Rapid Diagnosis of Pneumococcal Meningitis
Implications for Treatment and Measuring Disease Burden

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Background: Streptococcus pneumoniae is the leading cause of childhood pneumonia and meningitis worldwide. Isolation of this organism, however, is uncommon in resource-poor countries, in part because of extensive use of prior antibiotics. A rapid, highly sensitive immunochromatographic test (ICT) for S. pneumoniae was evaluated for the diagnosis of meningitis.

Methods: Cerebrospinal fluid (CSF) from 450 children with suspected meningitis was tested with ICT, and results were compared with CSF culture, latex agglutination test (LAT), and/or polymerase chain reaction (PCR). Serial CSF specimens from 11 patients were also evaluated for duration of positive results during effective antimicrobial therapy.

Findings: All 122 cases of pyogenic pneumococcal meningitis positive either by culture (N = 87) or PCR (N = 35) were positive by ICT, yielding 100% (122 of 122) sensitivity. All purulent CSF specimens from patients with meningitis caused by other bacteria by culture (N = 149) or by LAT (N = 48) or those negative by culture, LAT and LytA and thus of unknown etiology (N = 20), and normal CSF specimens (N = 104) were negative by ICT. Thus the specificity of ICT also was 100% (321 of 321), although negativity of ICT was not confirmed by PCR, if it was positive for other organisms either by culture or LAT. Serotyping of S. pneumoniae strains revealed 28 different serotypes, indicating that outcome of ICT are independent of diverse capsular serotype of pneumococcus. Antigen was detected by ICT for at least 10 days after presentation, and 1 was still positive on day 20, which was longer than for either LAT or PCR.

Interpretation: ICT for pneumococcal antigen in CSF is 100% sensitive and specific in diagnosing pyogenic pneumococcal meningitis and can detect ~30% more pneumococcal meningitis cases than with culture alone. The simplicity of the test procedure and the longevity of CSF antigen detection suggest the potential utility of ICT to estimate the true burden of pneumococcal disease, as for Haemophilus influenzae type b using data from meningitis, and to guide selection of appropriate antibiotic treatment, especially in resource-poor countries with widespread prehospital antimicrobial use.

Key Words: immunochromatographic test, meningitis, pneumococcus, pneumonia, polymerase chain reaction, Streptococcus pneumoniae

Pneumococcal pneumonia and meningitis are responsible for an estimated 800,000–1 million child deaths each year. Meningitis frequently leads to chronic sequelae and incurs substantial direct and indirect costs. Streptococcus pneumoniae as an etiology of pneumonia largely goes undetected given that blood cultures lack sensitivity for detecting the organism because most patients with pneumonia are not bacteremic. Pooled data from lung aspirate studies, performed mostly in developing countries, indicate that 55% of infections are caused by bacterial pathogens, predominantly S. pneumoniae, Haemophilus influenzae and Staphylococcus aureus. Lung puncture is not performed routinely, however, because of the invasive nature of the procedure and difficulty in obtaining ethical clearance. Therefore evaluation of pneumococcal meningitis cases might be useful to indirectly estimate the overall burden of pneumococcal pneumonia, similar to the use of the rate of meningitis due to H. influenzae type b (Hib) for estimating total Hib disease burden. Isolation of pneumococcus, however, is uncommon in meningitis cases that occur in locations with limited resources and where the burden of disease is the greatest. This is partly the result of an absence of standard microbiology procedures, which, in turn, makes clinicians reluctant to perform lumbar punctures. Pneumococcal isolation is also jeopardized by prior antibiotic therapy which, in Bangladesh, is the rule rather than an exception. Thus the true burden of pneumococcal diseases is still unknown in many developing countries, including Bangladesh.

