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Comparative study of different microscopic techniques and culture media for the isolation of dermatophytes

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Abstract

PURPOSE: To evaluate the usefulness of two different microscopic techniques and three different culture media for the identification and isolation of dermatophytes from clinical samples. METHODS: Skin, hair and nail samples from 260 clinically suspected cases of dermatophytosis were screened by direct microscopic examination using 10% potassium hydroxide (KOH) with and without 40% dimethyl sulphoxide (DMSO) mounts. All the samples were inoculated for culture in Sabouraud dextrose agar (SDA), dermatophyte test medium (DTM) and ready to use enriched dermatophyte medium (EDM). RESULTS: Fungal elements were detected in 157 samples by both the methods but faster and better visualization was noted with 40% DMSO added to 10% KOH. Fungi were recovered from SDA, DTM and EDM in 96.5%, 98.3% and 85.3% of the cases respectively. CONCLUSIONS: When performing direct microscopic examination of skin, hair and nail for dermatophytes, addition of 40% DMSO to the KOH mount gives better and faster results. The efficiency of SDA and DTM was found almost equal and slightly better than EDM. The EDM, although quite efficient with 85.3% isolation rate, requires further evaluation as its ready to use format makes the application and microscopy much easier and faster.

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Full Text

Fungal infections are very common in man. They are assuming greater significance both in developed and developing countries particularly due to advent of immunosuppressive drugs and disease.[1] Hot and humid climate in the tropical and subtropical countries like India makes dermatophytosis or ringworm a very common superficial fungal skin infection. Dermatophytosis is caused by dermatophytes, a group of keratinophilic fungi that require long incubation period to grow. The clinical presentation, though very typical of ringworm infection, is very often confused with other skin disorders particularly due to rampant application of broad-spectrum steroid containing skin ointments and creams leading to further misdiagnosis and mismanagement. Keeping in mind the need for correct, rapid and efficient laboratory diagnosis of dermatophytosis, the comparative evaluation of two clearing agents for microscopic examination and three different culture media for fungal isolation was undertaken.

Material and Methods

Samples were collected from 260 clinically suspected cases of ringworm infection between July 1999 and August 2000, attending the outpatient department of Skin and V.D. department of Shree Sayaji Rao General Hospital, Baroda, Gujarat. Suspected lesions were cleaned with 70% alcohol to remove the dirt and contaminating bacteria. Samples were collected in sterile paper, folded, labeled and brought to the laboratory for further processing.

For direct microscopy the sample collected was screened for the presence of fungal elements by two methods: 10% Potassium hydroxide mount (KOH) and 10% Potassium hydroxide (KOH) with 40% Dimethyl Sulfoxide Mount (DMSO) mixed in equal proportion.

10% KOH Mount

A drop of 10% KOH was kept on a clean, grease free glass slide. The sample (hair, skin and nail clipping) was placed in the KOH drop and slide passed through a burner flame to hasten keratolysis. When keratolysis softened the sample, a clean glass cover slip was kept on the sample and pressed, preventing the formation of air bubbles.

The sample was kept in KOH for a variable duration ranging from 5 minutes to 30 minutes, depending upon the thickness of the scales and examined every 5 minutes. Each slide was thoroughly examined for the presence of filamentous, septate, branched hyphae with or without arthrospores crossing the margins of the squamous epithelial cells of the skin. In case of hair, type and arrangement of the spore was noticed to name it as ectothrix or endothrix type of infection.

10% KOH with 40% DMSO

The sample was processed in the fashion similar to KOH mount except that sample was kept on a slide with 10% KOH with 40% DMSO. The slide was not passed through flame and was screened for the presence of fungus within 5 minutes.

Culture

For primary isolation of dermatophytes following media were used:

a. Sabouraud Dextrose Agar (SDA) with antibiotics (Himedia)

b. Dermatophytes Test Medium (DTM) with supplement (Himedia)

c. Enriched Dermatophytes Medium (EDM) (InTrayTM DM Biomed Diagnostics, San Jose, California, USA.)

The EDM medium contains soytone, carbohydrate, growth stimulants, antimicrobial agent including cycloheximide, color indicator and agar in distilled water dispensed in the form of a layer of media in circular window covered with a protective seal at one corner of the tray. In the EDM, sample was inoculated by pulling back the lower right corner adjacent to the clear window (around the size of a rupee coin) until the protective seal over the agar was completely visible. The seal was removed by pulling the tab and was discarded. Hair, nail or skin scraping were inoculated on the surface of the medium with the sterile inoculating loop or forceps. Pressing together the edges of the lid against the plastic tray resealed the tray.

