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Note

Meningococcal carriage rates in healthy individuals in Japan determined using Loop-Mediated Isothermal Amplification and oral throat wash specimens

Hideyuki Takahashi [a], Masae Haga [b], Tomimasa Sunagawa [c], Takehito Saitoh [c], Takeru Kitahara [d], Sohkichi Matsumoto [d], Makoto Ohnishi [a, *]

[a] Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan
[b] Healthcare Center, Hokkaido University of Education, Hokkaido, Japan
[c] Department of Infectious Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan
[d] Department of Bacteriology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

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ABSTRACT

The detailed epidemiology of meningococcal diseases in Japan has yet to be determined and, moreover, the healthy carriage rate is also unknown. In this study, to obtain insight into the carriage rate of *Neisseria meningitidis* in healthy individuals in Japan, we developed a new method to detect the *N. meningitidis*-specific *ctrB* gene, one of the genes encoding enzymes for capsule synthesis, by Loop-Mediated Isothermal Amplification (LAMP) and examined the meningococcal carriage rate by using self-collected oral throat wash specimens from 836 students at a university. Examination by LAMP showed that 7 out of 836 samples were positive for *N. meningitidis* DNA, and the results were also verified by the nested PCR method for the meningococcus specific *ggt* gene. The *N. meningitidis* carriage rate in healthy individuals was estimated to be 0.84%. Moreover, we further confirmed by the nested-PCR-based serogroup typing method that 5 of the positive samples belonged to serogroup Y, 1 belonged to group B and 1 was unidentifiable. Considering the epidemiology for meningococcal diseases in Japan, the carriage rate and the serogroup profile seem to be consistent with low incidence of meningococcal diseases and serogroup distribution of clinical meningococcal isolates in Japan, respectively.

Meningococcal diseases are a serious problem throughout the world, particularly in sub-Saharan Africa [1]. In Japan, the low incidence of the meningococcal meningitis (<0.02 cases/100,000/year) is reported [2], and approximately 40 cases of invasive meningococcal diseases are reported annually that meet the criteria for meningococcal bacteraemia and meningitis (from the data of Infectious Diseases Weekly Report, http://www.nih.go.jp/niid/ja/allarticles/surveillance/239-idwr/data/5249-idwr-sokuho-data-j-1452.html). The low incidence might relate to the minimal attention given to meningococcal diseases in Japan, where the detailed epidemiology of the disease has yet to be determined. Also the carriage rate in healthy individuals is unknown, although it might be associated with incident rate.

The surveillance of meningococcal carriers is hard to conduct because of the difficulty in obtaining consent for pharyngeal swabbing from volunteers. To overcome the problem, we developed an alternative method for examining meningococcal carriage by using oral throat wash specimens and a meningococcal DNA detection method with Loop-Mediated Isothermal Amplification (LAMP). Because the collection of oral throat wash specimens is an easier and less invasive sampling method than swabbing, healthy people are more likely to cooperate with the surveillance.

In this study, *ctrB* gene was selected for the target of LAMP assay. The *ctrB* gene encodes an enzymes involved in *Neisseria meningitidis* capsule synthesis [3]. The gene was preserved only among encapsulated *N. meningitidis*, but neither in noncapsulated *N. meningitidis* nor the related *Neisseria* spp. [3,4]. In addition, the neighbouring gene, *ctrA*, which is also involved in capsule

* Corresponding author. Department of Bacteriology I, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo, 162-8640, Japan. Tel.: +81 3 5285 1111x2022; fax: +81 3 5285 1163.
E-mail address: ohnishi7@nih.go.jp (M. Ohnishi).

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synthesis, has been applied to detect *N. meningitidis* DNA specifically in CSF by LAMP assay [5]. The LAMP reaction was performed with Loopamp DNA amplification kit and fluorescent detection reagents (Eiken Chemical Co. Ltd.) and contained 1.6 μM of each of the primers FIP and BIP, and 0.8 μM of primers F3 and B3 (Table 1). After adding the DNA sample, the mixture was incubated at 65 °C for 60 min and amplification was confirmed by emission under a UV lamp. The LAMP system was able to specifically detect 100 bacteria and 1 pmol of DNA of *N. meningitidis* H44/76 kindly provided from National Institute of Public Health in Norway (Fig. 1A). As expected the *ctrB* LAMP detection assay did not produce any positive results from other *Neisseria* spp, ATCC strains of *Neisseria gonorrhoeae* ATCC 49226, *N. flavesence* ATCC 13120, *N. denitrificance* ATCC 14686, *N. elongata* ATCC 25295, *N. canis* ATCC 14687, and *N. cinerea* ATCC 14685, and clinical isolates in our collection, *N. lactamica* NIID85, *N. mucosa* NIID16 and *N. sicca* NIID17 (Fig. 1A).

Oral throat wash specimens were collected from 836 volunteers who were students at a University between the ages of 18 and 23. Most volunteers lived in their own houses and not in dormitories. The specimens were obtained after the volunteers gargled with 20 ml saline for approximately 10 s. The specimens were collected in 50 ml tubes and stored at 4 °C until being further purified. Collection of the specimens was approved by the medical research ethics committee of the National Institute of Infectious Diseases for the use of human subjects (Approval No. 442 on September 25, 2013). The specimens were centrifuged (19,000 × g) for 30 min, the resultant pellet was treated with a DNeasy Blood & Tissue kit (Qiagen, Inc.), and the DNA was eluted with 100 μl of elution buffer. We examined meningococcal carriage rates by using 1 μl DNA samples from the oral throat wash specimens. Seven samples were presumed to be positive at first screening.