Alternate methods to diagnose pneumococcal meningitis in cases with prior antibiotic use include detection of antigen by latex agglutination test (LAT), or the LytA gene by polymerase chain reaction (PCR). Antigen detection by LAT, however, is expensive, and the sensitivity varies depending on the commercial assay. Moreover PCR, although considered a standard, is still not widely available for rapid diagnosis, especially in resource-poor settings.
In recent years, the immunochromatographic test (ICT) (Binax NOW Streptococcus pneumoniae test; Binax Inc., Portland, ME) was developed with the aim to detect pneumococcal antigen in urine collected from patients with invasive pneumococcal disease.10 This rapid assay, unlike other serotype-specific tests, detects the C-polysaccharide molecule found in the cell wall of all pneumococci.10,11 A major limitation of this test, however, is that children in areas with high nasopharyngeal colonization rates often have positive urinary antigen tests in the absence of any clinical manifestations of pneumonia or bacteremia.12-14 In contrast, the same assay used for detection of antigen in cerebrospinal fluid (CSF) was found to be 95-100% sensitive and 100% specific for pneumococcal meningitis when compared with CSF culture.15,16 Although studies have demonstrated the potential utility of this test for the detection of pneumococcal meningitis, major limitations of studies to date include small numbers of culture-positive cases, mostly from adults, and discrepancies between ICT and culture-negative results were not validated (eg, with PCR).

The present study aimed to (1) determine whether ICT detects pneumococcal antigen in urine from healthy children in Bangladesh, (2) investigate the utility of ICT of CSF for the diagnosis of pneumococcal meningitis cases with the use of CSF culture and/or PCR as the standard, (3) elucidate whether outcome of ICT varies with pneumococcal serotype and (4) evaluate the duration of positive tests with LAT, ICT and PCR during effective antimicrobial therapy.

**METHODS**

For study aim 1, urine specimens were collected from healthy children 2-9 months old who presented for routine immunizations at Dhaka Shishu (Children) Hospital. For study aims 2-4, patients younger than 5 years of age with clinical suspicion of meningitis were enrolled. Patients found to have pyogenic meningitis (CSF containing ≥100 leukocytes/mm<sup>3</sup> with >50% neutrophils and/or growth of the organism in culture) were subsequently followed. Another group of patients with febrile convulsions and/or CSF with 0-5 cells, negative culture, normal protein and glucose levels and no evidence of meningitis on follow-up were included as a control group. Equivocal cases with cell count from 6 to 99/mm<sup>3</sup> and negative culture were excluded. Serial CSF specimens were obtained from some patients as routine practice of clinical consultants to document a declining cell count before discharge or per protocol for a concurrent World Health Organization (WHO)-sponsored clinical study of meningitis.17,18

Study procedures were approved by the Bangladesh Institute of Child Health Ethical Review Committee, and written informed consent was obtained from each patient’s guardian who accompanied the child to hospital.

Specimens of CSF were evaluated biochemically and cultured following standard procedures as described previously.17,19 LAT (Remel, Inc., Lenexa, KS) and ICT were performed according to the manufacturers’ instructions. ICT was done on CSF samples by dipping a swab into the CSF specimen, then inserting it into the test device. Reagent A, a buffer solution, was added from a dropper bottle, and the device was closed, bringing the sample into contact with the test strip. Pneumococcal antigen present in positive samples is captured by immobilized S. pneumoniae antibody, which reacts to bind conjugated antibody, forming the sample line. Immobilized goat anti-rabbit IgG also captures a visualizing conjugate, forming the control line. All test results (positive and negative) are read visually at 15 minutes. The test was interpreted by the presence or absence of visually detectable pink to purple lines. A positive specimen produced both a sample line and a control line. A negative result only produced the control line, and the test was considered invalid if the control line did not appear.

LAT was performed on pyogenic CSF samples by mixing 40 μL of boiled urine or CSF and 1 drop of test latex on the specified circle of the supplied slide and then mixing on a rocking device for 3 minutes. LAT was done for detection of antigens of S. pneumoniae, Hib, Neisseria meningitidis ACY W135, group B Streptococcus and Escherichia coli K1/N. meningitidis B. Two independent laboratory personnel read the test results; S.K.S., who was blinded to other laboratory results, interpreted discrepant results. The LAT was interpreted as negative if it was clearly nonreactive or equivocal (±). To reduce costs, LAT was done on the first day only if the Gram stain result was negative or equivocal. An aliquot of CSF was preserved, and LAT was performed on the second day if there was no growth from CSF.18

