Serotyping Pneumococcal Meningitis Cases in the African Meningitis Belt by Use of Multiplex PCR with Cerebrospinal Fluid[▽]

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Received 18 July 2009/Returned for modification 1 October 2009/Accepted 3 December 2009

We reformulated a multiplex PCR algorithm for serotyping of pneumococcal meningitis directly on cerebrospinal fluid (CSF). Compared to established methods on isolates, CSF-based PCR had at least 80% sensitivity and 100% specificity. In regional meningitis surveillance, CSF-based PCR increased the serotype information yield from 40% of cases (isolate testing) to 90%.

Because invasive pneumococcal disease (IPD) causes an enormous burden in sub-Saharan Africa (12), pneumococcal conjugate vaccines will be introduced in this region over the next few years (5). To facilitate evidence-based vaccine introduction and to describe serotype-specific vaccine impact on IPD and potential serotype replacement, serotype-specific IPD surveillance is needed. However, currently there are three obstacles to this in sub-Saharan Africa: first, IPD surveillance usually relies on meningitis, as surveillance of pneumonia is technically difficult (14), while cerebrospinal fluid (CSF) is obtained from a high percentage of suspected bacterial meningitis cases in national surveillance systems (15). Second, serotyping relies on the Neufeld Quellung reaction, which is technically and financially challenging as it requires highly skilled technicians. Third, serotyping is performed on pneumococcal isolates and is therefore limited to CSF from cases seen in reference hospitals without antibiotic pretreatment. To avoid the latter two obstacles, we aimed to develop a technique of PCR for serotyping pneumococcal meningitis cases directly on CSF and to establish this procedure for IPD surveillance in Burkina Faso and Togo.

Serotyping was performed using the sequential multiplex PCR method (SM-PCR) described by Pai et al. (3, 4, 10) with a regional algorithm adapted to the distribution observed in Burkina Faso and Togo during the last few years, in which 29 different serotypes were grouped into seven PCRs (details available from authors). After a cycle of freezing (−20°C) and thawing (5 min at 100°C), CSF samples (200 to 500 µl) or 1 ml of bacterial suspension was submitted to centrifugation (10 min at 12,000 rpm). The DNA-containing supernatant was collected in a new tube for PCR testing. All PCRs included

four to five primer pairs for specific serotypes (5- to 8-µl reaction volume) and positive internal control primers (*cpsA*), with a total reaction volume of 25 µl (6, 10). Because reactions combining primers for serotype 38 and *cpsA* systematically yield negative results for both PCR products (2), all *cpsA*-negative samples were submitted for two separate reactions with each primer. Results were interpreted as “SM-PCR non-typeable” (PNT) if SM-PCR was negative in the serotype-specific reactions and positive in the *cpsA* controls and as “SM-PCR negative” if the serotype-specific reactions as well as the *cpsA* controls were negative. In cases with a positive reaction with primers that cross-reacted between several serotypes (e.g., 6A/6B), we did not attempt to further discriminate between serotypes. The Neufeld Quellung reaction on isolates was performed using antisera provided by the Statens Serum Institute (Copenhagen, Denmark) (7).

After satisfying testing of the adapted SM-PCR algorithm on isolates of a variety of serotypes (data not shown), we studied the performance of SM-PCR on CSF (CSF SM-PCR). For this, we used pairs of CSF specimens and isolates from meningitis cases from Burkina Faso and Togo (1, 11, 16). The presence of pneumococcal, meningococcal, or *Haemophilus influenzae* DNA in CSF samples was confirmed by multiplex PCR targeting the *lytA*, *ctrA*, or *bexA* gene, respectively (8, 11, 13). CSF aliquots had been stored at −80°C since collection.

Among 58 pneumococcal meningitis cases, CSF SM-PCR results matched with the Quellung reaction in 46 cases and with SM-PCR in 51 cases (Table 1). Four cases due to serotypes 2, 13, 46, and 48 were found to be PNT, and eight cases were CSF SM-PCR negative. In addition, 10 CSF samples from etiology-negative, meningococcal or *Haemophilus influenzae* meningitis cases were found to be SM-PCR negative. The resulting serotyping sensitivities of CSF SM-PCR compared to Quellung reaction were 79.3% (95% confidence interval [CI], 66.6% to 88.8%) and 87.9% (95% CI, 76.7% to 95.0%) compared to SM-PCR on isolates. If

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[▽] Published ahead of print on 9 December 2009.