The SDA and DTM were inoculated in duplicate; one incubated at 300C and other at 370C for 3 weeks while EDM was inoculated in single and incubated at room temperature (18-250C) as per manufacturers instructions. SDA was taken as standard media for primary isolation and other two were compared with it. Isolation of dermatophytes was confirmed by gross morphology of growth, typical microscopic characteristics, supplemented with hair perforation and slide culture as and when needed. To compare the efficiency of the three media used for primary isolation of dermatophytes, Chi square test and standard error of difference between two proportions was applied.

Results

A comparison of the direct microscopy and culture results is shown in [Table:1]. It is evident from the table that out of total 260 samples examined, 157 (60.38%) showed the evidence of fungal elements on direct microscopy, out of which 106 turned out to be positive on culture. Ten samples, which were culture positive, were negative on microscopic examination, making a total of 116 (44.61%) samples culture positive. Thus out of 260 sample studied 144 (55.3%) did not show evidence of the fungi either on direct microscopy or culture.

When doing direct microscopic examination it was observed that DMSO produced rapid clearing of keratin and faster visualization of fungal hyphae as all the samples could be examined within 5 minutes compared to plain KOH which required 10 to 15 minutes for complete clearing of keratin. When compared for nail clippings 10 minutes were adequate with DMSO and more than 30 minutes was required with plain KOH mount. The detection rate of fungal elements by both the methods remained same.

As seen in [Table:2], 97 samples were culture positive on all the three media while 15 were positive on both SDA and DTM. Two cultures were isolated only on DTM and two only on EDM.

Fungi were isolated from SDA in 112 (96.55%), DTM in 114 (98.27%) and EDM in 99 (85.34%) of 116 cases. While the difference between SDA

and DTM was statistically not significant, it was significant between SDA and EDM.

Discussion

Aqueous potassium hydroxide (KOH) has been used as a clearing agent for direct demonstration of fungi in skin, nail or hair scrapings[1] but addition of dimethyl sulphoxide as described by Rebell et al[2] in 1971 was found to be a better preparation over plain KOH.[2],[3],[4] Addition of DMSO permits rapid clearing of keratin and almost immediate examination of sample without warming of slide.[4] It also prevents rapid drying of the fluid and thus is a better option. KOH preparation tends to absorb carbon dioxide from air and form carbonate crystals thus reducing the effective hydroxide.[4] Also, hydroxide preparation tends to saponify when gently heated thus forming fat globules in the slide and reducing effective visualization of fungal hyphae. The faster keratolysis by addition of DMSO is probably due to increased transport of chemicals through the stratum corneum.[5],[6]

The metabolic end products of dermatophytes are such that an increase in the alkalinity of the surrounding medium is noticed in contrast to saprophytic fungi, which make the medium acidic.[7],[8] The early release of alkali is supposed to be of importance in the attack of keratin by the fungus.[9] This property has been used to prepare media using indicators for isolation of dermatophytes for rapid presumptive identification, particularly useful for non-mycologist as results can be evaluated simply by colour change in the medium without detailed knowledge of morphology. Ink blue agar, DTM and EDM are the media prepared using indicators.[8] Taplin et al,[10] who introduced the DTM, found it to be a better preparation over the mycosal agar, where as we have found DTM to be as efficient as SDA in primary isolation of dermatophytes (p=>0.05).

The comparative evaluation of the isolation of dermatophytes on SDA and DTM has been reported by Yavuzdemir who found no significant difference in the isolation rates of these media.[11] The effectiveness of SDA was 93.5% and that of DTM was 95.4% in his study of 225 samples. We found SDA to be 96.55% and DTM 98.27% effective in isolation of dermatophytes. The efficiency of SDA and DTM was found almost equal and slightly better than EDM. The isolation rate for EDM is 85.3% which though significantly lower than SDA and DTM is not really bad considering other advantages of EDM over SDA and DTM such as it is ready to use and it makes the application and microscopy much easier and faster. It is easy to handle, inoculate, incubate and store. The microscopic examination can be done by direct visualization of the EDM in tray under microscope, thus preventing exposure to infectious material. The main advantage of using EDM is a very short incubation period. The dermatophytes grew within a day or two in this study. The maximum incubation period was of ten days whereas SDA and DTM gave positive results on culture after a week and required to be incubated at least for three weeks before being reported as negative. Thus a rapid diagnosis can be made with EDM. However, it requires further evaluation using more number of samples.

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