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False-Negative PCR Result Due to Gene Polymorphism: the Example of *Neisseria meningitidis*▽

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Early treatment of meningococcal meningitis is mandatory but may negate the cerebrospinal fluid culture. Etiological diagnosis then mainly relies on PCR. Here, we report a case of false-negative results for real-time PCR for a *Neisseria meningitidis* serogroup B isolate with a polymorphism in the *ctrA* gene.

Bacterial meningitis is life threatening, and rapid treatment is mandatory. A number of culture-negative meningococcal disease cases have been observed in many countries due to increasing use of preadmission antibiotics. Therefore, detection of meningococcal DNA by PCR is widely used for patients with suspected meningococcal meningitis and negative cerebrospinal fluid (CSF) cultures (2). In this context, we read with great interest the report by Cavrini et al. (3) on multiple nucleotide substitutions in two isolates of *Neisseria meningitidis* serogroup C causing false-negative detection, with a real-time PCR targeting the *ctrA* gene.

We encountered a similar problem with a clinical isolate of *N. meningitidis* serogroup B not detected by our real-time PCR targeting the *ctrA* gene. For 5 years, we have been using meningococcal DNA detection by PCR for patients with suspected meningococcal meningitis and negative CSF cultures, as PCR increases the number of confirmed cases (4, 6). We use the primers and probe described by Corless et al. (4), also used by Cavrini et al. and by several other groups (6, 9, 10, 15, 16). This primer and probe combination was tested for its analytical specificity on many *N. meningitidis* strains (9, 15). Before introducing it in our diagnostic laboratory, this real-time PCR was evaluated on a collection of 37 CSF samples (11 positive for *N. meningitidis*, 10 positive for *Streptococcus pneumoniae*, 3 positive for *Listeria monocytogenes*, 1 positive for *Haemophilus influenzae*, 6 positive for other bacteria, and 6 negative for any bacteria) with specificity and sensitivity of 100%.

Then, from 2005 to July 2010, a total of 419 CSF samples received with a diagnostic of meningitis were examined by PCR. During these 5 years, 5 samples were PCR and culture positive for *N. meningitidis*, whereas 17 were positive by PCR but negative by culture. Among the remaining 397 PCR-negative CSF samples, one specimen was documented as being falsely negative with the *N. meningitidis ctrA* PCR.

The patient, a 2-year-old boy, presented to the hospital with meningitis and petechiae. He was already being treated with amoxicillin *per os*. Blood cultures were drawn, and ceftriaxone and dexamethasone were administered intravenously before CSF puncture. Gram-negative diplococci were seen on Gram staining of CSF, but the culture remained sterile. The real-time PCR was done on CSF and was surprisingly negative. Then, a broad-spectrum 16S RNA PCR was performed (8), and *N. meningitidis* DNA was detected in the CSF. Meanwhile, the blood cultures became positive for *N. meningitidis* serogroup B susceptible to beta-lactams. The clinical progression was favorable, and the child was sent home after 5 days of hospitalization.

The blood isolate was identified as *N. meningitidis* by an API NH test strip (bioMérieux, Geneva, Switzerland) and serogrouped using the Pastorex meningitis antiserum panel (Bio-Rad, Marnes-la-Coquette, France). It was more precisely typed at the Swiss Reference Center of Meningococci. The serogroup was defined with an agglutination test, the serotype and subtype were defined with a dot enzyme-linked immunosorbent assay (dot-ELISA) method (7), and the multilocus sequence type was defined by sequencing seven housekeeping genes (1). This strain was confirmed to belong to serogroup B, B:−:P1.2,5, and ST-269. However, when the DNA, extracted from the blood culture isolate, was tested by the real-time *ctrA N. meningitidis* PCR, the result was again negative without PCR inhibition.

Following these results, the complete *ctrA* gene was amplified with one primer located in the operon for capsule biosynthesis (*siaA*), NM_SEQ1F (5′-TCTGGTACCTGTAATGCAAAGAATTC-3′), and another primer in the *ctrB* gene, NM_SEQ2R (5′-ATTACCGTAGGGATAATCACCGTTAC-3′), in order to include the entire *ctrA* gene. On the agarose gel, a single band was observed with a much larger size (2,322 bp) than the one obtained with positive-control *N. meningitidis* isolates (1,485 bp). The segment was sequenced with the primers used for amplification as well as with the following internal primers: NM_NM-F (5′-GCTGCGGTAGGTGGTTCAA-3′) and NM_NM2-R (5′-ACCACGGCGCAACAAAATA-3′). Analysis of the obtained sequence revealed interesting features. (i) The *ctrA* gene (bp 1005 to 2180) exhibited only 97% identity with all published available *N. meningitidis ctrA* gene sequences, except the 2 strains recently described by Cavrini et al. (3); our strain also presented a strong polymorphism on the reverse primer and a single nucleotide substitution on the probe, which precluded PCR amplification. (ii) Another fea-

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ture was an insertion segment, IS*1301* (837 bp), in the *sia*/*ctr* intergenic region (12).

To assess the frequency of such polymorphism, we tested by our real-time PCR our collection of *N. meningitidis* clinical isolates since 2000 ($n = 38$) and none exhibited this polymorphism. Another attempt was to focus on the *N. meningitidis* strains belonging to the ST-269 complex. These strains are very rare in Switzerland, less than one isolate being found per year, but they account for 6% of all isolates described in the EU-MenNet project (1). The four serogroup B, ST-269 isolates collected from 2004 to 2009 in Switzerland were tested by the *ctrA* real-time PCR, and the result was negative for 2 of them. Those 2 strains belong to the same serotype and serosubtype, B:−:P1.2,5, as does the strain isolated from the index case reported above. Both strains exhibited 100% identity with the index *ctrA* sequence isolate as demonstrated by sequencing the polymorphic 3′-end region of the *ctrA* gene (567 bp).

To check whether we could have missed additional *N. meningitidis* DNA-positive CSF samples with negative culture results, a newly designed PCR was applied to 93 CSF samples collected from January 2009 to July 2010 with the same forward primer but a new reverse primer, NM_NM2-R (5′-ACCACGGCGCAACA AAATA-3′), and a new probe, NM_NM2-P (5′-VIC-CATTGC CACGTGTTAGCTGCACAT-BHQ-3′), corresponding to the polymorphism. None was positive, suggesting that this polymorphism is relatively rare. Nevertheless, we now use both *ctrA* primers/probes (the one reported by Corless et al. [4] and the one with the NM_NM2-R as reverse primer and the NM_NM2-P probe) in order not to miss additional cases exhibiting such a polymorphism.

In conclusion, isolates with polymorphism or closely related isolates may generate false-negative or false-positive PCR results, especially when a single gene is targeted, as demonstrated here as well as previously for *Chlamydia trachomatis* (14), *Streptococcus pneumoniae* (5, 11, 17), and *Bordetella pertussis* (13). To target at least two different genes may overcome this risk. Microbiologists should be aware of this problem and should alert the scientific community when such events occur.

Nucleotide sequence accession number. The sequence of the complete *ctrA* gene has been submitted under accession number HQ156899.

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