Rev. Inst. Med. Trop. Sao Paulo
2016;58:60
http://dx.doi.org/10.1590/S1678-9946201658060

ORIGINAL ARTICLE

COMPARISON OF METHODS TO IDENTIFY *Neisseria meningitidis* IN ASYMPTOMATIC CARRIERS

Camila F. RIZEK(1), André Machado LUIZ(2), Gracilene Ramos de ASSIS(1), Silvia Figueiredo COSTA(1), Anna Sara LEVIN(1) & Marta Heloisa LOPES(2)

SUMMARY

Neisseria meningitidis is a cause of several life-threatening diseases and can be a normal commensal in the upper respiratory tract of healthy carriers. The carrier rate is not well established especially because there is no standard method for the isolation of *N. meningitidis*. Therefore, the aim of this study was to compare identification methods for the carrier state. Two swabs were collected from 190 volunteers: one was cultured and the other had DNA extracted directly from the sample. The Polymerase Chain Reaction (PCR) was performed to determine species and serogroups and compared the results between the methods. PCR for species determination used two pairs of primers and when there was only one amplicon, it was sequenced. The culture technique was positive in 23 (12.1%) subjects while the direct extraction method was positive in 132 (69.5%), $p < 0.001$. Among the 135 subjects with positive *N. meningitides* tests, 88 (65.2%) were serogroup C; 3 (2.2%) serogroup B; 5 (3.7%) were positive for both serogroup B and C, and 39 (28.9%) did not belong to any of the tested serogroups. In this study, PCR from DNA extracted directly from swabs identified more *N. meningitidis* asymptomatic carriers than the culture technique.

KEYWORDS: Carrier state; *Neisseria meningitidis*; PCR; Asymptomatic carriage.

INTRODUCTION

Neisseria meningitidis is a Gram-negative diplococcus of global distribution. It is a cause of several life-threatening diseases in all age groups[1,2]. Based on its capsular polysaccharide, *N. meningitidis* is classified into twelve different serogroups, but only five of them cause the majority of invasive infections worldwide: A, B, C, W and Y[3]. In Brazil, where meningococcal disease is endemic with 8 cases per 100,000 inhabitants and an average fatality rate of 20%, the most frequent serogroups are C and B[4]. Besides the potential to cause several invasive illnesses, *N. meningitidis* is also a normal commensal in the upper respiratory tract of healthy carriers. Therefore, these carriers can constitute a reservoir of the microorganism. However, the relationship between invasive infection and the carrier state is not completely clear: it appears that the population with higher rates of invasive disease (infants and school-age children) is not the same population that is more frequently colonized by the microorganism (adolescents and young adults)[3].

In the clinical diagnostic setting, it is difficult to identify *N. meningitidis* using standard methods: culture and microscopy[2,5]. These methods have low positivity rates and are time consuming. According to Salgado *et al.* (2013), the identification occurs in only 50% of the cases, and there is also the possibility of cross-reactions using immunological methods[6]. Worldwide, these methods have a sensitivity of only 40-63%[2]. Several recent studies indicate that diagnoses based on PCR are more trustworthy and faster[1,2,5,6,7,8]. PCR for meningococcal identification has been standardized by different authors and has been employed in some references laboratories. The most frequent gene used in the amplification test is the *ctr*A for species identification and *sia*D for serogroup determination[1,5]. To study the carrier state, the conventional methods are even more problematic. The samples used to evaluate colonization are not free of contaminants: nasopharyngeal swabs often carry other groups of commensal bacteria and eukaryotic cells. The quantity of *N. meningitidis* cells in carriers is probably lower than in clinical samples from patients with invasive disease, thus, making cultures have an even lower sensitivity. Due to these considerations, cultures can underestimate the carrier state rate and lead to an incomplete understanding of the epidemiology of this pathogen[9].

