzed using STATA for Mac version 12.1 (StataCorp LP 2012).

**Results**

From the 1013 children enrolled across the four centers included in this analysis, a total of 2023 T or U pharyngeal swabs were obtained. There was minor variation in the distribution of participants by age and gender across the study sites (Table 1). Five percent of the swabs collected (95% CI: 4.1-6.0%) were positive for *N. meningitidis*. Two U swabs were collected from five children and two other children had missing information on the swab type; these were excluded from the analysis. Paired T and U samples from 1006 children were analyzed; 6.9% (95% CI: 5.4-8.6%) of children were positive on *N. meningitidis* carriage by at least one swabbing method. Prevalence of carriage by age group (50.7% of carriers were aged 5-10 years old, 46.4% were aged 11-15 years old) and sex (49.3% of carriers were female) were similar.

Prevalence of *N. meningitidis* carriage from the T swab was 5.2% (95% CI: 3.9-6.7%) compared with a prevalence of 4.9% (95% CI: 3.6-6.4%) from the U swab (McNemar’s Test for paired samples, p=0.6) (Table 2). Concordance between the two methods was 96.3% and the kappa was 0.61 (95% CI 0.50-0.73), indicating good agreement beyond chance between the two methods. However in 2.0% (95% CI: 1.2-3.1%) of children only the T swab was positive for carriage, and in 1.7% (95% CI: 1.0-2.7%) only the U swab was positive. Both swabs were positive in 3.2% (95% CI: 2.2-4.5%). Prevalence of carriage was higher in Mali than the other countries (Figure 1, supporting material Table S1).

Prevalence of carriage based on the first swab, regardless of the collection method, was 5.0% (95% CI: 3.7-6.5%) compared with prevalence from the second swab of 5.1% (95% CI: 3.8-6.6%) (McNemar’s Test for paired samples, p=0.9). Twenty
Table 1. Summary of characteristics of the samples collected overall and by center.

|                     | Ethiopia | Mali | Niger | Senegal | Total |
|---------------------|----------|------|-------|---------|-------|
| Number of Schools   | 2        | 1    | 5     | 7       | 15    |
| Dates of Survey     | 12/2009-1/2010 | 6/2009 | 11/2009 | 10-11/2009 | -     |
| Age                 | 5-10 years | 102  | 122   | 157     | 145   | 526   |
|                     | 11-15 years| 148  | 128   | 102     | 105   | 483   |
| Sex                 | Male      | 125  | 125   | 113     | 117   | 480   |
|                     | Female    | 125  | 125   | 150     | 133   | 533   |
| Swabbing Method     |           |      |       |         |       |       |
| Alternated          | Per 25 children | Per 20 children | - | - | - |
| Participants Enrolled | 250     | 250  | 263   | 250     | 1013  |
| T Swabs Collected   | 248      | 250  | 262   | 246     | 1006  |
| U Swabs Collected   | 249      | 250  | 264   | 254     | 1017  |

* Three swabs from two children were missing their T or U status and were excluded from this analysis.

doi: 10.1371/journal.pone.0078336.t001

Table 2. Overall concordance between the two swabbing methods.

| Paired Pharyngeal Swab Samples from All Participants | T Method |                  |                  |                  |
|-----------------------------------------------------|----------|------------------|------------------|------------------|
|                                                     | Positive | Negative         | Total            |                  |
| U Method                                            |          |                  |                  |                  |
| Positive                                           | 32 (3.2%)| 17 (1.7%)        | 49 (4.9%)        |                  |
| Negative                                           | 20 (2.0%)| 937 (93.1%)      | 957 (95.1%)      |                  |
| Total                                               | 52 (5.2%)| 954 (94.8%)      | 1006             |                  |

Comparison of swabbing the posterior pharynx behind the uvula (U) or swabbing the posterior pharynx behind the uvula plus one tonsil (T) to determine carrier status.

doi: 10.1371/journal.pone.0078336.t002

Methods for Identifying N. meningitidis Carriers

five per cent of swabs collected were ordered T then U. Of the 50 first swabs that were positive, 44% were T swabs and 56% were U swabs. Of the 51 second swabs that were positive, 59% were T swabs and 41% were U swabs. While 50 children were identified as carriers based on the first swab, an additional 19 children (an additional 38%) were identified as carriers based on the result of the second swab.

