Filtration of Cerebrospinal Fluid Improves Isolation of Mycobacteria

Clinical diagnosis of tuberculous meningitis (TBM) is difficult due to the disease’s inconsistent clinical presentation. Furthermore, owing to the paucibacillary nature of cerebrospinal fluid (CSF), traditional techniques for detecting acid-fast bacilli have limitations. Hence, isolating mycobacteria from CSF by culture is a challenge. There is a dire need to improve, accelerate, and enhance isolation of the bacteria for the prompt diagnosis of TBM. To enhance the isolation rate, concentration by centrifugation/immunomagnetic enrichment, followed by inoculation of specially designed media, and incubation of culture tubes in different automated systems have been utilized (with systems such as BACTEC-460 and MGIT-960, etc.). Here we describe a simple method for concentrating mycobacteria that may be present in the CSF sample and increasing the sensitivity of detection of mycobacteria by conventional and/or automated systems.

In this procedure, 0.5 to 2 ml of CSF specimen was filtered through a sterile 0.45-μm filter (Millipore Corp., Bedford, MA). The CSF container was washed twice with 1 ml of sterile normal saline. The wash fluid along with the sample were loaded into a sterile syringe. The entire fluid volume was filtered. The membrane was removed aseptically. The residue present on the membrane was used for inoculating Lowenstein-Jensen (LJ)/Mycobacterium growth indicator tube (MGIT 960) medium with polymyxin B-ampicillin B-nalidixic acid-trimethoprim-azlocillin according to the manufacturer’s instructions (BD BACTEC MGIT bar-coded 7-ml tubes; BD BACTEC MGIT 960 supplement kit). The filter membrane in our laboratory has been routinely divided into two parts; one part was used for the isolation of mycobacteria and the remaining portion for the isolation of DNA for PCR-based diagnosis (8).

CSF samples (n = 125) were collected from patients who were clinically suspected of having TBM, admitted to the neurology ward at All India Institute of Medical Sciences, New Delhi. The 50 sample residues inoculated into MGIT tubes were incubated at 37°C in the BACTEC MGIT 960 instrument (BD MGIT 960 system for mycobacteria testing) and monitored continuously. The growth of 75 sample residues smeared on LJ slants was checked at weekly intervals. On average, growth on LJ medium was detected after 3 to 4 weeks of incubation at 37°C. However, in the case of the MGIT liquid culture, on average, growth was detected after 10 days. Six mycobacterial (6/75; 8%) isolates were obtained from LJ medium slants. However, 12 mycobacteria (12/50; 24%) isolates were recovered from MGIT tubes. The isolates from culture-positive MGIT tubes were subcultured onto LJ medium and processed for identification by using classical biochemical and molecular techniques (5, 11). The efficiency of the isolation increased threefold in liquid medium compared to that in solid medium.

This method is simple, it entraps mycobacteria, and it takes a brief time to process each sample. This method of filtering CSF samples for isolation gives a better level of sensitivity than that reported previously (Table 1). The isolation rate on MGIT 960 is 24% by the present method of filtration compared to that of 7.4% (9), 18.36% (7), or 11.6% (7a) as reported previously. Using LJ medium, the method showed an isolation rate of 8%, which was higher than the rate range 4.3 to 6.5% reported previously by several investigators, (6, 7, 9, 10).

REFERENCES

TABLE 1. Comparative analysis of culture sensitivity in CSF specimens by various studies

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Source (yr), reference</th>
<th>% Mycobacterial isolates found (no. of mycobacterial isolates/total no. of clinical samples) in the indicated medium</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LJ</td>
</tr>
<tr>
<td>1</td>
<td>Chua et al. (2005), 3</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Venkatasamy et al. (2007), 10</td>
<td>4.3 (101/2,325)</td>
</tr>
<tr>
<td>3</td>
<td>Tortoli et al. (1999), 9</td>
<td>6.5 (168/2,567)</td>
</tr>
<tr>
<td>4</td>
<td>Rishi et al. (2007), 7</td>
<td>4 (2/49)</td>
</tr>
<tr>
<td>5</td>
<td>Lang et al. (1998), 6</td>
<td>5.9 (58/84)</td>
</tr>
<tr>
<td>6</td>
<td>Baker et al. (2002), 1</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>Johansen et al. (2004), 4</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>Bonington et al. (2000), 2</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>Present study</td>
<td>8 (6/75)</td>
</tr>
</tbody>
</table>

*a ND, not done.
*b Culture sensitivity was determined from growth in BACTEC-460 in combination with solid Middlebrook 7H11 medium.
*c Culture sensitivity was determined from growth in MGIT 960 in combination with LJ medium.


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