TABLE 1. Comparison of serotyping by Neufeld Quellung reaction with sequential multiplex PCR with CSF and bacterial isolates in an evaluation of 58 pneumococcal meningitis cases

Serotype according to Quellung reaction (<i>n</i>)	Resulting serotype by SM-PCR ^a	No. of cases yielding result from:	
		CSF	Isolates
1 (22)	1	20	22
	Negative	2	
2 (1)	PNT	1	1
3 (1)	3	1	1
4 (1)	4	1	1
6A (2)	6A/6B	2	2
6B (1)	6A/6B	1	1
7C (1)	7C	1	1
7F (1)	7F	1	1
12B (1)	12A/B/F	1	1
12F (4)	12A/B/F	4	4
13 (1)	PNT	1	1
14 (2)	14	2	2
18A (1)	18A/18B/18C/18F	1	1
18C (2)	18A/18B/18C/18F	2	2
19F (2)	19F	2	2
23F (5)	23F	5	5
25F (5)	25A/25F/38	2	5
	Negative	3	
35B (1)	35B		1
	Negative	1	
38 (1)	25A/25F/38		1
	Negative	1	
46 (1)	PNT	1	1
48 (1)	PNT	1	1
NT ^b (1)	Negative	1	1

^a PNT, nontypeable by sequential multiplex PCR; negative, negative in serotype-specific reactions and *cpsA* control.

^b NT, nontypeable.

PNT cases were excluded (due to serotypes not included in the PCR primer set), the sensitivities were 85.2% (95% CI, 72.9% to 93.4%) and 87.0% (95% CI, 75.1% to 94.6%), respectively. Specificity of CSF SM-PCR compared to PCR targeting the *lytA* gene was 100% (95% CI, 69.2% to 100.0%).

In the context of a technology transfer to Burkina Faso, we then evaluated the added value of CSF SM-PCR for serotype-specific IPD surveillance in the African meningitis belt. We used a systematic collection of 136 pneumococcal meningitis cases from surveillance carried out in Burkina Faso and Togo during 2007 and 2008 (1, 11, 16), for which either both CSF and isolates (*n* = 52), CSF only (*n* = 81), or isolates only (*n* = 3) were available. Among all 136 meningitis cases included, 90% were successfully serotyped by CSF SM-PCR, compared to 40% and 38% by Quellung reaction or SM-PCR on isolates. Serotyping by any technique showed the following serotype distribution: 1 (53%); 12A/B/F (10%); 25F/38/25A (8%); 14 (7%); 18A/B/C/F (4%); 23F (4%); 6A/B (3%); 3 (2%); and 4, 5, 7F, 13, 19F, 35B, 48, and nontypeable (NT) (1% each).

This is the first report that SM-PCR can be used directly on CSF samples, providing a serotyping tool with good sensitivity and high specificity. Some CSF samples had false-negative SM-PCR results despite a positive result for the *lyt* gene. This reduced sensitivity is probably due to insufficient DNA content in the CSF sample; it may be improved by using real-time PCR,

which, however, in turn, would increase the technical demands of the method.

Serotyping by SM-PCR in general has limitations, such as missing the distinction between some serotypes or within serogroups or restriction to a panel of preselected serotypes (3, 10). However, the missed serotypes in our evaluation (2, 45 and 46; 10% of cases) were relatively rare serotypes, which together may represent only 4% of IPD cases in African children (9). Both issues indicate that SM-PCR should be completed by sporadic Quellung testing of invasive isolates to update the regional algorithm.

Apart from a relatively simple molecular biology platform, SM-PCR serotyping has few technical requirements and can easily be transferred to institutions in sub-Saharan Africa. We suggest that SM-PCR is at its greatest value in resource-poor countries if used directly with CSF. CSF is readily available from meningitis cases, while bacterial isolates require rapid sample transport to skilled laboratories and the absence of antibiotic pretreatment, both of which are the exception. In these settings, CSF SM-PCR substantially increases the number of pneumococcal meningitis cases with available serotype information. It therefore represents a useful tool for pneumococcal serotype surveillance in sub-Saharan Africa.

This work was funded by the PneumoADIP.

We thank Bernard Beall, Gloria Maria Carvalho, and Bradford Gessner for helpful discussion and technical advice.

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