In developing countries, surveillance of *N. meningitidis* carrier state is extremely important to understand the epidemiology of invasive disease and to manage vaccination programs. However, in these countries the clinical diagnosis is underestimated because of the large use of cultures and the high proportion of negative results[9,10]. Although there is no consensus in this matter, Esposito *et al.* (2013)

(1) University of *São Paulo*, School of Medicine, Department of Infectious Diseases, Laboratory of Bacteriology (LIM-54). *São Paulo*, *SP*, Brazil. E-mails: camilarizek@usp.br; gracilenebio@yahoo.com.br; costasilviaf@ig.com.br; gcih.adm@hc.fm.usp.br
(2) University of *São Paulo*, School of Medicine, Department of Infectious Diseases, *São Paulo, SP*, Brazil. E-mails: andremluiz@gmail.com; mahlopes@usp.br
Correspondence to: Camila F. Rizek. E-mail: camilarizek@usp.br

claim that invasive meningococcal disease occurs mostly in previously asymptomatic carriers, especially the ones carrying the pathogen in their upper respiratory tract. Therefore, the study of the carrier state could be useful to determine the risk of invasive disease. The authors emphasize that these results can be affected by the technique used in the microbiological identification and that more studies comparing methods for identification are needed[11]. There is also a lack of studies that evaluate the identification of this pathogen using DNA extracted directly from swabs. Therefore, the aim of this study was to compare methods to identify *N. meningitides* asymptomatic carriers: DNA extracted directly from swabs and conventional culture methods.

MATERIAL AND METHODS

Samples

Two nasopharyngeal swabs were collected from 190 healthy volunteers (medical students aged 20-24 years) in the year 2010. One swab was cultured in Thayer-Martin medium and the other was submitted to DNA extraction within four hours after collection.

Cultures

Cultivated plates were incubated at 35 ± 2 ºC with 5 - 10% CO_2 and examined at 24 h and 48 h. If no growth was observed after 48 h, they were considered negative for pathogenic *Neisseria*. The plates that presented presumptive colonies of *Neisseria* spp. were submitted to biochemical tests: Gram stain, oxidase test and sugar fermentation (glucose, sucrose, fructose, lactose and maltose). Then, the DNA from the colonies was extracted using the commercial kit Illustra™ Bacteria Genomicprep Mini Spin (GE Healthcare Life Science, Pittsburgh, USA), according to the manufacturer's instructions.

Direct DNA extraction

DNA extraction directly from swabs was performed using the kit DNeasy Blood & Tissue Kit (QIAGEN Inc., Hilden, Germany) adapted according to Taha *et al.* (2005)[12]. The comparison between the positivity of the two tests was perfomed by the McNemar test by means of the Stata software, version 13 for Windows (StataCorp LP College Station, Texas, USA).

Species identification

Both non-cultivated and cultivated samples were submitted to PCR for species identification using two pair of primers: for the *ctr*A gene and for the *crg*A gene (Table 1). The reactions were performed under the following conditions: 3 minutes at 94 °C, 36 cycles of 40 seconds at 94 °C, 30 seconds at 55 °C and 20 seconds at 72 °C, with a final extension of 10 minutes at 72 °C[12,13]. The PCR master mix used was prepared as follows: 2 µL of template DNA in a 23 µL of the mix containing 10 mM Tris/HCl (pH 8.8), 4 mM $MgCl_2$, 200 µM of each dNTP, 100 nM of each primer and 0.5U Taq DNA polymerase. In all reactions, we used a positive control sample: *Neisseria meningitidis* serogroup C (07/318 NIBSC – UK EM 63QG) and a negative control. If both genes were positive, the sample was classified as *Neisseria meningitidis*. If only the non-capsular gene (*crg*A) was positive, the PCR product was sequenced. Nucleotide sequencing was performed using the MegaBACE 1000 (ABI 3730 DNA Analyser; Applied Biosystems, Alameda, CA, USA). Sequences were analyzed using the Sequence Analyzer software with the Base Caller Cimarron 3.12. Genetic sequences were compared with the BLAST database available on http://www.ncbi.nlm.nih.gov/blast/.