Discussion

In our direct comparison of the two most commonly employed methods for collecting pharyngeal swabs to identify N. meningitidis carriers, we found that swabbing the posterior pharynx behind the uvula and one tonsil or swabbing the posterior pharynx alone provided similar estimates of the prevalence of carriage. Furthermore, our analysis demonstrates that the collection of two swabs regardless of method identified a higher proportion of carriers than collecting a single swab. Since both of the swabbing methods underestimated the prevalence of carriage, misclassification of carrier status is likely a concern in carriage studies regardless of which method of sampling is used. Our results suggest that future studies could minimize the potential for misclassification by collecting two swabs.

Previous studies have shown that though meningococcal bacteria live on the surface of the tonsils and can be cultured from swabbing the surface, swabbing alone underestimates the true prevalence because the bacteria can also reside below the surface of the mucous membranes [21]. Previous research has also shown that swabbing behind the uvula alone identifies carriers more often that swabbing the tonsils alone [17]. Our results are consistent with this finding because both of the swabbing methods compared here included swabbing the posterior pharynx behind the uvula. In a previous study in the United Kingdom (UK), collecting two sequential swabs using the U method produced a very high level of concordance for carrier status (98%) [22]. Comparing sequential U swabs in the absence of a true gold standard means that the concordance can remain high even if the sensitivity of the screening method is low. However the increase in the observed prevalence following the second swab was minimal in the UK study compared to our study where a 38% increase in yield was observed. A limitation of the present study is that we did not obtain two consecutive swabs from the same site, although both swabbing methods included sampling from the posterior pharynx. Although our results suggest that future studies could minimize the potential for misclassification by collecting two swabs, the cost implications of double swabbing for prevalence studies would be significant and the extent of added benefit is worthy of further study. An analysis of the epidemiological aspects of these data including the age-specific prevalence, evaluation of risk factors for carriage in schoolchildren, and serogroups of the meningococci identified is underway and will be the focus of future work.

The specificity of either method for detecting carriers is likely to be high because the probability of identifying N. meningitidis following a multi-stage analysis that includes both culture and molecular tests in the absence of the bacterium is very low. While statistical methods to determine the specificity and sensitivity of two tests in the absence of a gold standard have been developed [23-26], these methods could not be applied because they rely upon the assumption that the two methods are independent, require knowledge about at least one of the tests, need prior information about the prevalence of the condition in the population, or require more than two tests.

The prevalence of meningococcal carriage in this pilot study varied considerably between centers. We concluded at the time that the very low carriage observed in most centers reflected a condition in the population, or require more than two tests.

The prevalence of meningococcal carriage in this pilot study varied considerably between centers. We concluded at the time that the very low carriage observed in most centers reflected a condition in the population, or require more than two tests.
The first documented *N. meningitidis* carriage prevalence study in Africa was conducted in 1915 among British soldiers stationed in Sudan [4,27], but researchers have long recognized that the reliability of the results of early studies may be questionable due to the difficulty of culturing, isolating, and identifying the bacteria, leading to misclassification [4]. Yet, nearly a century later, no gold standard for the collection and analysis of swab samples for identifying meningococcal carriers has been established. A true gold standard is needed to evaluate the sensitivity and specificity of different methods, to monitor newly emerging phenotypes and genotypes, to implement epidemiological studies to assess risk factors and the impact of vaccines on carriage, and to better understand the relationship between carriage and immunity. Methods that maximize the sensitivity of detecting carriers would be most informative in this regard. Techniques such as polymerase chain reaction (PCR) for directly identifying *N. meningitidis* from the swab could improve sensitivity by eliminating the need for culture-based methods, and are worthy of further investigation.

**Supporting Information**

**Table S1.** Center-specific concordance between the two swabbing methods. Comparison of swabbing the posterior pharynx behind the uvula (U) or swabbing the posterior pharynx behind the uvula plus one tonsil (T) to determine carrier status by center.