PCR to detect the LytA gene was performed in culture-negative, ICT-positive CSF specimens using the primers described earlier: 5'-694 TGAAGCGGA TT A TCACTGGC713'; and 5'-966GCTAAACTCCCTG TATCAAGCG94s-3'. In brief, DNA was extracted from 200 μL of CSF with the QIAmp kit (Qiagen, Hilden, Germany), and a 5-μL aliquot was placed in a PCR mixture containing 2.5 μL of 10× reaction buffer, 2.0 μL (2.5 mM) of each deoxynucleotide triphosphate, 0.125 μL (5 units/μL) of Taq polymerase, 0.025 μL of LytAs, 0.025 μL of LytAr (0.1 μM) and 20.3 μL of ΔH<sub>2</sub>O (Takara Bio, Inc., Shiga, Japan). DNA was denatured in a PX2 thermal cycler (Thermo Electron Corp., Needham Heights, MA) at 94°C for 2 minutes followed by 30 seconds at 94°C, 55°C and 72°C per cycle for 30 cycles. Further extension was done at 72°C for 10 minutes. After amplification, 10 μL of PCR product were electrophoresed on a 3% agarose gel (Invitrogen, Carlsbad, CA). For interpretation, PCR product from CSF was compared with the 273-bp LytA product of a previously characterized S. pneumoniae isolate.

Pneumococcal strains were serotyped by the capsular swelling procedure (quellung reaction) with antipneumococcal omni, pool, type or group and factor sera (Statens Seruminstitut, Copenhagen, Denmark) as described previously.21 Nontypable S. pneumoniae strains were screened out using omni sera at the first step of serotyping.

CSF specimens were tested for presence of antibiotic following the standard procedure described previously.23 In brief, a blank disc (Oxoid, Hampshire, United Kingdom) was placed on the lawn of a pansensitive organism (Micrococcus luteus ATCC 9341), and then the disc was soaked with 10 μL of CSF. The plate was incubated overnight at 37°C, and any
zone of inhibition around the disc was considered positive for the presence of an antibiotic capable of crossing the blood-brain barrier.

The standard for determination of sensitivity of ICT was positive culture or presence of LytA in culture-negative specimens. For specificity, the comparison standard was positive culture for other organisms besides *S. pneumoniae*, culture-negative specimens positive by LAT for other organisms, normal CSF or specimens negative by all tests, including LytA. Data were double entered, validated and analyzed by Epi Info 6.1.

**RESULTS**

**Urine.** Pneumococcal antigen was detected by ICT in urine specimens from 51% (102 of 200) of healthy children.

**Cerebrospinal Fluid.** Of 512 patients younger than 5 years old evaluated by lumbar puncture for clinical suspicion of meningitis at Dhaka Shishu Hospital from 2001 to 2004, 346 (68%) were enrolled with features of pyogenic meningitis; 62 cases had equivocal features of meningitis and were excluded; in 104 cases, meningitis was ruled out, and these were included as controls (Fig. 1). The mean and median ages of

![Diagram](image_url)

**FIGURE 1.** Study profile demonstrating results of CSF testing stratified by baseline CSF cell counts. TLC indicates total leukocyte count; Cult, culture; Neg, negative; Pos, positive; SPN, *S. pneumoniae*; Nmen, *N. meningitidis*; +ve, positive; *, 7 cultures were not available for further tests.
the 450 enrolled cases were 9 and 7 months, respectively, with 90% of the cases in the first year of life. Age distribution was similar in pyogenic cases and in cases with normal CSF. There was a marked predominance of boys (59% (302 of 512)).

Figure 1 also shows the results of CSF specimens evaluated by culture, ICT, LAT and PCR for LytA. Among 346 pyogenic meningitis cases, 236 (68%) yielded growth in culture. Presence of antibiotic was detected in 32% (111 of 346) of pyogenic CSF specimens, including 9% (22 of 236) and 81% (89 of 110) of culture-positive and culture-negative groups, respectively. Among culture-positive cases, antibiotics had been used before lumbar puncture in no instance (0 of 87) of infection with S. pneumoniae, whereas in 14% (18 of 125) of cases of H. influenzae meningitis, prior antibiotic use was detected.