Table 1
Primers used for the identification of species and serogroups of *N. meningitidis*

Gene	Primer sequence (5'-3')
*crg*A	GCTGGCGCCGCTGGCAACAAAATTC CTTCTGCAGATTGCGGCGTGCCGT
*ctr*A	GCTGCGGTAGGTGGTTCAA TTGTCGCGGATTTGCAACTA
orf-2 (serogroup A)	CGCAATAGGTGTATATATTCTTCC CGTAATAGTTTCGTATGCCTTCTT
*sia*D (serogroup B)	GGATCATTTCAGTGTTTTCCACCA GCATGCTGGAGGAATAAGCATTAA
*sia*D (serogroup C)	TCAAATGAGTTTGCGAATAGAAGGT CAATCACGATTTGCCCAATTGAC
*sia*D (serogroup W)	CAGAAAGTGAGGGATTTCCATA CACAACCATTTTCATTATAGTTACTGT
*sia*D (serogroup Y)	CTCAAAGCGAAGGCTTTGGTTA CTGAAGCGTTTTCATTATAATTGCTAA

Serogroup identification

For all samples that were positive for *N. meningitidis*, a PCR was performed to evaluate the serogroup. Each of the serogroups A, B, C, Y and W was tested with specific primers (Table 1). The reaction was performed as follows: 5 minutes at 94 °C, 35 cycles of 40 seconds at 94 °C, 30 seconds at 55 °C and 20 seconds at 72 °C, with a final extension of 10 minutes at 72 °C (Taha 2000, Maiden & Pollard 2001). The PCR master mix was prepared as follows: 3 µL DNA template in a 22 µL mix containing 10 mM Tris/HCl (pH8.8), 4 mM $MgCl_2$, 200 µM of each dNTP, 250 nM of each primer and 0.5 U of Taq DNA polymerase. For each reaction, we used positive and negative controls. The positive controls were *N. meningitidis* serogroup A (ATCC 13077; *N. meningitidis* serogroup B (ATCC 13090); *N. meningitidis* serogroup C (07/318, NIBSC – UK EM 63QG) and *N. meningitidis* serogroup W-135 (ATCC 35559). As negative controls ultrapure water was used instead of DNA. Based on the PCR product size, serogroups B, C and Y were tested separately; and serogroups A and W were tested together.

Ethical approval

This study was approved by the Institutional Ethics and Research Commitee (CAPPesq) of the *Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo,* protocol number 0554/10.

RESULTS

Culture samples

A total of 380 swabs were collected (two from each volunteer). One of the swabs was used for DNA extraction (190 swabs) and the other was

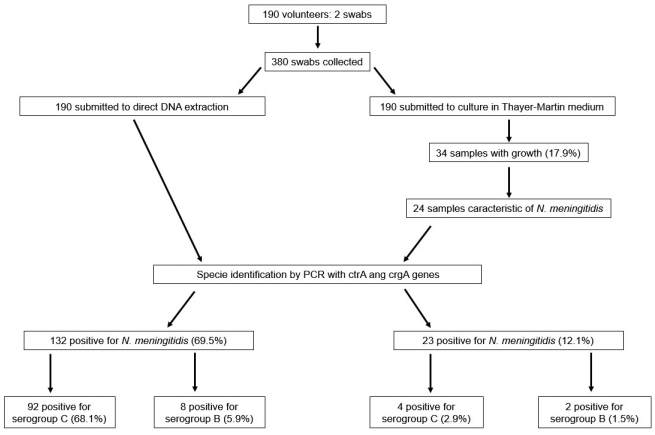

Fig. 1 - Specie and serogroup identification of *N. meningitidis* of 190 volunteers using DNA direct extraction from swab and swab culture.

cultured in selective medium (190 swabs). From the total of 190 cultured swabs, 34 samples (17.9%) were positive (Fig. 1).