**Acknowledgements**

We thank the national and local health authorities who supported the implementation of this research, the dedicated field staff who contributed to the data collection at each of the sites, and the participants without whom this research would not have been possible. In particular, we would like to acknowledge the efforts of (in alphabetical order) Medina Abdramane Yacoub, Lagaré Admaou, Genet Amare, Olubisi Amodu, Abraham Aseffa, Mohammed Askira, El Hadji Konko Ciré Ba, Omeiza Beida, Tigist Beyene, Rahamatou Moustapha Boukari, Akalifa Bugri, Rokia Dembele, Fatoumata Diallo, Mariétou Dieng, Saacou Djibo, Abakar Djimasngar, Ecole du Fleuve de Dijoroni Para Teachers, Jean-Pierre Gami, Toussaint Gnonwa, Fadima C. Haidara, Rawleigh Howe, Kevin Ibeh, Bassira Issaka, Mahamadou M. Keita, Alemayehu Kifle, Boulotigam Kodbesse, Tsehaynesh Lemma, Ali Elhadj Mahamane, Halima B Mainassara, Hiwot Mamo, Fantanesh Messele, Adane Mihrret, Wude Mihrret, Amadou Moussa,
Author Contributions
Conceived and designed the experiments: NEB JMS. Contributed to interpretation of the data: NEB JMS CT.

