Original Article

Studies on microscopic technique and culture on Sabouraud's dextrose agar medium for diagnosis of dermatophytes infection.

Khan AH[1], Khan SMAH[2], Sarker MR[3], Hussain RF[4],
Ahmed MU[5], Joarder Y[6], Hasan MB[7],

Abstract:

Dermatophytoses (a fungal infection of the skin, hair and nail, usually caused by dermatophytes) constitutes an important public health problem because of its high prevalence and associated morbidity but not life-threatening. Three genera of dermatophytes are recognized based on the site and pattern of fungal invasion. Dermatophytes are the predominant pathogenic mould, but yeasts (especially Candida albicans) and non-dermatophytic moulds may also be implicated. For accurate diagnosis of dermatophytoses requires microscopic demonstration and isolation and identification by culture. This study evaluates the usefulness of microscopic technique and culture for the isolation and identification of dermatophytes from clinical samples. Thirty samples were included in this study for detection of fungal elements by both methods but sensitivity of microscopic demonstration and culture were 60.0% and 66.7% respectively. As the sensitivity of microscopic demonstration (60.0%) is almost equal to the isolation and identification rate (66.7%), requires further evaluation in large scale as its ready to use format makes the application and microscopy much easier and faster.

Key word: *Dermatophytes, Sabouraud's dextrose agar medium, Hyphae.*

Introduction

Fungal infections are very common in human. 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, Bangladesh makes dermatophytosis or ringworm a very common superficial fungal skin infection 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

1. Dr. Md. Abul Hossain Khan, Associate Professor & Head, Department of Microbiology, Khwaja Yunus Ali Medical College, Enayetpur-Sirajgonj.
2. Dr. S.M. Atique Hasan Khan, Assistant Professor, Department of Dermatology and Venerology, Khwaja Yunus Ali Medical College, Enayetpur-Sirajgonj.
3. Dr. Mahbub Rashid Sarker, Pathologist, Department of Laboratory Services, Khwaja Yunus Ali Medical College, Enayetpur-Sirajgonj.
4. Dr. Rubiyat Farzana Hussain, Assistant Professor, Department of Community Medicine, Khwaja Yunus Ali Medical College, Enayetpur-Sirajgonj.
5. Dr. Mejbah Uddin Ahmed, Associate Professor, Department of Microbiology, Enam Medical College, Saver-Dhaka.
6. Dr. Yasmin Joarder, Assistant Professor, Department of Dermatology and Venerology, Northern International Medical College, Dhanmondi-Dhaka.
7. Dr. Md. Bulbul Hasan, Assistant Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.

spectrum steroid containing skin ointments and creams leading to further misdiagnosis and mismanagement. So, rapid and efficient laboratory diagnosis of dermatophytoses, the comparative evaluation of microscopic examination and isolation and identification by culture on Sabouraud's dextrose agar (SDA) medium for fungal isolation was undertaken.

Material and Methods

This cross sectional study done in the department of laboratory services of KYAMCH and samples were collected from 30 clinically suspected cases of ringworm infection on skin between March 2010 and February 2011, attending in the outpatient department of Skin and V.D department of Khwaja Yunus Ali Medical College Hospital, Enayetpur, Sirajgonj. Suspected lesions were cleaned with 70% alcohol to remove the dirt and contaminating bacteria. Samples were collected in sterile black paper and then folded, labeled and brought to the laboratory for further processing. For direct microscopy the collected sample was screened for the presence of fungal elements by 20% Potassium hydroxide mount (KOH) and then processed for culture on SDA medium.

20% KOH Mount method

A drop of 20% KOH was kept on a clean, grease free glass slide. The sample (skin scraping) was placed in the KOH drop on slide and wait for 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 30 minutes to 120 minutes, depending upon the thickness of the scales and examined every 15 minutes interval. 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.

Culture

For primary isolation of dermatophytes, SDA with antibiotics (Oxoid, UK) medium was used. Skin scraping was inoculated on the surface of the medium with the sterile inoculating loop or forceps. The SDA was incubated at 30oC upto three weeks. Isolated dermatophytes were identified by gross morphology of growth, typical microscopic characteristics of hyphae after stained with lactophenol cotton blue and with hair perforation test. To compare the microscopic finding and culture on SDA by Chi-square test and standard error of difference between two proportions was applied.

Results

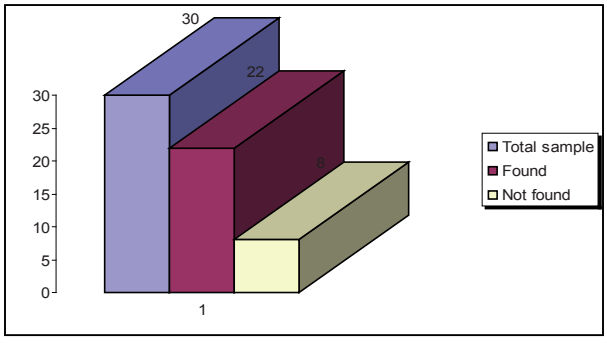
Direct microscopic finding is shown in Table 1 and Figure 1. It was evident from the table that out of total 30 samples examined, 22 (73.3%) showed the evidence of fungal elements on direct microscopy, among them all were skin specimens.