Twenty-four of the 34 isolates presented positive results in the oxidase test and were then tested for sugar fermentation. Six of the 24 isolates showed a typical phenotype for *Neisseria meningitidis* and 18 showed a non-typical phenotype. The 24 samples had their DNA extracted and tested by PCR for the identification of species and serotypes, and 23 were positive for *N. meningitidis*. Five of the 23 positive samples were only positive for the *crg*A gene and were submitted to sequencing, showing a 100% match with *N. meningitidis*. Thus, in summary, evaluating the 190 cultures, 23 (12.1%) were positive for *N. meningitidis* (Table 2).

When the PCR results performed using DNA extracted directly from clinical samples are used as the gold standard, as it was the case in the present study, the culture method would have a sensitivity and specificity of 15% and 95%, respectively. The positive and negative predictive values would be 87% and 33%, respectively.

Species identification for non-cultured samples

From the total of 190 samples, 132 (69.5%) had their DNA extracted directly from the swab and were positive for *N. meningitidis* by PCR. Nineteen of them were confirmed after the amplicon sequencing because they had only amplified the *crg*A gene. From the 132 positive samples, 20 were from culture-positive subjects and the remaining 112 samples were obtained from culture-negative subjects (Table 2). However, three samples that were culture-positive were negative by PCR (Table 2). In summary, the culture method was positive in 23 (12.1%) subjects while PCR performed with DNA extracted directly from swabs was positive in 132 (69.5%), $p < 0.001$ (Fig. 1).

Serotyping

Among the 135 *N. meningitidis* positive samples (from non-cultured and/or cultured samples) there were no positive results for serogroups A, Y or W. Eighty-eight samples (65.2%) were positive for serogroup C; 3 (2.2%) for serogroup B; 5 (3.7%) were positive for both serogroup B and C and 39 (28.9%) did not belong to any of the tested serogroups. If we consider only the *N. meningitidis* identified by each method, PCR performed with DNA directly extracted from swabs was able to detect 92 (68.1%) samples belonging to serogroup C, while using the culture the identification occurred in 4 (2.9%) samples. Regarding the serogroup B, the direct DNA extraction identified eight (5.9%) positive samples and the culture only two (1.5%) samples. From the samples identified as serogroup C by the direct DNA extraction amplification, ten of them were identified by culture and did not belong to the tested serogroups (Fig. 1).

Among the samples from volunteers that were positive for serogroup C, one sample could only be serotyped by culture. One volunteer that was positive for serogroup B by the culture method was found to carry the serogroups B and C by PCR (direct extraction method from swabs).

DISCUSSION

In this study, we found that DNA extracted directly from swabs identified more *N. meningitidis* positive subjects than the culture method. Several studies have pointed out that molecular methods provide a rapid result with high sensitivity and specificity. Some of them used the same targets as in this study, while others used two pairs of primers combining the capsular gene *ctr*A with another non-capsular gene[2,5,15,16,17]. In invasive meningococcal disease, studies have demonstrated that blood cultures could only recover *N. meningitidis* in 50% of the cases and this proportion decreases if the patient had used antibiotics[2]. As mentioned before, samples from carriers have a smaller quantity of *N. meningitidis* cells than clinical samples from patients with invasive disease, thus, causing the culture method to present an even lower sensitivity. On the other hand, there are very few studies comparing methods to evaluate the carrier state using DNA extracted directly from swabs[18]. Culture methods are still considered the gold standard although several recent studies have shown that there are many problems using only this technique[19,20].

Culture methods are important to analyze clinical samples because

Table 2
Results of *N. meningitidis* identification in cultured and non-cultured (*PCR) samples of nasopharyngeal swabs from 190 healthy volunteers

	Positive *N. meningitidis* PCR* with DNA extracted directly from swabs (n: 132)	Negative *N. meningitidis* PCR* with DNA extracted directly from swabs (n: 58)
Culture-positive samples for *N. meningitidis* (n = 23)	20	3
Culture-negative samples for *N. meningitidis* (n = 167)	112	55

*PCR: polymerase chain reaction

they identify *Neisseria meningitidis* and at the same time evaluate the antimicrobial susceptibility to guide treatment. However, this is not so important when evaluating the carrier state. Some of the reasons why cultures fail are poor sampling techniques; improper specimen storage, transportation and bacterial autolysis[19]. Because of these problems, some studies consider PCR (or RT-PCR) to be the gold standard to calculate sensitivity and specificity[20].