Overall 122 (35% of 346) cases of pneumococcal meningitis were identified, including culture-positive cases (N = 87) plus culture-negative cases in which PCR was positive, that is, detection of LytA (N = 35). Thus sensitivity of culture for identifying S. pneumoniae was 71% (87 of 122). All cases in which S. pneumoniae was recovered by culture of CSF (N = 87) or identified by LytA (N = 35) were positive by ICT within ≤2 minutes. The sensitivity of ICT therefore was 100% (122 of 122) for detection of S. pneumoniae. In contrast, all CSF specimens were negative by ICT from meningitis cases that were culture-positive for other organisms (N = 149), which showed no growth in culture but were identified as H. influenzae (N = 41) or N. meningitidis (N = 7) by LAT of CSF, or were normal (ie, had normal cytology, biochemistry and negative culture) (N = 104). For these cases, however, LytA was not done to confirm negativity for pneumococcal genome. In addition, all specimens that were negative by all tests, including LytA (N = 20), were also negative by ICT. Thus the specificity of ICT under these conditions was also 100% (381 of 381).

Among 35 culture-negative pyogenic CSF samples positive by ICT and LytA for S. pneumoniae, LAT was positive in 30 but falsely negative in 5 cases.

Among 87 pneumococcal isolates from CSF culture, 63 were available for serotyping. Testing revealed 28 different serotypes with a relative predominance of 2 (N = 7), 5 (N = 7), 33 (N = 5) and nontypable (N = 6) strains. Because LAT was conducted selectively, based on first day Gram stain results, only 41 pneumococcal culture-positive cases had a simultaneous assay for LAT and, in contrast to ICT, which was universally positive in these cases, 6 (15%; 6 of 41) CSF specimens did not show any agglutination. Among these falsely LAT-negative cases, 3 were nontypable whereas the others were types 1, 12A and 20.

Markers for S. pneumoniae were followed in serial CSF specimens from 11 patients from whom ≥3 specimens were available with sufficient quantity to run the assays, LAT, PCR and ICT, in addition to cytology and biochemistry. All were positive by ICT for at least 10 days after the initiation of appropriate treatment, and 1 patient was still positive on day 20 (the specimen available with longest duration) (Table 1). All patients survived on first line antibiotic therapy. LytA gene was undetectable by PCR by day 19–20 of treatment, whereas LAT was negative as early as day 10.

**DISCUSSION**

*S. pneumoniae* and Hib are the predominant causes of meningitis and pneumonia in Bangladesh.17,18 Recent studies showed an increasing trend of nonsusceptibility of *H. influenzae* strains.18 On the other hand, *S. pneumoniae* strains have remained susceptible to penicillins, and most other drugs for more than 1 decade.17,19,25 Therefore rational empiric treatment of these 2 organisms differs; thus rapid etiologic diagnosis would be of great interest to rationalize empiric therapy.

Our detection of *S. pneumoniae* antigen in urine specimens from one-half (51%) of healthy children by ICT is similar to the finding of others12-14 and is most likely a result of the high pneumococcal nasopharyngeal colonization rates25 and high prevalence of pneumonia in our population.26 Therefore antigen detection in urine by ICT is not useful for predicting the presence of disease in Bangladeshi children.

In contrast, this study showed that the ICT test was 100% sensitive for identification of *S. pneumoniae* in pyogenic CSF with very high specificity compared with the nonpneumococcal cases proved by culture and/or LAT and/or LytA. The findings of this study confirm the results of the previous studies with a small number of meningitis cases.15,16 Our data demonstrate that ICT remained ICT-positive even when the organism, which had originally been recovered from culture, was eradicated and CSF become culture-negative (Table 1). This finding was further strengthened by the investigations with serial CSF specimens from 11 culture-positive cases. Persistent detection of *S. pneumoniae* antigen by ICT for at least as long as the presence of LytA substantiates the high sensitivity of ICT in detecting pneumococcal C-polysaccharide antigen while concurrently demonstrating the longevity of antigen detection in CSF from children with meningitis. There has not been any previous research on the duration of antigen presence in CSF samples in subjects with pneumococcal meningitis. Antigen detection in urine by ICT remains positive for as long as 89 days in adults with community-acquired pneumococcal pneumonia.27,28 This is possibly caused by the release of small fragmented molecules of C-polysaccharide, such as teichoic acid, at the time of growth of pneumococci in the body. Nevertheless the high false positive rate of ICT in urine tests obviates its utility for pneumonia case detection in adults who have had recent episodes of lower respiratory tract infections. False positive urinary antigen tests can occur with even greater frequency in children as a result of nasopharyngeal colonization and/or recent pneumococcal respiratory tract infections.

