Techniques for Obtaining Specimens for Culture to Confirm Onychomycosis

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A study was conducted to determine whether fungal culture results in cases of suspected onychomycosis differ depending on the location from which the specimen is obtained. Specimens were taken from the nail plate, subungual debris, and nail bed in 30 patients with clinical evidence of onychomycosis. Cultures from the subungual debris were more likely to be positive for dermatophytes, nondermatophytic molds, and yeasts than were cultures from the deeper nail bed or nail plate. (J Am Podiatr Med Assoc 90(8): 394-396, 2000)

Onychomycosis, or fungal infection of the nail, is caused by dermatophytes (tinea unguium),1 nondermatophytic molds, or yeasts. The clinical presentation of onychomycosis has been well described by Zaias,2 who lists five types of the infection: distal or lateral subungual, superficial white, proximal subungual, candidal, and total dystrophic onychomycosis. The last type constitutes the long-term manifestation of the other types.

Distal or lateral subungual onychomycosis is the most common form. The nail plate must be lifted away from the nail bed in order for organisms to invade the nail. This can occur through acute or repetitive trauma or as a result of hyperkeratotic buildup at the hyponychium. Dermatophytes are associated with hyperkeratosis of the soles, including the area of the hyponychium; thus nail infection generally begins as tinea pedis and subsequently progresses to onychomycosis.2

Two types of culture medium are used to confirm onychomycosis. Both types may contain an antibiotic such as chlortetracycline, chloramphenicol, or gentamicin. However, cycloheximide, an antifungal agent that inhibits nondermatophytic molds, may or may not be added. Sabouraud dextrose agar, Littman oxgall agar, and inhibitory mold agar have no added cycloheximide, while dermatophyte test medium (DTM), mycologic agar, and mycobiotic agar contain cycloheximide.3

Reported estimates of the prevalence of specific fungal organisms in cases of onychomycosis range from 30% to 99% for dermatophytes and 2% to 57% for nondermatophytic molds and yeasts.2, 4-11 This wide variation in the prevalence of organisms reported in different studies may be due to the fact that there are a variety of techniques for obtaining specimens for culture. Another factor in this variation may be noncomparable patient populations; for example, patients may be from different geographic regions or have significant age differences. Fingernails and toenails may be included in some studies, whereas others may include specimens from only one anatomical area. Laboratory techniques may vary across studies, with some laboratories using nonselective media and others using selective media.

Dermatophytes have long been considered the primary pathogens in onychomycosis because they are keratinolytic, while nondermatophytic molds and yeasts are not. These latter organisms live on partially denatured keratin and intracellular cement and have

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been viewed as secondary invaders of the nail, acting only after the onset of disease or trauma. Support for this view is provided by a study by Ellis et al. that showed that the presence of nondermatophytic fungal organisms in addition to dermatophytes did not affect outcomes of treatment with oral terbinafine.

Numerous methods of obtaining specimens for culture in cases of distal and lateral subungual onychomycosis have been described. Specimens may be obtained from three areas: 1) the nail plate, in the form of scrapings, clippings, or particles produced by electrical debridement; 2) the superficial nail bed (subungual debris; it is usually recommended that the debris be obtained from the proximal or leading edge of the presumed fungal involvement); and 3) the deeper nail bed after removal of the overlying debris. The presumption of those recommending the second or third method is that the pathogenic organisms primarily invade skin (tinea pedis) and only secondarily affect the nail plate.

Some authors are adamant that a particular method of obtaining nail specimens for culture be used. The current authors, however, were unable to find any studies in the literature that compare results of cultures using different techniques for obtaining specimens.

The purpose of this study was to determine whether culture results from specimens obtained from toenails with clinical evidence of distal or lateral subungual onychomycosis vary depending on the location from which the specimen is taken. If the culture results did vary, the authors also wanted to determine whether any particular specimen location was more likely to be associated with positive cultures. Of special interest was whether dermatophytes, the presumed pathogens, were more likely to grow in specimens taken from a particular location.

Materials and Methods

The study population consisted of 30 patients (25 men and 5 women) recruited from the general podiatry clinic at the California College of Podiatric Medicine in San Francisco. The age range of subjects was 28 to 84 years, with an average age of 60. Each patient had received a clinical diagnosis of distal or lateral subungual onychomycosis. Informed consent was obtained from all patients prior to the beginning of the study.