The carrier state found in other studies varied from 6% to 20%[11,18,21,22]. These studies used a wide variety of methods to detect *N. meningitidis* and, in some studies the methods were not even described. If we consider that most studies used culture techniques, our rate using the same method (12.1%) was similar. However, our carriage rate using DNA directly extracted from the swab was much higher. In Brazil, one study performed during an outbreak in an oil refinery, found a carrier rate of approximately 21.5%. In this study among the 104 positive results, 95 were detected by culture and real-time PCR, one was detected by culture alone, and eight were detected only by real-time PCR. It is important to emphasize that they did not use DNA extracted directly from the samples, but from previously cultured microorganisms[23].

Other aspects can also be considered to understand the differences. First, several factors affect the carrier state dramatically, such as age, environment and time spent in high risk environments such as military or university settings[22]. Second, a true gold standard has not yet been established. Besides, using only PCR results, we might still underestimate the real carriage rate because of false negative results caused by inhibitors that can interfere with PCR, such as DNA from other species or chemical factors that could be present in the swab[24]. Thus, our three culture-positive subjects that were negative by PCR using DNA extracted directly from swabs probably do not represent false-positive results of the culture, but false-negative results of PCR.

Another variability of the molecular method is the target of choice. Three articles published in 2015[15,16,17] evaluated which region would be better for the molecular identification of *N. meningitidis* (from invasive disease and carriers). Among the tested genes, *ctr*A, *crg*A, *por*A, 16S rRNA and *sod*C were included. The best results were obtained for the *ctr*A gene used together with the *por*A gene. However, neither of these studies used sequencing of the *amplicon* for the non-encapsulated microorganisms as presented herewith. Another difference was that the isolates used in these three studies were from a bacteriological collection previously cultivated and not from DNA extracted directly from swabs.

It has been reported that it may be difficult to detect *N. meningitidis* carriers directly from swabs regarding non-capsulated strains[11]. However, only one pair of primers (*ctr*A) was used, while in our study, we used two pairs of primers (*ctr*A and *crg*A). If only the non-capsule gene was amplified, the *amplicon* was sequenced to determine the species. The *crg*A gene is a constitutive gene involved in regulation of *Neisseria* adhesion to target cells and is not exclusive of *N. meningitidis*, therefore, if there is no amplification of the *ctr*A gene (capsular gene of *N. meningitidis*), the *crg*A *amplicon* should be sequenced.

Even though there are few studies on the detection of *N. meningitidis* carriers using DNA extracted directly from the carrier sample, in the case of other species this has been already established[25,26,27,28]. This technique has been used for *Staphylococcus aureus* infections in which several authors stated that cultures are time-consuming and less trustworthy than DNA extracted directly from biological samples, especially considering asymptomatic carriers[25,26,28]. Another difference between the two methods evaluated in the present study was that PCR performed with DNA samples extracted directly from clinical samples was able to identify more than one serogroup in the same sample, as occurred in five of the volunteers, while the culture method was most likely to retrieve only one serogroup. This may lead to important epidemiological information. In addition, with PCR performed with DNA extracted from swabs, a high percentage of the *N. meningitidis* belonging to serogroup C, which is an important group for invasive disease was identified in this study.

Some limitations of the study are the specific population (medical students) that was evaluated and the limited number of samples. It is also important to highlight that the high positivity rate of the molecular test might be a result of detection of non-viable microrganisms[8]. This may, at least partially, explain the high prevalence of positive samples detected by PCR performed with DNA extracted directly from swabs in the present study. Although this is undeniably a more practical approach and may generate important information, more studies are needed to establish the gold standard for the identification of *N. meningitidis* carriers.