Table 1: Distribution of microscopic findings among the specimen

Microscopic findings	Specimen(Skin scraping)
Found	22(73.3%)
Not found	8(26.7%)
Total	30(100%)

Chi-Square 11.250, df 1 and Significance. 0.001

Figure 1: Distribution of microscopic findings among the specimen



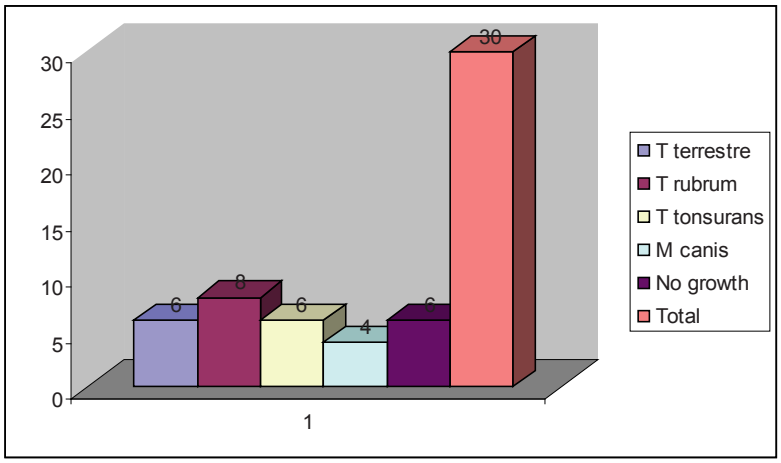
Twenty four specimens among the 30 turned out to be positive on culture after 14 days at 25o C to 30o C temperature incubation shown in table 2 and figure 2. Six samples, which were culture negative, on the other hand eight specimens were negative on microscopic examination, making a total of 24(80.0%) samples culture positive. Thus out of 30 sample studied 6(20.0%) did not show evidence of the fungi on culture and 26.7 % on direct microscopy. Among the growth of dermatophytes Trichophyton rubrum, Trichophyton terrestre, and Trichophyton tonsurans were the top of the list.

Table 2: Distribution of Isolated fungi among the specimen

Sl. No	Fungal species	Specimen (Skin scraping)
01	*Trichophyton terrestre*	6(25.0%)
02	*Trichophyton rubrum*	8(25.0%)
03	*Trichophyton tonsurans*	6(25.0%)
04	*Microsporum canis*	4(8.3%)
05	No growth	6(16.7%)
06	Total	30(100%)

Chi-Square 15.000, df 4 and level of Significance is 0.005

Figure 2: Distribution of Isolated fungi among the specimen



Discussion

Aqueous potassium hydroxide (KOH) has been used as a clearing agent for direct demonstration of fungi in skin scrapings[1] but addition of culture as described by Rebell in 1991 was found to be relatively better than microscopy[2]. KOH preparation tends to absorb carbon dioxide from air and form carbonate crystals thus reducing the effective hydroxide as well as, hydroxide preparation tends to saponify when gently heated thus forming fat globules in the slide and reducing effective visualization of fungal hyphae[3].

The metabolic end products of dermatophytes were such that an increase in the alkalinity of the surrounding medium was noticed in contrast to saprophytic fungi, which make the medium acidic[4,5]. The early release of alkali was supposed to be of importance in the attack of keratin by the fungus[6] .This property has been used to prepare media using indicators for isolation of dermatophytes for rapid presumptive identification on SDA in primary isolation of dermatophytes (p=>0.05). The comparative evaluation of the isolation of dermatophytes on SDA and direct microscopy of KOH preparation has been reported by Yavuzdemir who found no significant difference between these two methods[7]. The effectiveness of SDA was 80.0% and that of direct after 20% KOH preparation was 73.3% in his study of 30 samples. The efficiency of SDA and direct KOH preparation was found almost equal. The isolation rate for dermatophytes which though

significantly higher than microscopy but later technique was ready to use and it makes the application and microscopy much easier and faster, on the other hand isolation on SDA gave positive results on culture after two weeks and required to be incubated at least for four weeks before being reported as negative. Thus a rapid diagnosis of dermatophytosis can be made with microscopic demonstration of fungal element after 20% KOH preparation. However, it requires further evaluation using more number of samples.

Acknowledgements

We thank Trustee Board, KYAMCH, Enayetpur-Sirajgonj for providing assistance in the form of Enriched dermatophyte media (SDA) for this study.

References

1. Chandra J. A Textbook of Medical Mycology. (Mehta Publishers, New Delhi) 1996; 67-79.

2. Rebell G, Taplin D. Dermatophytes. Their recognition and identification. 2nd ed. (Miami University Press, Miami) 1970.

3. Mehta JP, Deodhar KP, Mehta VR, Chapnekar PM. A study of dermaotmycosis in Bombay. Indian J Pathol Microbiol 1977; 20(1):23-31.

4. Stockdale PM. Nutritional requirements of the dermatophytes. Biological reviews 1952:84-100.

5. Baxter M. The use of Ink Blue in the identification of dermatophytes. J Invest Dermatol 1964; 23-25.

6. Goldfarb NJ, Hermann F. A-study of pH changes by molds in culture media. J Invest Dermatol 1956; 27:193-201.

7. Yavuzdemir S. Comparative evaluation of the isolation of the dermatophytes by direct laboratory evidence and MSDA with MDTM culture media. Microbiol Bulletin 1992; 26(4):367-72.