Three specimens were taken from the involved nail area of each patient. A specimen from the nail plate was taken by first cleaning the dorsal surface of the nail with alcohol and then scraping the area with a scalpel blade. A nail nipper was used to obtain small clippings of the plate at the proximal leading edge of the nail. The nail was then trimmed back to reveal the nail bed. A specimen of subungual debris was obtained with a curette placed as far proximally as possible without causing the patient discomfort. Finally, a specimen from the deeper nail bed was obtained by scraping the bed with a scalpeld blade after removal of the overlying debris.

Each specimen was implanted into Sabouraud dextrose agar and DTM. Cultures were evaluated for up to 6 weeks after implantation. A Gram’s stain was performed on smooth-textured colonies to determine whether they consisted of yeast or bacteria. Species of dermatophytes, nondermatophytic molds, and yeasts were identified by direct microscopy.

Results

Twenty-five of the 30 patients (83%) had cultures that were positive for fungal organisms (Table 1). Nineteen of these 25 patients (76%) had cultures that were positive for dermatophytes. Specimens taken from the subungual debris produced significantly more fungal organisms than did specimens taken from the nail plate or the deeper nail bed (P < .01, using chi-square goodness-of-fit analysis for frequency distribution). This was true of dermatophytes, nondermatophytic molds, and yeasts (Fig. 1). In 18 of the 19 patients with cultures positive for dermatophytes, the dermatophytes were found in the specimens from the subungual debris. In the remaining patient the dermatophyte was found only in the specimen taken from the nail plate.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton rubrum</td>
<td>16</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>5</td>
</tr>
<tr>
<td>Trichophyton tonsurans</td>
<td>1</td>
</tr>
<tr>
<td>Penicillium</td>
<td>3</td>
</tr>
<tr>
<td>Scopulariopsis</td>
<td>2</td>
</tr>
<tr>
<td>Alternaria</td>
<td>1</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>1</td>
</tr>
<tr>
<td>Chrysosporium</td>
<td>1</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>1</td>
</tr>
<tr>
<td>Rhodotorula</td>
<td>1</td>
</tr>
<tr>
<td>Scytalidium dimidiatum</td>
<td>1</td>
</tr>
<tr>
<td>Yeast (non–Candida albicans)</td>
<td>4</td>
</tr>
</tbody>
</table>

Note: The number of patients is more than 30 because the culture results of some patients revealed the presence of more than one organism.
Discussion

These results show that positive fungal cultures are significantly more likely to be obtained with specimens taken from the subungual debris, as compared with those from the nail plate or the deeper nail bed area. Given that distal and lateral subungal onychomycosis begins as tinea pedis and that the nail plate is dry and thus inhospitable to living organisms, it is not surprising that cultures of the nail bed are more likely than those of the nail plate to show growth of organisms. However, the question remains as to why cultures from the superficial nail bed (subungual debris) are more likely than those of the deeper nail bed to show growth of organisms. The authors believe that there are two possible explanations for this.

First, there is an abundance of specimen material in the nail debris, as compared with the deeper nail bed, once the overlying debris has been removed. The more specimen material that is obtained, the greater the likelihood of viable organisms being transferred to the culture media. Second, fungal organisms in the subungual debris are further removed from the body’s immune defenses than those in the area of the deep nail bed and are thus more likely to remain viable.

It is interesting to note that the proportion of nondermatophytic molds and yeasts increases as the specimen becomes more superficial. In specimens from the deep nail bed, 23% of the organisms found were nondermatophytes, compared with 38% and 45% for the subungual debris and the nail plate, respectively. These findings support the theory that nondermatophytic molds and yeasts are contaminants rather than the pathogenic organisms responsible for the primary infection in most cases of onychomycosis.

Summary

The current study is the only study in the literature that compares techniques for obtaining specimens for fungal culture in cases of onychomycosis. The results show that specimens taken from the subungual debris are more likely than specimens taken from the deeper nail bed or the nail plate to be positive for dermatophytes as well as nondermatophytic molds and yeasts. The authors hope that this information will be helpful in reducing the need for multiple cultures, thereby decreasing the cost of patient care. Additionally, improving techniques for obtaining specimens for fungal culture will increase the accuracy and reliability of fungal culture results, ultimately enhancing patient care.

References