ACKNOWLEDGMENTS

This study was supported by *Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP),* process number 2011/13290-1.

REFERENCES

1. de Filippis I, do Nascimento CR, Clementino MB, Sereno AB, Rebelo C, Souza NN, et al. Rapid detection of *Neisseria meningitidis* in cerebrospinal fluid by one-step polymerase chain reaction of the nspA gene. Diagn Microbiol Infect Dis. 2005;51:85-90.

2. Munoz-Almagro C, Rodriguez-Plata MT, Marin S, Esteva C, Esteban E, Gene A, et al. Polymerase chain reaction for diagnosis and serogrouping of meningococcal disease in children. Diagn Microbiol Infect Dis. 2009;63:148-54.

3. Gasparini R, Comanducci M, Amicizia D, Ansaldi F, Canepa P, Orsi A, et al. Molecular and serological diversity of *Neisseria meningitidis* carrier strains isolated from italian students aged 14 to 22 years. J Clin Microbiol. 2014;52:1901-10.

4. Tauil MC, Carvalho CS, Vieira AC, Waldman EA. Meningococcal disease before and after the introduction of meningococcal serogroup C conjugate vaccine. Federal District, Brazil. Braz J Infect Dis. 2014;18:379-86.

5. McKenna JP, Fairley DJ, Shields MD, Cosby SL, Wyatt DE, McCaughey C, et al. Development and clinical validation of a loop-mediated isothermal amplification method for the rapid detection of *Neisseria meningitidis*. Diagn Microbiol Infect Dis. 2011;69:137-44.

6. Salgado MM, Gonçalves MG, Fukasawa LO, Higa FT, Paulino JT, Sacchi CT. Evolution of bacterial meningitis diagnosis in São Paulo State-Brazil and future challenges. Arq Neuropsiquiatr. 2013;71:672-6.

7. Pedro LG, Boente RF, Madureira DJ, Matos JA, Rebelo CM, Igreja RP, et al. Diagnosis of meningococcal meningitis in Brazil by use of PCR. Scand J Infect Dis. 2007;39:28-32.

8. Wu HM, Cordeiro SM, Harcourt BH, Carvalho M, Azevedo J, Oliveira TQ, et al. Accuracy of real-time PCR, Gram stain and culture for *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* meningitis diagnosis. BMC Infect Dis. 2013;13:26.

9. Sacchi CT, Fukasawa LO, Gonçalves MG, Salgado MM, Shutt KA, Carvalhanas TR, et al. Incorporation of real-time PCR into routine public health surveillance of culture negative bacterial meningitis in São Paulo, Brazil. PLoS One. 2011;6:e20675.

10. Nhantumbo AA, Cantarelli VV, Caireão J, Munguambe AM, Comé CE, Pinto GC, et al. Frequency of pathogenic paediatric bacterial meningitis in Mozambique: the critical role of multiplex real-time polymerase chain reaction to estimate the burden of disease. PLoS One. 2015;10:e0138249.

11. Esposito S, Zampiero A, Terranova L, Montinaro V, Peves Rios W, Scala A, et al. Comparison of posterior pharyngeal wall and nasopharyngeal swabbing as a means of detecting the carriage of *Neisseria meningitidis* in adolescents. Eur J Clin Microbiol Infect Dis. 2013;32:1129-33.

12. Taha MK, Alonso JM, Cafferkey M, Caugant DA, Clarke SC, Diggle MA, et al. Interlaboratory comparison of PCR-based identification and genogrouping of *Neisseria meningitidis*. J Clin Microbiol. 2005;43:144-9.

