Printcultures for Postmortem Microbiology

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ABSTRACT

A modified printculture method for postmortem bacteriology was compared to the traditional tissue homogenate inoculum method. The two methods were comparable in their recovery rate of bacterial isolates. However, printcultures is an easy and rapid method; thus, it is superior to the traditional technique for postmortem culture. Histologic examination of most postmortem lung specimens with no bacterial growth showed no evidence of pneumonia or bronchitis, suggesting that the lack of growth is a reliable indicator of no bacterial infection.

Introduction

The traditional microbiology protocol for processing postmortem tissue specimens has involved its homogenization and subsequent inoculation onto multiple media. Several attempts have been made to modify this protocol in order to improve the correlation between postmortem culture and clinical diagnosis. Quantitative postmortem lung cultures have been useful in associating bacterial isolates with pneumonia and bronchitis. Cryostat sections of tissues, mounted onto agar surfaces, have recovered not only the organisms found by traditional methods, but have aided in their localization in tissues. Such precise localization has allowed for the distinction between possible surface contamination and actual bacterial penetration deep into the tissue. Recently, lung printcultures performed on frozen lung sectioned by a sledge microtome showed similar results. In this study, a modified printculture technique is reported using unfrozen postmortem tissues sectioned with a sterile scalpel blade.

Methods

Postmortem tissues were collected following the procedures of DeJong et al and submitted to the Microbiology Laboratory within two hours after necropsy. Specimens were kept at 4°C until processed. Most specimens were processed within two hours and all by 12 hours of receipt.
A sterile set of forceps and blade was used for processing each tissue. The tissue was sliced in half, and two printcultures were made onto the following media: sheep blood agar, chocolate agar, MacConkey agar, Schaedler agar, and two colistin-nalidixic acid agar plates. The same tissue was then minced and homogenized in a sterile glass tissue grinder in 1.0 ml sterile saline. A second set of plates and one tube of thioglycolate broth were each inoculated with 0.1 ml of the homogenized tissue. The tissue suspension and one of the two tissue imprints (figure 1) were streaked for single-colony isolation.

To evaluate the reliability of culture results, histologic sections of lung tissues were examined by one of us (TS) to determine if pneumonia, bronchitis, or other infectious process was present.

Results

Of the 65 spleens and 73 lungs processed, 42 and 33, respectively, showed no growth (table I). Seven spleen and seven lung specimens grew only in thioglycolate broth. Sixteen spleen cultures exhibited organism growth on solid agar media. In all 16 cases, spleen imprints yielded the same organisms in the same quantities as with the tissue homogenate inocula. Of the 33 lungs showing growth on solid media, two homogenates yielded rare to few organisms not seen with lung imprints. Both cases showed no evidence of inflammation in lung tissues histologically. On the other hand, one lung printculture demonstrated two colonies of Escherichia coli not found by the traditional tissue suspension. This case showed evidence of bronchopneumonia in tissue section.

Of the 33 lung specimens with bacterial growth on all media, 18 showed histological evidence of inflammation

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Spleen</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>65</td>
<td>73</td>
</tr>
<tr>
<td>No growth</td>
<td>42</td>
<td>33</td>
</tr>
<tr>
<td>Growth in thioglycolate only</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Growth on agar media</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>Tissue homogenate method</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Printculture method</td>
<td>16</td>
<td>31</td>
</tr>
</tbody>
</table>
(Table II). Two of the seven lung specimens, with bacterial growth only in thioglycollate broth, demonstrated histologic evidence of inflammation. On the other hand, two of the 33 specimens with no growth on all media showed evidence of lobar pneumonia or bronchopneumonia.

Discussion

Printculture of tissue sections is an easy method for culturing postmortem lung and spleen specimens. It can localize the tissue site of the organism isolated to aid in interpreting its role as a possible etiologic agent of infection or as a possible surface contaminant. The results from this study demonstrate that the detection of bacterial isolates from the printculture inoculum method was comparable to the traditional tissue homogenate method. Both methods yielded essentially the same semi-quantitative recovery of all isolates. Three specimens differed in their recovery of bacterial isolates by the two inoculum methods. This difference, however, appears to be the result of low numbers of organisms present in these tissues. Interestingly, one of the three discrepancies was the result of growth by the printculture method and no growth by the homogenate method. Thus, tissue suspensions may further dilute the low numbers of organisms present in the tissue, resulting in their absence on growth media.

<table>
<thead>
<tr>
<th>Culture Results</th>
<th>Total Number</th>
<th>Number with Evidence of Pneumonia/ Bronchitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>Growth in thioglycollate only</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Growth on agar media</td>
<td>33</td>
<td>10</td>
</tr>
</tbody>
</table>

The printculture method used in this study was simplified from methods previously described. Meer and Hunt cultured microtome sections of tissue by actually laying 25 to 30 micron thick frozen tissue sections onto agar surfaces with sterile forceps. Zanen-lim and Zanen described culturing postmortem lung specimens by pressing the cut surface of the frozen tissue sectioned with a sledge microtome. Our method differs in that fresh unfrozen tissues were sliced with disposable sterile scalpel blades. Each tissue was imprinted twice on each of the chosen agar surfaces, followed by streaking one tissue imprint. This allows not only localization of organisms in the tissue imprint but also allows for single colony isolation in the streaked printculture (figure 1). The tissue imprint pattern was reproducible on the six agar plates used.

Organisms were isolated in 35 percent of spleen and in 55 percent of lung specimens processed. This high percentage of isolation from postmortem tissues has been observed by other investigators and appears to reflect that the viscera of man are not sterile.

Only half the lung specimens showing growth on solid media had histologic evidence of infection. This incidence parallels the reported 30 to 50 percent lack of correlation between post- and antemortem cultures. In contrast, a good correlation was observed between tissues with no growth and the lack of inflammation seen on histological sections. Thus, a negative postmortem culture result is a reliable indicator of no bacterial infection. Knapp and Kent noted in quantitative lung cultures that cases with more than $10^5$ organism per cc of lung tissue were generally associated with clinical evidence of pneumonia or bronchitis and those with less than $10^6$ organism per cc with no pneumonia or bronchitis. In the semiquantitative lung cultures in this series, such an association
between the magnitude of organisms isolated and histologic evidence of infection was not found.

In summary, the printculture technique appears to be a suitable substitute for the traditional postmortem tissue homogenate method. The former method is easy to perform, less time-consuming, and may aid in the interpretation of culture results based on the location of bacterial colonies.

References


