Isolation of Mycobacterium ulcerans from Swab and Fine-Needle-Aspiration Specimens

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Received 11 November 2010/Returned for modification 16 December 2010/Accepted 4 March 2011

For cultivation of Mycobacterium ulcerans from clinical specimens, we optimized the release of bacteria from swabs, as well as decontamination and cultivation on supplemented medium. Nevertheless, the proportions of positive cultures, 41.7% (5/12) for fine-needle-aspiration (FNA) samples and 43.8% (49/112) for swab samples, were lower than those we have previously observed for excised tissue specimens.

Buruli ulcer (BU), a necrotizing disease caused by the environmental mycobacterium Mycobacterium ulcerans, is the third most important mycobacteriosis globally, after tuberculosis and leprosy (1, 9, 11). Laboratory diagnosis of BU is performed by the microscopic detection of acid-fast bacilli, cultivation of the pathogen from clinical specimens, identification of characteristic histopathological changes, and detection of M. ulcerans-specific DNA by PCR (10). The isolation of M. ulcerans from clinical samples is a slow and difficult process due to many factors that have been previously well documented (9, 10). Traditionally, BU case management has been wide surgical excision of BU lesions to remove infected tissues followed by skin grafting. Isolation of the causative agent therefore required reliably on tissue samples from the surgical interventions (4, 7, 12). Hence, we previously optimized M. ulcerans cultivation from such tissue specimens and achieved recovery rates of >75% (7, 12). However, in 2006, the World Health Organization (WHO) introduced a combination of rifampin and streptomycin or amikacin for 8 weeks as a first-line treatment for all patients. Nevertheless, the proportions of positive cultures, 41.7% (5/12) for FNA samples and 43.8% (49/112) for swab samples, were lower than those we have previously observed for excised tissue specimens (10), and clinical diagnosis was confirmed by a positive IS2404 PCR laboratory test (7). To prevent overgrowth by other bacteria, swabs were collected in a 15-ml conical tube (BD) containing 5 ml semisolid transport medium (4, 12) with 2% antibiotics (10). Within 24 h, samples (on ice) were transported from the participating health facilities of the Ga District (Amanasam District Hospital, Obom Health Centre, and Kojo Ashong Community Clinic) to the cultivation facility of the Noguchi Memorial Institute for Medical Research (Legon, Ghana) for analysis. Ethical clearance for the study was obtained from the institutional review board of the Noguchi Memorial Institute for Medical Research. One or two swab specimens were taken from the undermined edges of ulcerative lesions. In the latter case, the two samples were pooled together in 2 ml of phosphate-buffered saline (PBS) and vortexed for 2 min in 50-ml tubes containing glass beads (3-mm diameter; Merck, Germany) to disperse as much as possible all bacteria attached to the swab into the PBS. Five hundred microliters of bacterial suspension was used for DNA extraction with the QiAamp DNA minikit (Qiagen, Hilden, Germany). IS2404 PCR analysis was performed as described previously (7).

We evaluated three different decontamination procedures for the efficiency of decontamination, as well as for M. ulcerans recovery rates. Aliquots of suspensions prepared from 20 swab specimens of 20 PCR-positive patients were decontaminated in parallel using the Petroff method (12), the oxalic acid method (12), or a double decontamination method (the oxalic acid method followed by the Petroff method). After decontamination, specimens were inoculated onto two slants of Lowenstein-Jensen (LJ) medium. With either Petroff or oxalic acid single decontamination, 10/20 (50%) of the specimens were culture positive (Table 1). Contamination was observed with only one (5%) or two (10%) of the cultures after oxalic acid or Petroff decontamination, respectively. After double decontamination, 19/20 (95%) of the samples were culture negative; M. ulcerans growth was recorded for only one sample (Table 1). This find-

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† Published ahead of print on 16 March 2011.
TABLE 1. Result obtained with the different decontamination procedures that were evaluated in this study

<table>
<thead>
<tr>
<th>Decontamination method</th>
<th>No. (%) of cultures</th>
<th>Culture positive (n = 20)</th>
<th>No bacterial growth (n = 20)</th>
<th>Contaminated (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalic acid method</td>
<td></td>
<td>10 (50)</td>
<td>9 (45)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Petroff method</td>
<td></td>
<td>10 (50)</td>
<td>8 (40)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Double decontamination</td>
<td></td>
<td>1 (5)</td>
<td>19 (95)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

\* A culture was considered positive if at least one tube gave an isolate that was confirmed as *M. ulcerans* and contaminated if all tubes inoculated were overgrown by other bacteria.