To confirm positive results of LAMP assay, DNA was examined by PCR for *ggt* gene, which is the specific gene for *N. meningitidis* [6,7]. In this study we improved the previously established PCR method for *ggt* [7] as a nested PCR protocol. Primary PCR of the nested PCR was performed as follows: the PCR mixture (25 μl) consisted of 0.2 mM dNTP, 1 U of Ex Taq DNA polymerase (Takara Bio Inc.), 1 μM of each primer, ggt-nested-1 and -2, and 1 μl of DNA sample in 1× attached buffer of the Ex Taq Kit. PCR reaction was performed as follows; the first denaturation cycle at 95 °C for 5 min, followed by 25 cycles, each consisting of 30 s of denaturation at 94 °C, 30 s of annealing at 55 °C, 30 s of extension at 72 °C. The secondary PCR was also performed as the primary PCR using with ggt-29 and ggt-20 (Table 1) as primers, and 1 μl of primary PCR product as a template. Products were visualized by resolution on a 2% agarose gel followed by staining with ethidium bromide. The nested PCR showed similar sensitivity and specificity as the LAMP for *ctrB* (Fig. 1B), although *N. gonorrhoeae* showed positive result because *N. gonorrhoeae* possesses homologue of meningococcal *ggt* gene [7]. All seven LAMP-positive samples were *ggt* positive, suggesting that 7 of the 836 students (0.84%) were healthy carriers of *N. meningitides*.

We further examined the serogroup by using a nested-PCR serogroup typing method, which was modified on a previously reported method [8]. PCR mixture and reaction was the same with PCR for *ggt*, except for primers used (Table 1). *N. meningitidis* H44/76 (serogroup B) as well as NIID1 (serogroup A), NIID3 (serogroup C), NIID5 (serogroup Y) and NIID93 (serogroup W) *N. meningitidis*

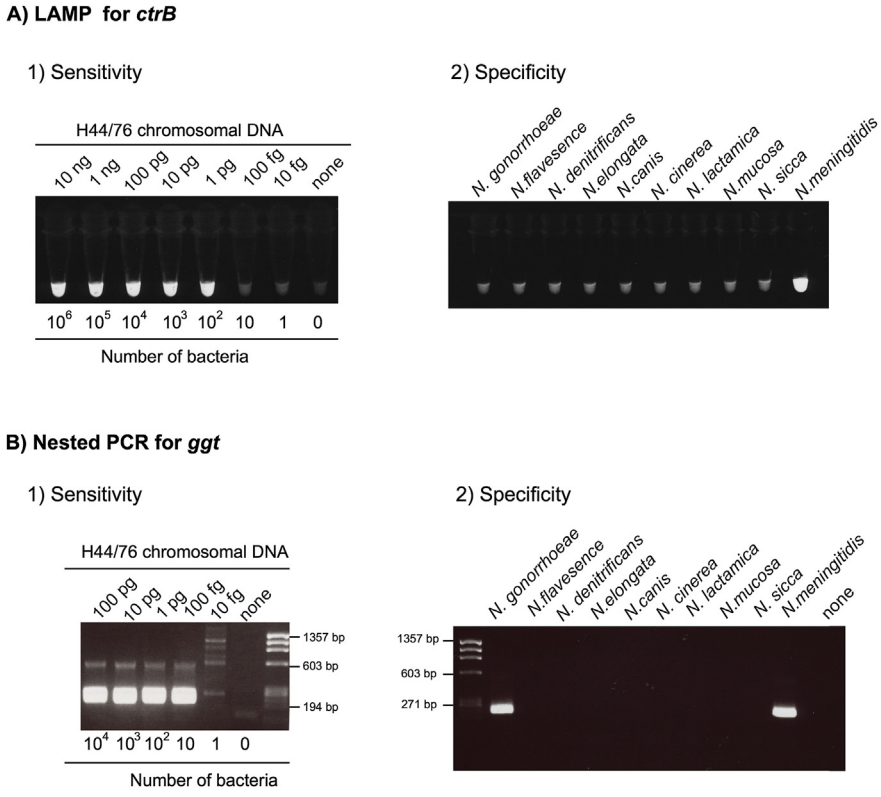

Fig. 1. Sensitivity and specificity of the detection system by A) LAMP for *ctrB* gene and B) nested PCR for *ggt* gene. For LAMP assay, amplified products were visualized by addition of fluorescent detection reagents (Eiken Chemical) and PCR amplified products were separated on 2% agarose gels and visualized by etidium bromide staining. 1) Chromosomal DNA of *N. meningitidis* strain (H44/76; serogroup B) was used as a template at the indicated amount. The corresponding number of bacteria for each DNA amount are shown below. 2) Ten ng of chromosomal DNA from *Neisseria* species were used as a template. *N. gonorrhoeae* (ATCC 49226), *N. flavesence* (ATCC 13120), *N. denitrificance* (ATCC 14686), *N. elongata* (ATCC 25295), *N. canis* (ATCC 14687), *N. cinerea* (ATCC 14685), *N. lactamica* (NIID85), *N. mucosa* (NIID16), *N. sicca* (NIID17) and *N. meningitidis* (H44/76) were used.