In this series of patients, prior antibiotic therapy, based on the presence of antibiotic in CSF specimens at sufficient levels to inhibit bacterial growth, was found in one-third of patients with pyogenic meningitis. Detection of prior antibiotic use in no cases of pneumococcal meningitis identified by culture (0 of 87) but in 14% (18 of 125) of cases in
TABLE 1. Test Results of Serial CSF Specimens From 11 Patients

<table>
<thead>
<tr>
<th>Patient/ Sample</th>
<th>Antibiotic Given</th>
<th>Outcome of Treatment</th>
<th>Hospital Day</th>
<th>Culture</th>
<th>LAT</th>
<th>PCR for LytA</th>
<th>ICT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ceftriaxone</td>
<td>Sequelae</td>
<td>0</td>
<td>Streptococcus pneumoniae</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Ampicillin and chloramphenicol</td>
<td>Cured</td>
<td>0</td>
<td>Streptococcus pneumoniae</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Ampicillin and chloramphenicol</td>
<td>Sequelae</td>
<td>0</td>
<td>Streptococcus pneumoniae</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Ceftriaxone</td>
<td>Sequelae</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Ceftriaxone</td>
<td>Cured</td>
<td>0</td>
<td>No growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

which Hib was cultured from CSF suggests that reliance only on culture positivity in this population, in which most strains of S. pneumoniae are penicillin-susceptible, can specifically lead to an underestimation of the burden of pneumococcal disease. The ICT, however, can be used to successfully detect pneumococcal meningitis in these culture-negative cases.

Pneumococcal serotypes among the Bangladeshi population are diverse. Nevertheless, in this series, all 28 different serotypes of 25 serogroups gave positive results with ICT. In contrast, 15% (6 of 41) of culture-positive cases and 14% (5 of 35) of culture-negative, LytA-positive cases were negative by LAT, thus implying a lower sensitivity of the later test.

Our study has a few limitations. (1) Culture-negative cases with 6–99 cells/mm³ were not subjected to ICT; therefore the utility of this test for detection of pneumococcal meningitis in patients with CSF white blood cell counts in this range (6–99/mm³) could not be discerned. Although all these cases were negative by culture, there might have been some pneumococcal cases as indirectly evidenced for Hib in the recent Lombock vaccine trial. (2) We also could not determine the sensitivity and specificity of LAT, although the available data suggest that its sensitivity is lower than that of ICT.

This study evaluated ICT with large number of cases using detection of LytA gene as a standard to confirm culture-negative and ICT-positive cases of pneumococcal meningitis and supported the preliminary findings of Samra et al. and Marcos et al. Although PCR for the pneumococcal gene was not applied to culture-negative, LAT-positive nonpneumococcal cases, ICT proved to be similar to detection of LytA by PCR for diagnosis of pneumococcal meningitis. PCR, however, requires advanced facilities and expertise and incurs higher costs. On the other hand, ICT can be done at the bedside or field level without any laboratory facility, and the device can be stored at room temperature. Given the simplicity of the test and the rapidity with which results become available, this can be used at hospitals of all levels, from primary to tertiary, for surveillance on the burden of pneumococcal meningitis. This information in turn could be used to estimate the burden of pneumococcal pneumonia as has been done for H. influenzae. In addition to disease burden, ICT might have paramount significance in determining the treatment of pneumococcal meningitis in Bangladesh and neighboring countries, where prior empiric treatment is common and isolation of S. pneumoniae is jeopardized even after a single dose of first line antibiotic because of its high susceptibility to penicillin.
REFERENCES