In conclusion, our results prove that noninvasive sampling procedures such as FNA and swabbing are useful not only for case confirmation by PCR but also for primary culture of *M. ulcerans*. This finding, especially in the case of FNA, is very important. As part of our laboratory diagnostic services for the Ghana Health Service, we routinely receive swab samples from clinical diagnosed Buruli ulcer patients for confirmation of clinical diagnosis by IS2404 PCR. For this purpose, samples are generally shipped as dry swabs; they usually reach the laboratory between 7 days and 1 month after collection. Of 9 of such IS2404 PCR-positive samples cultured after oxalic acid decontamination, 3 (33.3%) were culture positive and 6 (66.7%) yielded no bacterial growth. This preliminary analysis indicates that dry samples routinely used for PCR-based reconfirmation of clinical diagnosis may also be suitable for culture-based surveillance for the potential emergence of antibiotic-resistant strains in remote areas of Buruli ulcer endemicity.

The diagnostic value of FNA specimens is well documented with respect to PCR and microscopy (2, 5, 8); however, the ability to isolate *M. ulcerans* from FNA samples is crucial with regard to the current mode of treatment. An important goal of Buruli ulcer surveillance and control strategies is early case detection. It is therefore envisaged that FNA sampling of non-ulcerative lesions will gain increasing importance as a diagnostic approach. At the same time, the availability of *M. ulcerans* isolates will be paramount for surveillance of drug susceptibility, analysis of recurrences, and molecular epidemiological studies. Our results demonstrate that *M. ulcerans* isolates can be obtained at reasonable frequency from FNA samples when sample collection and transport are optimal. However, culture positivity rates observed in this report for FNA and swab samples were lower than our previously reported rate (>75%) for tissue specimens (12). Due to the apparent lower sensitivity of isolation of *M. ulcerans* from swab and FNA specimens as a result of the amount of collected specimen by these two sampling procedures, one is tempted to advocate punch biopsy as a more appropriate sampling procedure. However, we are of the opinion that due to the invasiveness of punch biopsy, a new study assessing the improvement in isolation rate when the number of swabbed samples taken is increased will be needed. In this analysis, at most two swabs were collected per lesion; it may therefore be possible to further increase the culture positivity rate by increasing the surface area sampled per swab and/or by increasing the number of swabs collected per lesion. While Eddyani et al. (5) used the double decontamination procedure to successfully solve the problem of contaminations associated with primary cultivation of *M. ulcerans*, it was too harsh in our hands; in contrast, single decontamination of swab samples yielded satisfactory results.

In conclusion, our results prove that noninvasive sampling procedures such as FNA and swabbing are useful not only for case confirmation by PCR but also for primary culture of *M. ulcerans*. This finding, especially in the case of FNA, is very important.

![FIG. 1. Laboratory diagnosis of preulcerative Buruli ulcer lesion by Ziehl-Neelsen staining of a smear prepared from an FNA sample. The black arrows point to acid-fast bacilli.](http://jcm.asm.org/)
important, as it allows the raising of isolates even from early cases, which will be beneficial for addressing a number of biological and epidemiological questions. This is very crucial in the case of *M. ulcerans*, for which current knowledge is very scant. Some of the questions that need to be addressed using strain collections are the mode of transmission and drug susceptibility pattern analysis.

We appreciate the contributions of Benjamin Anku, Isaac Lamptey, Enoch Aniagyei, Edwin Ampadu, nurses involved in BU management in the Ga West and South Districts of Ghana, and the participants. We received funding from the Stop Buruli Consortium.

REFERENCES