13. Taha MK. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. J Clin Microbiol. 2000;38:855-7.

14. Pollard AJ, Maiden MC, editors. Meningococcal disease: methods and protocols. Totowa: Humana Press; 2001.

15. Jones CH, Mohamed N, Rojas E, Andrew L, Hoyos J, Hawkins JC, et al. Comparison of phenotypic and genotypic approaches to capsule typing *Neisseria meningitidis* using invasive and carriage isolate collections. J Clin Microbiol. 2016;54:25-34.

16. Moreno J, Hidalgo M, Duarte C, Sanabria O, Gabastou JM, Ibarz-Pavon AB. Characterization of carriage isolates of Neisseria meningitidis in the adolescents and young adults population of Bogota (Colombia). PLoS One. 2015;10:e0135497.

17. Rojas E, Hoyos J, Oldfield NJ, Lee P, Flint M, Jones CH, et al. Optimization of molecular approaches to genogroup *Neisseria meningitidis* carriage isolates and implications for monitoring the impact of new serogroup B vaccines. PLoS One. 2015;10:e0132140.

18. Basta NE, Stuart JM, Nascimento MC, Manigart O, Trotter C, Hassan-King M, et al. Methods for identifying *Neisseria meningitidis* carriers: a multi-center study in the African meningitis belt. PLoS One. 2013;8:e78336.

19. Hassanzadeh P, Mardaneh J, Motamedifar M. Conventional agar-based culture method, and nucleic acid amplification test (NAAT) of the cppB gene for detection of *Neisseria gonorrhea* in pregnant women endocervical swab specimens. Iran Red Crescent Med J. 2013;15:207-11.

20. Collard JM, Wang X, Mahamane AE, Idi I, Issaka B, Ousseni M, et al. A five-year field assessment of rapid diagnostic tests for meningococcal meningitis in Niger by using the combination of conventional and real-time PCR assays as a gold standard. Trans R Soc Trop Med Hyg. 2014;108:6-12.

21. Jordens JZ, Williams JN, Jones GR, Heckels JE. Detection of meningococcal carriage by culture and PCR of throat swabs and mouth gargles. J Clin Microbiol. 2002;40:75-9.

22. Yazdankhah SP, Caugant DA. *Neisseria meningitidis*: an overview of the carriage state. J Med Microbiol. 2004;53:821-32.

23. Sáfadi MA, Carvalhanas TR, Paula de Lemos A, Gorla MC, Salgado M, Fukasawa LO, et al. Carriage rate and effects of vaccination after outbreaks of serogroup C meningococcal disease, Brazil, 2010. Emerg Infect Dis. 2014;20:806-11.

24. Liphaus BL, Cappeletti-Gonçalves-Okai MI, Silva-Delemos AP, Gorla MC, Rodriguez-Fernandes M, Pacola MR, et al. Outbreak of *Neisseria meningitidis* C in a Brazilian oil refinery involving an adjacent community. Enferm Infecc Microbiol Clin. 2013;31:88-92.

25. Francois P, Pittet D, Bento M, Pepey B, Vaudaux P, Lew D, et al. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. J Clin Microbiol. 2003;41:254-60.

26. Andriesse GI, van Rijen M, Bogaers D, Bergmans AM, Kluytmans JA. Comparison of two PCR-based methods and conventional culture for the detection of nasal carriage of *Staphylococcus aureus* in pre-operative patients. Eur J Clin Microbiol Infect Dis. 2009;28:1223-6.

27. Durant JF, Fonteyne PA, Richez P, Marot L, Belkhir L, Tennstedt D, et al. Real-time PCR and DNA sequencing for detection and identification of *Trichophyton rubrum* as a cause of culture negative chronic granulomatous dermatophytosis. Med Mycol. 2009;47:508-14.

28. Peterson LR, Liesenfeld O, Woods CW, Allen SD, Pombo D, Patel PA, et al. Multicenter evaluation of the LightCycler methicillin-resistant *Staphylococcus aureus* (MRSA) advanced test as a rapid method for detection of MRSA in nasal surveillance swabs. J Clin Microbiol. 2010;48:1661-6.

Received: 23 October 2015
Accepted: 11 March 2016