References

1. Greenwood B (1999) Manson Lecture. Meningococcal meningitis in Africa. Trans R Soc Trop Med Hyg 93: 341-353. doi:10.1016/S0035-9203(99)90106-2. PubMed: 10674069.
2. Heymann D, editor (2004) Control of Communicable Diseases Manual. Washington DC American Public Health Association.
3. Harrison LH, Trotter CL, Ramsay ME (2009) Global epidemiology of meningococcal disease. Vaccine 27 Suppl 2: B51-B63. doi:10.1016/j.vaccine.2008.10.030. PubMed: 19477562.
4. Trotter CL, Greenwood BM (2007) Meningococcal carriage in the African meningitis belt. Lancet Infect Dis 7: 797-803. doi:10.1016/S1473-3099(07)70288-8. PubMed: 18045562.
5. Leimkugel J, Hodgson A, Forgör AA, Pfliüger V, Dangy JP et al. (2007) Clonal waves of Neisseria colonisation and disease in the African meningitis belt: eight-year longitudinal study in northern Ghana. PLOS Med 4: e101. doi:10.1371/journal.pmed.0040101. PubMed: 17388665.
6. Kristiansen PA, Diomandé F, Wei SC, Ouldraogo R, Sangaré L et al. (2011) Baseline meningococcal carriage in Burkina Faso before the introduction of a meningococcal serogroup A conjugate vaccine. Clin Vaccine Immunol 18: 435-443. doi:10.1128/CVI.00479-10. PubMed: 21228139.
7. Kristiansen PA, Diomandé F, Ba AK, Sanou I, Ouldraogo AS et al. (2013) Impact of the Serogroup A Meningococcal Conjugate Vaccine, MenAfriVac, on Carriage and Herd Immunity. Clin Infect Dis 56: 354-363. doi:10.1093/cid/cis892. PubMed: 23087396.
8. Soriano-Gabarró M, Wolter J, Hogeia C, Vyse A (2011) Carriage of Neisseria meningitidis in Europe: a review of studies undertaken in the region. Expert Rev Anti Infect Ther 9: 761-774. doi:10.1586/eri.11.89. PubMed: 21905785.
9. LaForce FM, Ravenscroft N, Dinqarey M, Viviani S (2009) Epidemic meningitis due to Group A Neisseria meningitidis in the African meningitis belt: a persistent problem with an imminent solution. Vaccine 27 Suppl 2: B13-B19. doi:10.1016/j.vaccine.2009.04.062. PubMed: 19477559.
10. Lee CH, Kuo WC, Beri S, Kapre S, Joshi JS et al. (2009) Preparation and characterization of an immunogenic meningococcal group A conjugate vaccine for use in Africa. Vaccine 27: 726-732. doi:10.1016/j.vaccine.2008.08.065. PubMed: 19063929.
11. LaForce FM, Konde K, Viviani S, Pretiösi MP (2007) The Meningitis Vaccine Project. Vaccine 25 Suppl 1: A97-100. doi:10.1016/j.vaccine.2007.06.067. PubMed: 17521780.
12. Jódar L, LaForce FM, Ceccarini C, Aguado T, Granoff DM (2003) Meningococcal conjugate vaccine for Africa: a model for development of vaccines for the poorest countries. Lancet 361: 1902-1904. doi:10.1016/S0140-6736(03)13494-0. PubMed: 12788589.
13. Okobo BJ, Idoko OT, Adegbola RA (2009) Prospects and challenges with introduction of a mono-valent meningococcal conjugate vaccine in Africa. Vaccine 27: 2023-2029. doi:10.1016/j.vaccine.2008.11.092. PubMed: 19095025.
14. Discussion group on priorities for research on epidemic meningococcal disease in Africa (2013) Priorities for research on meningococcal disease and the impact of serogroup A vaccination in the African meningitis belt. Vaccine 31 (1453-1457).
15. Raghunathan PL, Jones JD, Tiendrebéogo SR, Sanou I, Sangaré L et al. (2006) Predictors of immunity after a major serogroup W-135 meningococcal disease epidemic, Burkina Faso, 2002. J Infect Dis 193: 607-616. doi:10.1086/499822. PubMed: 16453255.
16. MacLennan J, Kafatos G, Neal K, Andrews N, Cameron JC et al. (2006) Social behavior and meningococcal carriage in British teenagers. Emerg Infect Dis 12: 950-957. doi:10.3201/eid1206.051297. PubMed: 16707051.
17. Roberts J, Greenwood B, Stuart J (2009) Sampling methods to detect carriage of Neisseria meningitidis; literature review. J Infect 58: 103-107. doi:10.1016/j.jinf.2008.12.005. PubMed: 19167762.
18. the African Meningococcal Carriage Consortium (2013) Meningococcal carriage in the African meningitis belt. Trop Med Int Health 18: 968-976. doi:10.1111/jmi.12125. PubMed: 23681210.
19. Martin JE, Armstrong JH, Smith PB (1974) New system for cultivation of Neisseria gonorrhoeae. Appl Microbiol 27: 802-805. PubMed: 4207764.
20. Clopper C, Pearson E (1934) The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika: 404-413.
21. Sim RJ, Harrison MM, Moxon ER, Tang CM (2000) Underestimation of meningococci in tonsillar tissue by nasopharyngeal swabbing. Lancet 356: 1653-1654. doi:10.1016/S0140-6736(00)03162-7. PubMed: 11089827.
22. Cartwright KA, Stuart JM, Jones DM, Noah ND (1987) The Stonehouse survey: nasopharyngeal carriage of meningococci and Neisseria lactamica. Epidemiol Infect 99: 591-601. doi:10.1017/S0950268800066449. PubMed: 3123263.
23. Hui SL, Walter SD (1980) Estimating the error rates of diagnostic tests. Biometrics 36: 167-171. doi:10.2307/2530508. PubMed: 7370371.
24. Enae C, Georgiadis MP, Johnson WO (2000) Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. Prev Vet Med 45: 61-81. doi:10.1017/S0167-5877(00)00117-3. PubMed: 10802334.
25. Johnson WO, Gastwirth JL, Pearson LM (2001) Screening without a "gold standard"; the Hui-Walter paradigm revisited. Am J Epidemiol 153: 921-924. doi:10.1093/aje/kw9.9.921. PubMed: 11323324.
26. Georgiadis MP, Johnson WO, Gardner IA, Singh R (2003) Correlation-adjusted estimation of sensitivity and specificity of two diagnostic tests. J R Stat Soc C Appl Statist 52: 63-76. doi:10.1111/1467-9876.00389.
27. Chalmers A, O’Farrell W (1916) Preliminary remarks upon epidemic cerebrospinal meningitis as seen in the Anglo-Egyptian Sudan. J Trop Med Hyg: 101-116.